PROCEDINGS

NINETY-THIRD

ANNUAL MEETING

of the

UNITED STATES ANIMAL

HEALTH ASSOCIATION

RIVIERA HOTEL
LAS VEGAS, NEVADA

October 28–November 3, 1989
This book is dedicated in memory to the members of USAHA who passed away in 1989.

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<td>Dr. John F. Hudelson</td>
<td>Parachute, CO</td>
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<tr>
<td>Dr. Norman W. Kruse</td>
<td>Berthoud, CO</td>
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<tr>
<td>Dr. S. R. Nusbaum</td>
<td>Trenton, NJ</td>
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<tr>
<td>Dr. J. B. Thomas</td>
<td>Elgin, SC</td>
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<tr>
<td>Dr. D. K. Thorpe</td>
<td>Pierre, SD</td>
</tr>
<tr>
<td>Dr. David U. Walker</td>
<td>Morrisville, VT</td>
</tr>
</tbody>
</table>
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Dr. Lewis P. Thomas, Charleston, WV
Dr. Janice Webb, Washington, DC
Dr. Sharon Williams, Hyattsville, MD
Mr. Larry D. Woodson, Topeka, KS
<table>
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<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tr>
<td>1. Sept. 27-28, 1897†</td>
<td>Fort Worth, TX</td>
<td>*Mr. C P. Johnson, Springfield, IL</td>
<td>*Mr. D. O. Lively, Fort Worth, TX</td>
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<tr>
<td>2. Oct. 11-12, 1898</td>
<td>Omaha, NE</td>
<td>*Mr. C P. Johnson, Springfield, IL</td>
<td>*Mr. Taylor Riddle, KS</td>
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<td>3. Oct. 11-12, 1899†</td>
<td>Chicago, IL</td>
<td>*Mr. C P. Johnson, Springfield, IL</td>
<td>*Mr. Mortimer Levering, Lafayette, IN</td>
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<td>4. Oct. 2-3, 1900</td>
<td>Louisville, KY</td>
<td>*Mr. C P. Johnson, Springfield, IL</td>
<td>*Dr. E. T. Eisenman, Louisville, KY</td>
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<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, NY</td>
<td>*Dr. E. P. Niles, VA</td>
<td>*Dr. E. T. Eisenman, Louisville, KY</td>
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<td>6. Sept. 23-24, 1902</td>
<td>Wichita, KS</td>
<td>*Mr. W. H. Dunn, TN</td>
<td>*Mr. Wm. P. Smith, Monticello, IL</td>
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<td>7. Sept. 22-23, 1903</td>
<td>Denver, CO</td>
<td>*Mr. W E. Bolton, Woodward, OK</td>
<td>*Mr. Wm. P. Smith, Monticello, IL</td>
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<td>8. Aug. 23-24, 1904</td>
<td>St. Louis, MO</td>
<td>*Dr. J. C. Norton, AZ</td>
<td>*Mr. Wm. P. Smith, Monticello, IL</td>
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<td>9. Aug. 15-16, 1906</td>
<td>Guthrie, OK</td>
<td>*Mr. Wm. P. Smith, Monticello, IL</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
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<tr>
<td>10. Aug. 15-16, 1906</td>
<td>Springfield, IL</td>
<td>*Mr. M. M. Hankins, Quanah, TX</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
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<tr>
<td>11. Sept. 16-17, 1907</td>
<td>Richmond, VA</td>
<td>*Dr. D. F. Luckey, Columbia, MD</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>14. Dec. 5-7, 1910</td>
<td>Chicago, IL</td>
<td>*Dr. C. E. Cotton, St. Paul, MN</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>15. Dec. 5-6, 1911</td>
<td>Chicago, IL</td>
<td>*Dr. John F. Devine, Goshen, NY</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>16. Dec. 3-5, 1912</td>
<td>Chicago, IL</td>
<td>*Dr. Macyck P. Ravenel, Madison, IL</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>17. Dec. 2-4, 1913</td>
<td>Chicago, IL</td>
<td>*Dr. Peter F. Bahnsen, Atlanta, GA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>18. Feb. 16-18, 1914</td>
<td>Chicago, IL</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>19. Dec. 2-3, 1915</td>
<td>Chicago, IL</td>
<td>*Dr. J. I. Gibson, Des Moines, IA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>20. Dec. 5-7, 1916</td>
<td>Chicago, IL</td>
<td>*Dr. O. E. Dyson, Springfield, IL</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>21. Dec. 3-5, 1917</td>
<td>Chicago, IL</td>
<td>*Dr. J. G. Wills, Albany, NY</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>22. Dec. 2-4, 1918</td>
<td>Chicago, IL</td>
<td>*Dr. M. Jacob, Knoxville, TN</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>23. Dec. 1-3, 1919</td>
<td>Chicago, IL</td>
<td>*Dr. G. W. Dumphy, Lansing, MI</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>24. Nov. 29-Dec. 1, 1920</td>
<td>Chicago, IL</td>
<td>*Dr. S. F. Musseiman, Frankfort, KY</td>
<td>*Dr. D. M. Campbell, Chicago, IL</td>
</tr>
<tr>
<td>25. Nov. 28-30, 1921</td>
<td>Chicago, IL</td>
<td>*Dr. W. F. Crewe, Bismarck, ND</td>
<td>*Dr. D. M. Campbell, Chicago, IL</td>
</tr>
<tr>
<td>26. Dec. 6-8, 1922</td>
<td>Chicago, IL</td>
<td>*Dr. T E. Munce, Harrisburg, PA</td>
<td>*Dr. Theo. Burnett, Columbus, OH</td>
</tr>
<tr>
<td>27. Dec. 5-7, 1923</td>
<td>Chicago, IL</td>
<td>*Dr. W. J. Butler, Helena, MT</td>
<td>*Dr. Theo. Burnett, Columbus, OH</td>
</tr>
<tr>
<td>28. Dec. 3-5, 1924</td>
<td>Chicago, IL</td>
<td>*Dr. J. G. Ferneyhough, Richmond, VA</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>29. Dec. 2-4, 1925</td>
<td>Chicago, IL</td>
<td>*Dr. J. H. McNeil, Trenton, NJ</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>30. Dec. 1-3, 1926</td>
<td>Chicago, IL</td>
<td>*Dr. John R. Mohler, Washington, DC</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>31. Nov. 30-Dec. 2, 1927</td>
<td>Chicago, IL</td>
<td>*Dr. L Van Es, Lincoln, NE</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>32. Dec. 5-7, 1928</td>
<td>Chicago, IL</td>
<td>*Dr. C. A. Cary, Auburn, AL</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>33. Dec. 4-6, 1929</td>
<td>Chicago, IL</td>
<td>*Dr. Chas. O. Lamb, Denver, CO</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
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<tr>
<td>34. Dec. 3-5, 1930</td>
<td>Chicago, IL</td>
<td>*Dr. A. E. Wight, Washington, DC</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>35. Dec. 2-4</td>
<td>Chicago, IL</td>
<td>*Dr. J. W. Combs, Columbia, MD</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>34. Nov. 30-Dec. 2, 1932</td>
<td>Chicago, IL</td>
<td>*Dr. Peter Malcolm, Des Moines, IA</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>37. Dec. 6-8, 1933</td>
<td>Chicago, IL</td>
<td>*Dr. E. T. Faulder, Albany, NY</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>38. Dec. 5-7, 1934</td>
<td>Chicago, IL</td>
<td>*Dr. T. E. Robinson, Providence, RI</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>39. Dec. 4-6, 1935</td>
<td>Chicago, IL</td>
<td>*Dr. Edward Records, Reno, NV</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
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<tr>
<td>40. Dec. 2-4, 1936</td>
<td>Chicago, IL</td>
<td>*Dr. Walter Wisnicky, Madison, WI</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>41. Dec. 1-3, 1937</td>
<td>Chicago, IL</td>
<td>*Dr. R. W. Smith, Concord, NH</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
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<tr>
<td>42. Nov. 30-Dec. 2, 1938</td>
<td>Chicago, IL</td>
<td>*Dr. D. E. Westmoreland, Frankfort, KY</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
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<tr>
<td>43. Dec. 6-8, 1939</td>
<td>Chicago, IL</td>
<td>*Dr. J. L. Axby, Indianapolis, IN</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>44. Dec. 4-6, 1940</td>
<td>Chicago, IL</td>
<td>*Dr. H. D. Polk, Cheyenne, WY</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>45. Dec. 3-5, 1941</td>
<td>Chicago, IL</td>
<td>*Dr. E. A. Crossman, Boston, MA</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>46. Dec. 2-4, 1942</td>
<td>Chicago, IL</td>
<td>*Dr. I. S. McAdory, Auburn, AL</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>47. Dec. 1-3, 1943</td>
<td>Chicago, IL</td>
<td>Dr. W. H. Hendricks, Salt Lake City, UT</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
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<tr>
<td>48. Dec. 6-8, 1944</td>
<td>Chicago, IL</td>
<td>Dr. J. M. Sutton, Atlanta, GA</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>49. Dec. 5-7, 1945</td>
<td>Chicago, IL</td>
<td>Dr. C. U. Duckworth, Sacramento, CA</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>50. Dec. 4-6, 1946</td>
<td>Chicago, IL</td>
<td>*Dr. William Moore, Raleigh, NC</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>51. Dec. 3-5, 1947</td>
<td>Chicago, IL</td>
<td>*Dr. Will J. Miller, Topeka, KS</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>52. Oct. 13-15, 1948</td>
<td>Denver, CO</td>
<td>*Dr. Jean V. Knapp, Tallahassee, FL</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>53. Oct. 12-14, 1949</td>
<td>Columbus, OH</td>
<td>*Dr. T. O. Brandenburg, Bismarck, ND .</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>54. Nov. 1-3, 1950</td>
<td>Phoenix, AZ</td>
<td>*Dr. C. P. Molin, Denver, CO</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>55. Nov. 14-16, 1951</td>
<td>Kansas City, KS</td>
<td>*Mr. F. E. Molin, Denver, CO</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>56. Oct. 29-31, 1952</td>
<td>Louisville, KY</td>
<td>Dr. Ralph L. West, St. Paul, MN</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>57. Sept. 23-25, 1953</td>
<td>Atlantic City, NJ</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>58. Nov. 10-12, 1954</td>
<td>Omaha, NE</td>
<td>*Dr. T. C. Green, Charleston, WV</td>
<td>*Dr. L. Enos Day, Chicago, IL</td>
</tr>
<tr>
<td>59. Nov. 16-18, 1955</td>
<td>New Orleans, LA</td>
<td>Dr. H. E. Wilkins, Helena, MT</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
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<tr>
<td>60. Nov. 28-30, 1956</td>
<td>Chicago, IL</td>
<td>Dr. A. L. Brueckner, Baltimore, MD</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>61. Nov. 13-15, 1957</td>
<td>St. Louis, MO</td>
<td>Dr. G. H. Good, Cheyenne, WY</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, FL</td>
<td>Dr. John G. Milligan, Montgomery, AL</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
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<tr>
<td>63. Nov. 15-18, 1959</td>
<td>San Francisco, CA</td>
<td>Mr. F. G. Buzzell, Augusta, MB</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>64. Oct. 17-21, 1960</td>
<td>Charleston, WV</td>
<td>*Dr. J. R. Hay, Chicago, IL</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>65. Oct. 3-Nov. 3, 1961</td>
<td>Minneapolis, MN</td>
<td>Dr. A. P. Schneider, Boise, ID</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
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<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
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<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, NM</td>
<td>Dr. T. J. Grennan, Jr., Providence, RI</td>
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<tr>
<td>69. Oct. 25-29, 1965</td>
<td>Lansing, MI</td>
<td>Dr. J. W. Safford, Helena, MT</td>
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<td>70. Oct. 10-14, 1966</td>
<td>Buffalo, NY</td>
<td>Dr. C. L. Campbell, Tallahassee, FL</td>
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<td>71. Oct. 16-20, 1967</td>
<td>Phoenix, AZ</td>
<td>Dr. Grant S. Kaley, Albany, NY</td>
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<td>72. Oct. 6-11, 1968</td>
<td>New Orleans, LA</td>
<td>Dr. John F. Quinn, Lansing, MI</td>
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<td>75. Oct. 24-29, 1971</td>
<td>Oklahoma City, OK</td>
<td>Dr. M. D. Mitchell, Pierre, SD</td>
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<tr>
<td>76. Nov. 5-10, 1972</td>
<td>Miami Beach, FL</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
<td></td>
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<td>77. Oct. 14-19, 1973</td>
<td>St. Louis, MO</td>
<td>Dr. W. C. Tobin, Denver, CO</td>
<td></td>
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<tr>
<td>79. Nov. 2-7, 1975</td>
<td>Portland, OR</td>
<td>Dr. J. E. Andrews, GA</td>
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<td>80. Nov. 7-12, 1976</td>
<td>Miami Beach, FL</td>
<td>Dr. H. E. Goldstein, Columbus, OH</td>
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<tr>
<td>81. Oct. 16-21, 1977</td>
<td>Minneapolis, MN</td>
<td>Dr. A. E. Janawicz, Montpelier, VT</td>
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<tr>
<td><strong>82. Oct. 21-Nov. 3, 1978</strong></td>
<td>Buffalo, NY</td>
<td>Dr. L. E. Bartelt, Sacramento, CA</td>
<td></td>
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<tr>
<td>83. Oct. 28-Nov. 2, 1979</td>
<td>San Diego, CA</td>
<td>Dr. T. F. Zweigart, Raleigh, NC</td>
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<td>84. Nov. 2-7, 1980</td>
<td>Louisville, KY</td>
<td>Dr. B. W. Hawkins, Ontario, OR</td>
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<td>85. Oct. 11-16, 1981</td>
<td>St. Louis, MO</td>
<td>Dr. L. W. Hinchman, Indianapolis, IN</td>
<td></td>
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<tr>
<td>86. Nov. 7-12, 1982</td>
<td>Nashville, TN</td>
<td>Dr. G. B. Rea, Salem, OR</td>
<td></td>
</tr>
<tr>
<td>88. Oct. 21-26, 1984</td>
<td>Ft. Worth, TX</td>
<td>Mr. J. O. Pearce, Jr., Okeechobee, FL</td>
<td></td>
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<tr>
<td>89. Oct. 27-Nov. 1, 1985</td>
<td>Milwaukee, WI</td>
<td>Dr. David U. Walker, Montpelier, VT</td>
<td></td>
</tr>
<tr>
<td>90. Oct. 19-24, 1986</td>
<td>Louisville, KY</td>
<td>Dr. N. W. Kruse, Lincoln, NE</td>
<td></td>
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<tr>
<td>91. Oct. 25-30, 1987</td>
<td>Salt Lake City, UT</td>
<td>Dr. J. F. Hudelson, Denver, CO</td>
<td></td>
</tr>
<tr>
<td>92. Oct. 16-21, 1988</td>
<td>Little Rock, AR</td>
<td>Dr. J. A. Cobb, Atlanta, GA</td>
<td></td>
</tr>
<tr>
<td>93. Oct. 28-Nov. 3, 1989</td>
<td>Las Vegas, NV</td>
<td>Mr. P. E. Bradshaw, Griggsville, IL</td>
<td></td>
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</tbody>
</table>

+This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION
J. E. Slauter, D.V.M.
Jefferson City, Missouri

Our Father who art in heaven, hallowed be thy name.

Father, we as a group of your children, come before your throne of grace this evening in the holy and sacred name of your only begotten son, Jesus Christ

We pray that this gathering and the many meetings yet to take place this week may be blest with the presence of your Holy Spirit. May your Spirit enlighten our minds and lead each of us to new truths concerning the mysteries of your creation.

Because we lack wisdom, we turn to you, our Heavenly Father, Please bless the leaders of this great meeting with your divine wisdom.

Father, grant to us greater vision as we plan for the future, as we collectively focus our attention the needs of animal health in this great nation and throughout the world.

We humbly thank you for the many gifts and talents of the members of our association. For the opportunities for even greater service to our fellowman we praise you and thank you. May we always remember your commandments and the life and ministry of your Son, as we work together to help build a better world for our children and grandchildren who will someday be the leaders of our industry, our communities, and our nation.

In the name of Jesus, I place this gathering in your hands and pray your richest blessings upon each one.

In Jesus Christ's name, we pray.

Amen.

The following members of our association passed away since our last meeting.

DECEASED - 1989

1. Dr. M. D. Schneider—Knoxville, TN—December 12, 1988—Life Member.
2. Dr. Blaine McGowan—Davis, CA—January 15, 1989—Life Member.
7. Dr. Arnett Matchett—October 17, 1989.

Please bow your heads for a moment of silent prayer in memory of those who have passed away as well as those who are ill and stand in need of our prayers especially, Dr. David Walker and Dr. Jack Armstrong.

Amen.
WELCOME TO NEVADA

COMMISSIONER THALIA M. DONDERO

Good evening. It is my pleasure to be here to speak to you about population and economic growth in Nevada and some of the real challenges these factors present to local government.

As most of you know, Nevada now has over one million people living within the state.

Nevada is also one of the fastest growing areas in the United States and has been for most of the last thirty years.

This rapid increase in population reflects the growth in the tourism industry as well as the recent new business expansion in manufacturing.

In terms of population, over eighty-two percent of Nevadans live in two metropolitan areas centered in Reno and Las Vegas.

The largest population growth, however, has occurred here in Southern Nevada.

Clark County has grown from 463,087 in 1980 to over 649,000 in 1988, an increase of twenty-eight percent.

Conservative projections are that Clark County alone will exceed one million by 1995.

In western Nevada, greater Reno has also blossomed substantially from 193,623 in 1980 to 242,800 in 1988.

Reno expects to add another 100,000 residents by the year 2000.

Statewide in 1988, building was up twenty percent, personal income increased around nine percent, job growth rose five percent, and taxable sales jumped fifteen percent.

Because of the 498 new companies which have started up in the last four years, Nevada has jumped from tenth to eighth in the United States in a magazine survey ranking climates for entrepreneur activity and business growth.

The U.S. Department of Commerce also recently ranked Nevada twelfth in the nation for per capita income, which compares favorably with Arizona (29th), Iowa (43rd), and Utah (48th).

As most of us who live here know, there are many good reasons why Nevada is enjoying its phenomenal population and economic growth.

One of the reasons is our weather.

In Southern Nevada, people enjoy sunshine eighty-five percent of the year, with an average temperature of seventy-seven degrees and an average daily minimum of fifty-five degrees.

The western and northern parts of the State — particularly the Reno/Lake Tahoe area — have a more diverse climate that permits a diversity of excellent year-round recreational activities.
Another reason for the migration to Nevada is the vast amount of open space.

As an example, sixty-five percent of Clark County, Nevada's most populated area, is still undeveloped.

A third reason is Nevada's pro-business environment.

Because of gaming revenues, there are no corporate income, personal income, inventory, estate, franchise, or unitary taxes in Nevada.

A fourth reason is Nevada's strategic location.

Nevada is a natural stop-off point for travelers en route to Phoenix, Denver, Salt Lake City, Los Angeles, and San Diego.

Business travelers can fly from Las Vegas' ultramodern McCarran International Airport to any major city west of the Rockies in no more than two hours.

In western Nevada, Reno and Sparks started as towns where north-south and east-west railroads met, and today, rail lines still tie that area to the entire nation.

Reno also serves as an effective trucking hub along the Interstate 80 corridor from San Francisco to Salt Lake City and the I-5 corridor from Seattle to San Diego.

Fifth and last, Nevada has become much more diversified in recent years.

In the last five and one-half years, for example, the Nevada Development Authority (NDA) has recruited about 130 companies to Southern Nevada, including twenty-nine in just the final six months of 1988.

The Economic Development Authority of Western Nevada (EDAWN) has had similar success luring firms to the Reno/Sparks area.

The 133 recruits in Western Nevada during the last four years include Porsche, R. R. Donelley, Sherwin-Williams, and Chevron — twenty-four percent of the new operations in 1988 were foreign.

One of the best selling points of the Reno/Sparks area, of course, is the lifestyle.

In 1988, the American Economic Review rated Washoe County as having the fifth best quality of life in the United States.

Nevertheless, despite all of the talk in Nevada about diversification, there is no denying that gaming and tourism are still the horses that pull the economic wagon.

For example, the Golden Nugget's new property on the Las Vegas Strip, called The Mirage, is a $575 million resort that will set new standards for desert opulence.

Construction is expected to be completed by this December.

Just two miles south, Circus-Circus has broken ground on Excalibur, a $290 million European Middle Ages castle resort.
Scheduled for mid-1990 completion, Excalibur will have 4,000 rooms and a 100,000-square-foot casino.

And Hilton Corp. is building a 2,006-room hotel/casino in Laughlin, the once obscure little town on the Colorado River.

Located just ninety miles from Las Vegas at the southern tip of the State, Laughlin has been the talk of the gambling world for the last five years. Showing yearly gaming profit increases in the thirty percent range, Laughlin has attracted all of the big players.

Circus-Circus owns two properties there, the Edgewater and the Colorado Belle, and last year the Golden Nugget purchased the Nevada Club casino in Laughlin from Del Webb Corp. and is building a 1,000-room tower next to the casino.

In all, some 13,000 new rooms currently are under construction at fifteen hotel/casinos in Las Vegas and Laughlin.

In fact, as fast as new hotels are built in Las Vegas and Laughlin and new rooms added to existing properties, the convention and tourism industries are filling them up.

Room occupancy for the year was eighty-four percent in Las Vegas and an astounding ninety-six percent in Laughlin.

In total, a record seventeen million people visited Las Vegas in 1988 and spent an all-time high of $8.8 billion on rooms, gaming, and other goods and services.

Of those visitors, 1.7 million were convention delegates who attended more than 550 conventions and trade shows, making Las Vegas the fifth hottest convention city in the United States.

Statewide gaming revenues for Fiscal Year 1988 climbed 9.8 percent to a record $4.07 billion.

Though less startling than in Las Vegas and Laughlin, the gaming news in Reno is also significant.

And if current efforts to upgrade existing facilities are any indication, the Reno area intends on achieving even greater successes in the future.

In 1988, two refurbished hotel/Casinos — The Riverboat and the Ponderosa — reopened after being closed for several years; the Virginian casino also set up shop; and two others changed ownership and are undergoing remodeling.

The Eldorado has a $45 million development under way, including a 25-story 400-room tower and new casing and convention areas.

In its $50 million project, the Sands Regent plans to add 600 rooms, 16,000 square-feet of convention space, and 30,000 square-feet of casino space over the next six years.

At this point, I think it is very clear that Nevada will continue to rank as one of America's fastest growing areas and will continue to enjoy great economic prosperity for many years to come.
How well we manage this population growth and economic prosperity, of course, will determine the level of the quality of life we have in Nevada.

We must plan carefully for the future in order to accommodate the tremendous influx of new residents and handle the challenges they will create.

In Clark County alone, for example, we are having to deal with increased airport traffic, which in 1988 increased to over fifteen million passengers, an increase of 17.6 percent over the previous year.

Recently prepared forecasts predict that by the year 2007, McCarran will have grown to the size of today's Los Angeles International Airport with more than forty-two million passengers.

Preparing to meet these future demands is a high priority of the County as evidenced by a recent $172 million airport revenue bond issue which will finance a five-year capital improvement program.

We also face similar challenges in moving automobile traffic in, about, and out of our area.

That is why in 1987 the County authorized $85 million in street improvement bonds to construct road improvements. This program is now being implemented, but there remains some question as to whether this program will make significant differences in the face of the tremendous growth pressures facing the County.

That is also why we are presently at work developing a clear-cut countywide plan for traffic and transportation.

The costs of an effective program, however, may well be in excess of one billion dollars.

Preliminary results from the beltway studies indicate that the final cost of a beltway around the south end of the Las Vegas Valley could cost from $15 to $30 million per mile by the time the project is completely studied, designed, and built.

Controlling air pollution is also a serious problem and will become more so as our population continues to grow, particularly among senior citizens.

For this reason, it is essential for us to look to develop newer, cleaner methods of transportation.

In response this winter the use of oxygenated gasoline will be mandated in all automobiles.

This gasoline burns much cleaner and should help to reduce the level of air pollution in the Las Vegas Valley.

Possibly one of the greatest challenges facing us, however, is water.

Living in the desert as we do, water is vital to our growth and well-being. Therefore, we must accomplish more in the area of developing water
conservation strategies and negotiating more equitable water allocations.

The Clark County Water Resource Strategy Report was prepared in response to these needs.

The major goals set forth in the Report include:

1. A reduction in per capita water use by twenty to twenty-five percent within five years;
2. Balance water supply and conservation concerns for quality of life concerns;
3. Clean up the legal, institutional, and regulatory gaps associated with return flow credits; and
4. The “Use-it-or-lose-it” nature of our State Water contracts which preclude conservation and wise-water management.

Energy providers and regulators are also facing enormous challenges as a result of the tremendous population growth in Nevada and must work to develop and construct more efficient power generating plants.

In addition, there are also great challenges in the areas of flood control, the installation of infrastructure facilities such as sewer and water lines, and additional fire, police, and social welfare and recreational services.

Lastly, we must continue to improve the quality of education in our State. Proficiency levels must be significantly improved, and school curriculums at the elementary, secondary, and college levels must be expanded to include more math, science, and high technology related courses.

Throughout Nevada there exists today, insufficient numbers of skilled and semi-skilled workers for some of the more technically-oriented industries which are fast coming to dominate the national employment picture.

This must be addressed if we expect to continue to compete in attracting new businesses.

There are many other important problems, of course, but I think these will give you an excellent idea of the challenges that lie ahead.

In closing, I hope I have given you a good idea of where Nevada is and where it is going, and of some of the major problems that are facing us.

And because these problems do and will continue to exist, I would like to end by encouraging each of you to take a strong interest in your government because the elected officials that you vote for and the decisions that they make will have a great deal of influence over every aspect of your lives for many years to come.

Thank you for your time.
RESPONSE TO WELCOME ADDRESS

Dr. Jim Williams
Denver, Colorado

Thank you, Commissioner Dondero. On behalf of the members and guests of the American Association of Veterinary Laboratory Diagnosticians and the United States Animal Health Association, I thank you for your warm welcome to the State of Nevada and the City of Las Vegas.

These organizations provide a medium where science, industry, and governments can work together to achieve common goals: protecting those industries to continue supplying the American public with a safe and wholesome supply of animals and animal products. We look forward to an enlightening and productive annual meeting. The excellent facilities of this hotel will undoubtedly help to bring this about; and with the many entertainment options available in Las Vegas, I'm sure that no one will want to go home before the work is done.

On behalf of the State of Colorado, I wish to extend an invitation to come early and stay late for the 1990 meeting in Denver. Livestock production is a major industry throughout Colorado. The state ranks fourth in cattle feeding, and has long been the number one lamb feeding state in the nation. A number of livestock organizations, among which are the National Cattlemen's Association and the American Sheep Industry Association, have their national headquarters in the Denver area. Denver is proud to be known as a "Cow Town", and as host to the National Western Stock Show, warmly welcomes the thousands of livestock producers, from across America and throughout the world, who visit the city during January of each year.

I think everyone will find that October is a terrific time to visit Denver and I encourage everyone take advantage of this opportunity to enjoy the hospitality and the many scenic attractions of the Rocky Mountain area.

I hope to see you all in the Mile High City next year.
MESSAGE OF THE INCOMING PRESIDENT OF THE AAVLD

Gavin L. Meerdink, D.V.M.

Urbana, Illinois

The topic of where we are and where we are going as an association reminds me of a comment made by my grandfather. He said “when you split wood, just concentrate on where to swing the axe. If you measure the pile you have split, you will become complacent. And too close a look at what is left to do will only breed frustration.”

Certainly, our organization has racked several cords of exemplary accomplishments on which I am sure Dr. Bergeland will elaborate. The Proceedings contain the first mention of several now recognized syndromes, and, of course, the Journal of Veterinary Diagnostic Investigation is well under way, thanks to its Editor, Dr. Crandell and the administrative efforts of several, particularly Dr. Bergeland. The AAVLD and AOAC have worked together in the interlaboratory method standardization process. At least three of our members have won the AOAC Associate Referee of the year award. We are now considering adding this review process into some other areas, namely serology. Assuredly, there are numerous other achievements, to numerous to mention. So I will stop here at this point (after all, we would not want to become complacent).

The fundamental purpose for the existence of the AAVLD is to help all of us become better diagnosticians and laboratories to improve our accountability, reliability, and assurance. This, of course, will continue to be our principal mission. Since becoming more involved in this organization over the last few years I’ve gained an interest in the inquiries received for information regarding a national animal disease database or other information surveillance source which might be available. You should know that AAVLD has discussed this issue with the National Academy of Sciences and center for Disease Control and others in the recent past.

The people in this room — representing the laboratories of US & Canada — log some 4000 cases per working day. Most diagnostic laboratories are at the core of animal disease information in their state and are centrally located between the practitioner and the academic institution. We hold an enormous information reserve.

We have learned to interpret the animal’s problems from the cells, cultures, and chemical values. And we have learned to make interpretations on herd status from studies of the individual animal. Perhaps we can also devise ways to use our data in a prospective manner to better manage disease prevention and eradication procedures, assist in the objectives of the National Animal Health Monitoring System, and ultimately, to again use animal information for the betterment of the human condition.

Admittedly, the task of compilation and analysis of national diagnostic animal disease data is immense. Let us not be frustrated by the log jam, but
rather explore where to swing the axe.

In the interest of brevity, I will stop here. I trust you are enjoying the meeting. Please contact me anytime throughout this next year, I want your opinion. THANK YOU for your attention.
REMARKS OF THE PRESIDENT OF AAVLD
Martin E. Bergeland
St. Paul, Minnesota

Distinguished Guests, Ladies and Gentlemen:

It has been a special privilege and honor for me to serve as president of the AAVLD this year.

It is a very good year for our Association, thanks to the many people who serve the AAVLD with unselfish dedication. Our membership has increased by about 150 members, to a total of over 660, plus about 50 library subscriptions to our Journal. There remains the potential for substantial broadening of our membership base, which will be a challenge for the future.

Volume 1 of our official publication, the Journal of Veterinary Diagnostic Investigation, has been completed and distributed, providing 372 pages of high quality papers. Already, the Journal is established as the premiere source of scientific information in our specialty of veterinary diagnostic medicine. Fifteen companies now are patrons of the Journal. Their sponsorship and support are greatly appreciated, and are essential to the continuing vitality of the publication. Credit for this splendid accomplishment goes to many people, but especially to Dr. Bob Crandell, the Editorial Board, and the many members who have prepared excellent manuscripts for publication.

Dr. Dan Goodwin again provided us with interesting and informative newsletters. This year we received a most useful publication on the laboratory diagnosis of swine dysentery, thanks to the efforts of Dr. Lorraine Hoffman and the members of the Swine Dysentery Committee.

In June we had the opportunity to participate in the Vth International Symposium of the World Association of Veterinary Laboratory Diagnosticians, which helped us to broaden our global perspective of diagnostic medicine. Our gracious Canadian hosts provided us with an unforgettable experience in Guelph, Ontario.

This 32nd annual meeting provides the opportunity to again reaffirm the mutually beneficial alliance and excellent working relationship that exists between our two associations. Dr. Shook, Ella Blanton and Linda Ragland have been helpful in so many ways.

Finally, a word of appreciation to several who have worked diligently throughout the year to carry out association activities and prepare for this meeting. Our new president, Gavin Meerdink, has arranged for an excellent scientific program. Harvey Gosser, Secretary-Treasurer and Donna Dare, Coordinator of meeting arrangements and exhibits have attended to the countless details that allow our association to function smoothly. We are all very indebted to you.

Thank you again.
ADDRESS OF THE PRESIDENT-ELECT
Max A. Van Buskirk, Jr., V.M.D.
Harrisburg, Pennsylvania

Distinguished guests, fellow members and friends of the United States Animal Health Association, I wish to express my gratitude for your confidence in electing me to the office of President-Elect. I am looking forward to serving you and I will do my best to meet your expectations and uphold the traditions of USAHA.

As your President-Elect, I believe it is my duty to seek ways and means to expand the effectiveness of USAHA. I believe it is fair to say that this organization has played a key role in shaping animal health programs since it (then the Interstate Association of Livestock Sanitary Boards) first met in 1897 in Fort Worth, Texas to inspect a vat for dipping cattle and sheep. To recite and do justice to our accomplishments since that time would take much too much time this evening. It is surely sufficient to say this organization is not broken and improvement, at most, is simply a matter of refinement to adjust to changing times.

Change management is critical to the success of any organization. You may be interested in comments made by Dr. J. V. Knapp in his President’s address to the 1948 annual meeting of the United States Livestock Sanitary Association:

“This Association was organized over 50 years ago by a group of men occupying positions very much the same as you and I occupy today. Then, as now, livestock producers of this country were faced with the problem of how to overcome economic losses occasioned by infectious and contagious diseases. Looking back, from our advantage of years and experience, we think their problems of cattle fever tick and scabies eradication were minor compared to the problems and losses confronting the livestock industry today. Since its organization in 1897, this Association has endeavored to correlate scientific information, sound thinking and practical experience in infectious livestock disease control and has become the agency which, through the years, has been largely responsible in formulating and conducting livestock sanitary disease control programs necessary to effect a profitable livestock industry and maintain a balanced agricultural economy.

How long this Association remains the effective agency it has been in the past will be determined by the manner in which it meets and solves the problems confronting it from year to year.”

Change is ongoing. Societal perceptions of animal stewardship are changing. The propriety of utilization of natural resources for food animal production is being challenged. Food safety remains a major issue despite tremendous progress. Environmentalists are insisting on proper management of infectious waste and animal waste in general. The ethics of genetic engineering is questioned. Economic and political determinants of food
animal production and marketing are international in scope. Our effectiveness in managing these complex issues depends in large part on the efficiency of our decision-making process and our ability to communicate. Information; planning; consensus; action; these elements are essential to accomplish change management and each depends on effective communication.

It is essential that our committees not only pursue ongoing agendas but also anticipate and react to emerging issues in their respective areas of responsibility. Our committees for transmissible diseases of poultry and for salmonellosis are conducting a joint session on Tuesday afternoon to address the matter of egg-associated *Salmonella enteritidis*. That session will include involvement by representatives of government and industry. It is an example of initiative that keeps this organization responsive to its constituency.

I believe that USAHA's committee system provides a sound structure for change management. When animal health problems appear, we bring together scientific, government and industry expertise to seek cost effective and politically acceptable solutions. We have tended, however, to be crisis oriented. Unless there are pressing issues, committee agendas sometimes are perfunctory and non-productive. Committee chairpersons need to inspire committee members to communicate—to identify and lift up for committee consideration important subjects that may not yet be issues.

Our State/Federal Relations Committee is one of our most important committees because it provides a mechanism for candid communication between USAHA and federal regulatory agencies. Unfortunately, only a few (representative) persons are involved and feedback to interested committees and the general membership does not occur until the next annual meeting. We need to find a way to relay to committees, in a timely manner, information presented or generated at the State-Federal Relations Committee meeting.

Perhaps the most serious problem with our committee system is scheduling meetings to maximize participation. Those states and organizations that can send only a few representatives are frustrated when important committees meet concurrently. Although the proceedings of our annual meeting provide an excellent record of committee activity, it is necessarily incomplete and largely historical in effect.

It goes without saying that communications is the lifeline of any organization. Those who belong to USAHA should know what this organization is all about. However, as USAHA grows larger—and we need to grow larger—communication with individual members will become more important and more difficult. Many state regulatory agencies still do not understand or appreciate the role USAHA plays in management of animal health in this country. Animal owners and the general public generally do not know USAHA exists, let alone appreciate what we do. We need to let industry and legislators, in particular, know who and what we are. As Phil
Bradshaw will likely tell you in his remarks, we have already taken a step to improve public relations by retaining Neal Black to assist us to publicize this meeting.

Failure to communicate effectively compromises any effort. Those of you who watch football have witnessed how crowd noise can stymie a team’s effectiveness. If a team cannot hear the signals—if organization (USAHA) members are not tuned to strategy and official policy—then the team will have difficulty executing plans and may fail to accomplish its objectives.

I began these remarks by proposing to seek ways and means to expand the effectiveness of USAHA. More specifically, I consider communications the key to finding those ways and means. If you will assist me by contributing advice, I will do the best I can to put that advice to work for the betterment of USAHA and animal health.

Thank you once again for the privilege of serving the office of USAHA President-Elect.

Max A. Van Buskirk, Jr., V.M.D.
October 30, 1989
REMARKS OF THE PRESIDENT

P. E. Bradshaw
Griggsville, Illinois

President Bergeland, fellow members, and guests. First, let me thank you for making this year a big success. Several of you helped write statements for me to present on behalf of your organization. For this a special thanks.

No organization of this size can function without good staff. We have an excellent staff: Ella Blanton, Executive Director, Linda Ragland, and Joyce Redman do a fine job and especially keeping the President straight this year, which was no small undertaking.

You have all served in volunteer positions, so you know what the family has to put up with. Tonight I have my family with me except my 15 year old son, who stayed home. He wouldn't miss school. He has only missed 1-1/2 days in 10 years. Let me introduce the rest of my family. The newest member is Brock, who is 4 weeks old, his sister Christy, 9 years, their mother and my oldest daughter, Cindy Willard and her husband Richard; Kasey, 2 years old, her mother and my youngest daughter Lisa Pruett and her husband Bobby; and my wife Linda, who keeps it all together.

Now let me turn to some observations I have made during my year as your President. Food producing animals make up approximately 52% of the total farm marketings. That equates to 78.8 billion dollars of livestock and products marketed. (U.S. Agricultural Outlook, September, 1989) This places no value on zoo animals, wild animals, or exotic animals, pets, etc. It is no small part of our economy and food supply.

Now, let us look at what we are faced with to protect this vitally, important part of our economy and food supply. There are several diseases to be eradicated in the near future. The U.S. Agricultural trade is increasing from all over the world. Mexico alone shipped 2-1/2 billion dollars worth of ag products to the U.S. in 1988. As U.S. citizens become more affluent and man continues to encroach on the natural habitat, more exotic animals will be brought into the U.S.; are we going to make the commitment necessary to protect the $78.8 billion dollar business?

On the domestic side, look at the number of diseases which affects a small percentage (under 10%) of the total animals and it has been agreed, if not proven, that they can be eradicated. Scrapie in sheep, PRV in swine, brucellosis in cattle, and highly pathogenic avian influenza in poultry, plus others, but we are still living with these diseases. Is the commitment there to eradicate the diseases from the U.S.?

Then there is the matter of new technology, monitoring what is developed, how it is used and by whom. This all leads to the underlying factor of environmental and food safety, which is an area that our organization has been and will become more involved in.
The committee structure, the diversity of the membership, and the free, open debate makes the USAHA a logical place to debate and recommend not only disease programs but new animal health technology.

Where will the funding come from to address these issues? In the years ahead this organization will increase programs and will need a larger budget.

A 78.8 billion dollar industry, 52% of total ag income, but what is the total commitment for Veterinary Services in the U.S.? It certainly is not 52% of the USDA budget or 52% of the state agricultural budgets, nor do I think it should be. It is time we in the animal health arena stand up and tell our story and be proud of our accomplishments and our future goals. Let’s not feel guilty to ask for 65–70 million dollars for brucellosis, 11 million dollars for scrapie, 4.5 million dollars for pseudorabies when other small segments of our economy ask for billions.

I have and will always be proud of the animal industry and this organization and thankful to have had the opportunity of serving as your President. Thank you.

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Dr. Max A. Van Buskirk, Jr., President-Elect, USAHA, presents a plaque to outgoing President, Mr. Philip E. Bradshaw for his contributions and outstanding leadership in 1989.
USAHA AWARD PRESENTATION
TO
MR. JOHN BARKLAY ARMSTRONG V
BY
DR. JAMES W. GLOSSER, ADMINISTRATOR
USDA, ANIMAL AND PLANT HEALTH INSPECTION SERVICE

October 30, 1989

This evening, it is we who are honored by the honoree. His is a lifetime of action and deeds, commitment and leadership — at the very core of America’s sound, successful advancement in animal health during the 21st century.

It is not required that the APHIS Animal Health Award, inaugurated in 1968, be presented annually. The prime criteria is to recognize an individual who has provided outstanding leadership — on both the State and National levels — and has, thereby, contributed significantly to the progress of cooperative State-Federal animal health efforts and to making America’s livestock and poultry populations the healthiest and most productive in the world. Tonight’s recipient says he has retired now. But we all know his exemplary public service and leadership to enhance our country’s animal health will continue — and that this tribute of federal recognition is long overdue!

At present, APHIS looks to our awardee for his wise counsel and expertise as Chair of the Animal Health Committee of the National Cattlemen’s Association and as Chair of the very important Brucellosis Committee of U.S. Animal Health Association. Indeed, his charismatic leadership during the past ten years of reorganization and modernization of our nation’s brucellosis program has been key to its present vitality. He has been a major force behind the recent successes in reducing the incidence of brucellosis in the United States, ensuring that the eradication program developed scientifically and efficiently while allowing the livestock industry to prosper.

As we continue to build on the past and enter the 1990s, there could be no more meaningful or appropriate setting than the (1989) annual meeting of AHA to present the U.S. Animal Health Award to John Barklay Armstrong V. For this is the premier animal health association of American Federal and State governments, producers, and academia — and it represents every sector of the “vineyards” in which John Armstrong has faithfully labored for a half century.

To quote an industry colleague, it is not an exaggeration to say that there is no one more deeply involved, skilled, and experienced in every facet of the livestock industry. “As a cow and calfman with both commercial and purebred cattle, he has followed the product to the feedlot, to the supermarket, even to the hands of the consumer, becoming knowledgeable about every single process.” His love for his industry is renowned.
John Barklay Armstrong V was born on December 23, 1919, into an historic Texas ranching family. Since his youth, he has contributed to the proud heritage of American breeding and livestock tradition. It is evident from childhood that he could take the reins firmly, with determination and a commanding purpose of direction — as a skilled horseman, rancher, and world-class polo player — and in promoting animal health in his state, and his industry nationally. To this day, one can be assured that “bunch quitters” do not leave his herd nor any committee that he chairs; and no stampedes occur when John Armstrong is “in the saddle.”

John graduated from Texas Military Institute and in 1941 from the University of Texas with a degree in economics and business administration. Immediately thereafter, he capably undertook the enormous responsibility of actually managing the Armstrong family ranch which fell to him at this young age, during the difficult years of World War II. Later, “on his own,” he moved to Alabama and became a leading breeder of Santa Gertrudis cattle.

Returning to Texas in 1964, until 1988, he helped oversee the John B. Armstrong Ranch, and the mammoth King Ranch holdings, including operations in Mississippi, Kentucky, Pennsylvania, and Florida, as well as King Ranch holdings in Spain, Morocco, Argentina, Brazil, Venezuela, and Australia. Indeed, his career, and his public service parallel 20th century progress in promoting animal health at the state, national, and increasingly important international levels!

Uncompromising in his dedication to advance American animal health, he has been a “human bridge” connecting the old with the new, traditional with contemporary values, industry with all levels of government and scientists. He is a lightning-rod and catalyst for policy-making, a consensus builder, a man with a larger-than-life resume.

I will mention but a few highlights to project the scope and expanse of his involvement. He was President of Santa Gertrudis Breeders International from 1957–59, Regional Vice President of the American National Cattlemen’s Association from 1959–1961, and the first Chairman of the Beef Industry Council of the National Live Stock and Meat Board from 1962 to 1969. He was a Director of the Texas and Southwestern Cattle Raisers Association from 1969 to 1974, serving as President in 1978 and 1979. And in 1979, he was recognized by PROGRESSIVE FARMER magazine as “Man of the Year” for his service to Texas Agriculture.

In 1975, he was appointed to the Texas Animal Health Commission. Some say this marked his entering “a second career.” Even before, he had successfully addressed the Texas cattle fever tick and screwworm problems. Now he would accept the challenge of carrying out these programs, and in addition the challenge to provide Texas with a workable, enforceable brucellosis program. It never mattered whether the issues were difficult or impossible; if the cause was just, he championed it, “turning around”
Key to John Armstrong's character and success is his personal philosophy best stated by the great Dr. Samuel Johnson whom he has always admired. "A man accustomed to vicissitudes is not easily dejected." Throughout his years as Chairman of the Texas Animal Health Commission, as a member of the Board of Directors of the National Cattlemen's Association, as Director of the Texas Livestock Marketing Association, and as Vice-chairman of the U.S. Polo Association, John's mastery of patience and summation of issues and views, his refusal to be discouraged, his humor and common sense, have stood all of us in good stead.

In 1983 the Livestock Publications Council awarded him its prestigious "Headliner Award," and a month later he became the sixth recipient of "The Gold Spur Award" for service to the livestock industry. He was again acclaimed by his peers when the National Cattleman's Association made him "Cattle Business Man of the Year for 1985." In 1986, the Alabama Cattlemen's Association honored him as an inductee to the "Alabama Livestock Hall of Fame" and, in 1987, he was inducted into the "International Stockmen's Hall of Fame" at the Houston Livestock Show.

What better can be said about the quality of John Armstrong's leadership than that he chose to "ride life" rather than "to graze in its shadow," to play the game fully, reasonably and fairly, to influence by action and example, by his handshake, by his innate sense of sportsmanship and "team spirit."

As a friend and colleague for whom I feel enormous personal and professional respect, John Armstrong personifies the litmus test of good practice and wisdom, ethics and fair play — uncompromising in his standards but always reasonable and open to science and new information. He is a man strengthened by yesterday's tradition, fortified by today's successes, with a passion for tomorrow — whose broad focus can envision new cooperative needs beyond the immediate horizon. And he has been known to pistol-whip the rigidity of regulatory mindset when necessary.

THE ARMSTRONG CHRONICLE states it well, John, "you are a living legacy of "sophisticated forward-tuned Armstrongs standing squarely in the present, ... "continuing to make their own marks for posterity," and, like your legendary great-grandfather John Barklay Armstrong, you have distinguished yourself "with skill and valor blended with a generous dash of charm and style," ... "fine southern upbringing," ... "a scrapper with the flair of class and gentility."

And so tonight APHIS is extremely privileged and proud to present the U.S. Animal Health Award to you — for all you have done to advance our nation's animal health and for the inspiration you have provided others to follow in your path. Our thanks, appreciation, and congratulations!
Dr. James W. Glosser, Administrator, APHIS, VS, USDA, presents the Animal Health Award to Mr. John B. Armstrong, V for his lifetime of action and deeds, commitment and leadership.

Dr. J. E. Slauter, President of the National Assembly of Chief Livestock Health Officials, presents the first National Assembly Award to Ms. Mabel Owen, Director, Division of Animal Industry of Massachusetts. The Award is given to an active regulatory official or an industry representative for outstanding service in animal health regulatory programs.
REPORT OF THE COMMITTEE ON NOMINATIONS

Elective Officers

President .......................... M. A. Van Buskirk, Jr., Harrisburg, PA
President-Elect ..................... P. L. Smith, Sacramento, CA
First Vice-President ............... J. L. Alley, Montgomery, AL
Second Vice-President ............. T. J. Hagerty, St. Paul, MN
Third Vice-President .............. J. B. Finley, Jr., Encinal, TX
Treasurer .......................... J. C. Shook, Mechanicsburg, PA

Regional Delegates

Northeast .......................... Dr. Everett S. Bryant, CN
Dr. Victor P. LaBranche, MA
North Central ....................... Mr. Don D. Gingerich, IA
Mr. Bill Gallagher, SD
South ................................ Mr. J. O. Pearce, Jr., FL
Mr. William C. Baisley, GA
West .................................. Mr. Olin H. Timm, CA
Dr. Richard H. McCapes, CA

RESOLUTIONS
United States Animal Health Association
Passed October 29–November 3, 1989
Las Vegas, Nevada

Resolution No. 1
Source: Committee on Brucellosis.
Subject Matter: Support of Rapid Completion Plan.
Resolution
RESOLVED that the UNITED STATES ANIMAL HEALTH ASSOCIATION be on record in support of the Rapid Completion Plan; that full funding be given to the Brucellosis Program for Fiscal Years ’91–’96; that additional funding be especially “earmarked” for the Rapid Completion Plan; that expenditures of these funds be used in depopulation, area and county testing, adult vaccination and epidemiology; and that a copy of this resolution be immediately forwarded to the Secretary of Agriculture.

Resolution No. 2
Source: Committee on Brucellosis.
Subject Matter: Use of the competitive enzyme-linked immunosorbent assay, (ELISA) as a supplemental test for brucellosis.
Resolution
RESOLVED to use and evaluate the cELISA as a supplemental test to aid in determining the disease or vaccination status of animals exposed to brucellosis as interpreted by the designated epidemiologist.
Resolution No. 3  
Source: Committee on Brucellosis.  
Subject Matter: Evaluation of the new experimental killed brucellosis vaccine in cattle under field conditions.  

Resolution  
RESOLVED to recommend that the new experimental killed brucellosis vaccine be experimentally evaluated in cattle in actual field conditions under the supervision of USDA, APHIS, Veterinary Services designated epidemiologists.

Resolution No. 4  
Source: Committee on Brucellosis.  
Subject Matter: The CITE (concentration immunoassay technology) be approved for use on nonvaccinated bovine females through marketing channels.  

Resolution  
RESOLVED that the Uniform Methods and Rules, and 9 CFR Part 78, be amended to allow the CITE test to be used on nonvaccinated, as well as vaccinated animals, as an official supplemental test.

Resolution No. 5  
Source: Committee on Brucellosis.  
Subject Matter: Quarantined feedlots in class free states.  

Resolution  
RESOLVED that the U.S. Animal Health Association recommend that USDA, APHIS change the UM&R and CFR to allow quarantined feedlots within class free areas.

Resolution No. 6  
Source: Committee on Brucellosis.  
Subject Matter: Continued funding for education and information.  

Resolution  
RESOLVED, that USAHA commend and encourage the continued funding for the development and dissemination of information and education by privately endowed organizations, extension services, governmental entities, educational institutions, industry leaders and livestock producers, with the ultimate goal of brucellosis eradication.

Resolution No. 7  
Source: Committee on Brucellosis.  
Subject Matter: Compensating livestock owners for losses from Yellowstone wildlife.
Resolution

RESOLVED, that USAHA support the enactment of a mechanism and the appropriation of funds to require Federal agencies in custody of wildlife to compensate livestock owners and other aggrieved entities for actual expenses and losses brought about by transmissible disease from such wildlife.

Resolution No. 8
Source: Committee on Brucellosis & Committee on Wildlife Diseases.
Subject Matter: *Brucella abortus* in wildlife of the Greater Yellowstone Ecosystem; Montana, Wyoming and Idaho.

Resolution

RESOLVED, that U.S.A.H.A. encourages resolution of conflicts of missions of Federal and State agencies for either preserving or eradicating *Brucella abortus*.

The responsible Federal and State agencies identify the major constraints to controlling or eradicating *Brucella abortus* in free-ranging bison and elk.

The responsible Federal and State agencies support research to optimize control or eradication of *Brucella abortus* in free-ranging wildlife through, but not limited to, vaccination, diagnosis, understanding its ecological role, and wildlife management practices.

Resolution No. 9
Source: Committee on Foreign Animal Diseases.
Subject Matter: Vesicular Stomatitis.

Resolution

RESOLVED the United States Animal Health Association strongly recommends to the United States Department of Agriculture that multidisciplined epidemiologic studies and research with application toward diagnosis, control, or prevention of VS be identified as a priority item for funding either within ARS and/or APHIS, USDA, and through CSRS competitive grants.

Resolution No. 10
Source: Committee on Parasitic Diseases and Parasiticides, Committee on Foreign Animal Diseases, Committee on Import-Export & Committee on Epizootic Attack.
Subject Matter: Tropical Bont Tick Caribbean Project.

Resolution

RESOLVED that USAHA is pleased by the progress made by USDA-
OICD on the information and evaluation component of the tropical bont tick project in the Caribbean.

USAHA is dismayed at the lack of progress by USDA-APHIS and USAID on the pilot eradication project on Antigua.

USAHA strongly urges USDA-APHIS and USAID without further delay meet and agree on the actions necessary to initiate as soon as possible the pilot tropical bont tick eradication program on Antigua.

Resolution No. 11
Source: Committee on Brucellosis.
Subject Matter: Authority to require collection of blood samples at slaughter.

Resolution
RESOLVED that USAHA support APHIS-VS in its request to FSIS for mandatory collection of blood samples from sows and boars at selected packing plants.

USAHA supports APHIS-VS in seeking authority to require collection of samples at slaughter in disease eradication programs, as well as the authority to require that packing plants provide suitable facilities for such collection.

Resolution No. 12
Source: Committee on Wildlife Diseases.
Subject Matter: Brucellosis and Tuberculosis — Wood Buffalo National Park.

Resolution
RESOLVED, the United States Animal Health Association supports the concept of elimination of the brucellosis and tuberculosis-infected hybrid wood bison x plains bison populations association with the Wood Buffalo National Park with subsequent replacement with disease-free, genetically pure wood bison from other areas.

Resolution No. 13
Source: Committee on Salmonella and Committee on Transmissible Diseases of Poultry and Other Avian Species.
Subject Matter: Salmonella Enteritidis phage type 4.

Resolution
RESOLVED that USAHA request USDA to develop a contingency plan for the eradication of Salmonella enteritidis Phage Type IV if it is introduced into poultry flocks in the U.S.
Resolution No. 14
Source: Committee on Salmonella and Committee on Transmissible Diseases of Poultry and Other Avian Species.
Subject Matter: Salmonella Enteritidis Task Force.
Resolution
RESOLVED that USAHA develop a Task Force in cooperation with USDA, Health and Human Services, State Agencies and the Poultry Industry to develop a control program for Salmonella enteritidis infection in the commercial egg industry.

Resolution No. 15
Source: Committee on Infectious Diseases of Cattle, Bison & Llama, Committee on Foreign Animal Diseases and Committee on Epizootic Attack.
Subject Matter: Importation quarantine for llamas and alpacas.
Resolution
RESOLVED that APHIS Dockets 880216, Change in Disease Status of Chile Because of Foot-and-Mouth Disease, and 89-116, Llamas and Alpacas Imported From Chile, should be amended to require a high-security 90-day quarantine at the Harry S Truman Animal Import Center with sentinel animals and all appropriate testing for llamas and alpacas imported from Chile. These special restrictions should be subject to review based on changing conditions and further research.

FURTHER, BE IT RESOLVED that 9 CFR, Section 94.11 should be reviewed and amended as necessary to address the risk to the United States created by the importation of live animals from such countries.

Resolution No. 16
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species.
Subject Matter: USAHA Sponsor Third International Symposium on Avian Influenza.
Resolution
RESOLVED that USAHA in cooperation with USDA and other governmental agencies, professional associations and poultry industry sponsor the Third International Symposium on Avian Influenza in 1991 or 1992 and the USAHA Committee on Transmissible Diseases of Poultry and Other Avian Species serve as the organizing group for the Third International Symposium on Avian Influenza similar to the role played for the previous symposia.
Resolution No. 17
Source: Committee on Foreign Animal Disease & Committee on Epizootic Attack.
Subject Matter: Importation of rabbits and rabbit byproducts.

Resolution
RESOLVED that the proper authorities of FDA-Public Health and/or USDA place an embargo on the importation of rabbits and rabbit products until measures are in place to protect our rabbit industries from Viral Hemorrhagic Disease of Rabbits and the USDA seek authority to control the importation of Rabbits and Rabbit Products.

Resolution No. 18
Source: Committee on Salmonella, Committee on Food Animal Hygiene and Committee on Transmissible Diseases of Poultry and other Avian Species.
Subject Matter: Foodborne Diseases and Biosecurity/Disease Prevention Programs.

Resolution
THEREFORE, BE IT RESOLVED that USAHA request USDA and HHS to make funds and other resources available to and by the appropriate government agency or agencies to:
1. Develop and disseminate information and program aids on disease costs and the economic advantages of biosecurity practices in food animal production.
2. Support projects to develop models/demonstrations, videotapes and other educational materials and programs to promote biosecurity in food animal production units.
3. Provide for further and continued development of action videotapes on safe food handling procedures.

This resolution should be directly forwarded, by USAHA, to administrators of the following federal agencies:
Agricultural Marketing Service
Agricultural Research Service
Animal and Plant Health Inspection Service
Communicable Disease Center
Cooperative State Research Service
Economic Research Service
Extension Service
Food and Drug Administration
Food Safety Inspection Service

Resolution No. 19
Source: Committee on Infectious Diseases of Cattle, Bison and Llama,
Committee on Foreign Animal Diseases and Committee on Epizootic Attack.

Subject Matter: Importation quarantine for llamas and alpacas.

Resolution

RESOLVED that llamas and alpacas from Chile (South America) be allowed to enter the United States only after 90-day quarantine in the high security Harry S Truman Animal Import Center with sentinel animals and all appropriate testing, and the APHIS Dockets 88-216 and 89-116 be amended to maintain this current level of protection. These protections should be subject to review based on changing conditions and further research.

Resolution No. 20
Source: Committee on Embryo Movement—Import/Export and Committee on Foreign Animal Diseases.

Subject Matter: Illegal movement of germplasm to Central America, Panama and Mexico.

Resolution

RESOLVED THAT USAHA commend the Central American countries, Panama and Mexico, for their efforts to eliminate an important risk for the introduction of FMD and other diseases and to urge that USDA/APHIS and international organizations support OIRSA in their efforts to facilitate the safe and economical (workable/feasible) importation of germplasm from South America and other FMD countries and to assist with the strengthening of the animal health systems of the OIRSA member countries.

Resolution No. 21 Withdrawn by Import/Export Committee.

Resolution No. 22
Source: Committee on Infectious Diseases of Horses, Committee on Foreign Animal Diseases, Committee on Epizootic Attack and Committee on Import/Export.

Subject Matter: Importation of Ringling Brothers Barnum and Bailey Show Stallions.

Resolution

RESOLVED, that the United States Animal Health Association urgently requests that this entire issue of the importation of the 38 Ringling Brothers Barnum and Bailey Show stallions be reviewed by the Secretary of Agriculture, recognizing that such a breach of communication could seriously impair not only the health of the equine population of the United States, but could also jeopardize the credibility and cooperative relationships of USDA with the states and industry.
Resolution No. 23
Source: Committee on Infectious Diseases of Horses & Committee on Import/Export.
Subject Matter: Establishment of Equine Staff Veterinarian Position in USDA.

Resolution
RESOLVED: The United States Animal Health Association strongly urges the United States Department of Agriculture to re-establish an equine staff veterinarian position which, among other equine-related duties assigned, would assume those responsibilities involving international equine disease monitoring and acting in such liaison capacities as would resolve existent problems of communication between USDA and the equine industry as well as state health regulatory officials.

Resolution No. 24
Source: Committee on Transmissible Diseases of Swine.
Subject Matter: Swine Dysentery.

Resolution
That the USAHA, LCI, the AASP and NPPC set up a committee to examine the feasibility and economics of the elimination of Swine Dysentery from individual herds of swine and to better publicize means of prevention and control.

Resolution No. 25
Source: Committee on Transmissible Diseases of Swine.
Subject Matter: Feral Swine Movement.

Resolution
The USAHA support the proposed addition to the Program Standards for pseudorabies eradication relating to feral swine movement and change in the Uniform Methods and Rules for Porcine Brucellosis eradication.

Resolution No. 26
Source: Committee on Transmissible Diseases of Swine and Committee on Foreign Animal Diseases.
Subject Matter: Blue Eye Disease.

Resolution
RESOLVED that the Transmissible Diseases of Swine Committee of USAHA recommends to USDA that Swine Blue Eye be immediately listed as an official foreign animal disease.
Resolution No. 27
Source: Committee on Environmental Residue.
Subject Matter: Mycotoxins.

Resolution

RESOLVED: A study should be funded wherein cooperating laboratories carry out the following investigations:
1. Veterinary clinicians investigate suspected cases of forage toxicosis and gather specimens clearly associated with animal illness or death.
2. Botanically trained mycotoxicologists establish the mycologic flora of selected samples.
3. Toxicological laboratories examine the suspect samples for toxic effects in laboratory animals associated with either the invasive characteristics of the fungi or the secondary metabolites elaborated (mycotoxins).
4. Toxicological laboratories will differentially extract (i.e., 2 or 3 predetermined solvent systems) selected specimens of suspect forage. Subsequent testing of extracts would include:
   a) Laboratory animal screening of extracts for biological effects.
   b) Histopathology studies.

Chemical characterization of suspect toxins would follow.

Resolution No. 28
Source: Committee on Animal Disease Surveillance and Animal Health Information Systems.
Subject Matter: NAHMS.

Resolution

RESOLVED: USAHA strongly supports NAHMS and urges appropriate funding.

The existing APHIS infrastructure be kept in place and the APHIS field force parallel the regional livestock density, and

The NAHMS program be structured to address user needs and the protocol and program reflect input from the appropriate resources.

Resolution No. 29
Source: Committee on Animal Disease Surveillance and Animal Health Information Systems.
Subject Matter: Resolution on the Implementation of NAHMS Program.

Resolution

RESOLVED that USAHA strongly urge USDA-APHIS to include multiple species simultaneously and that they explore options to obtain both regional and national disease rates for those species and the NAHMS
program design be done fully utilizing the lessons learned from the pilot projects.

Resolution No. 30
Source: Committee on Biologics. Failed to pass approval of General Assembly.

Resolution No. 31
Source: Committee on Pseudorabies.
Subject Matter: Feral Swine.
Resolution
RESOLVED: The USAHA recommend to USDA, APHIS, VS to establish regulations and standards to allow movement of feral swine to approved and regulated hunting preserves on a VS 1-27 permit.

Resolution No. 32
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species.
Subject Matter: U.S. Pullorum–Typhoid Clean State Classification in the Western United States.
Resolution
RESOLVED that State Veterinarians in the western region of the United States be encouraged to adopt regulations necessary to qualify their states for the National Poultry Improvement Plan Classification “U.S. Pullorum/Typhoid Clean.”

Resolution No. 33
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species.
Subject Matter: Mycoplasma gallisepticum (MG) and Mycoplasma symoviae (MS) HI and Serum Plate Antigens.
Resolution
RESOLVED that USAHA request USDA to develop more specific MG and MS Hemagglutination Inhibition (HI) and Serum Plate (SP) antigens and improved MG and MS Fluorescent Antibody (FA) conjugates. Also, produce reference antisera for MG and MS check testing.

Resolution No. 34
Source: Committee on Salmonella, Committee on Food Animal Hygiene and Committee on Transmissible Diseases of Poultry and Other Avian Species.
Subject Matter: Foodborne Diseases of Poultry and Biosecurity/Disease Prevention Programs.
Resolution
RESOLVED that USAHA request USDA and Health and Human Services and other resources available to and by the appropriate government agency or agencies to:

1. Develop and disseminate information and program aids on disease costs and the economic advantages of biosecurity practices in food animal production.
2. Support projects to develop models/demonstrations, videotapes and other educational materials and programs to promote biosecurity in food animal production units.
3. Provide for further and continued development of action videotapes on safe food handling procedures.

This resolution should be directly forwarded, by USAHA, to administrators of the following federal agencies:
- Agricultural Marketing Service
- Agricultural Research Service
- Animal and Plant Health Inspection Service
- Communicable Disease Center
- Cooperative State Research Service
- Economic Research Service
- Extension Service
- Food and Drug Administration
- Food Safety Inspection Service

Resolution No. 35
Source: Committee on Public Health and Environmental Quality.
Subject Matter: Research to establish incidence and prevalence of *E. coli* 0157:H7 in livestock and poultry.

Resolution
RESOLVED that the United States Animal Health Association strongly supports funding from the public and private sectors for epidemiologic research to establish prevalence and incidence of *E. coli* 0157:H7 in livestock and poultry and that this epidemiologic research and in conjunction with risk assessment, be utilized to develop strategies for control measures to minimize risk of human exposure to *E. coli* 0157:H7.

Resolution No. 36
Source: Committee on Aquaculture.
Subject Matter: Consideration of Commercial Aquaculture as Agriculture.

Resolution
RESOLVED: that USAHA requests that fish, amphibians, reptiles, invertebrates, and products of these animals that are produced through con-
trolled commercial aquacultural production (including commercial aquatic ranching) techniques, be officially recognized by the USDA and the USDI as agricultural commodities and that the US commercial aquaculture industry be officially recognized as an agricultural industry.

**Resolution No. 37**

Source: Committee on Import/Export.

Subject Matter: Fraudulent Certification of Export Shipments I/ER #7.

**Resolution**

RESOLVED that USAHA urge the state licensing boards of the 50 states to take the following action:

Upon notification by APHIS that the federal accreditation has been suspended for fraudulently certifying an export shipment. The state licensing boards should be encouraged to:

A. For the first incident, suspend the veterinarian's license to practice in that state for a period of not less than three (3) months.

B. For the second incident, suspend the veterinarian's license to practice in that state permanently, and officially notify the boards of the other 49 states and the Commonwealth of Puerto Rico of said action and the reasons for it.

**Resolution No. 38**

Source: Committee on Import/Export.

Subject Matter: Plum Island Animal Disease Center I/ER #1.

**Resolution**

RESOLVED: that USAHA show USDA its support for keeping diagnostic activities related to foreign animal diseases on Plum Island, vs. a mainland setting, except for the current arrangement with the National Veterinary Services Laboratories (NVSL), and support for the consolidation plan for PIADC as presented by USDA, ARS and APHIS.

USAHA recommends a special three-year appropriation of $15 million per year be requested for major renovation and improvements at PIADC, and a permanent appropriation increase of $3 million per year be requested for facility maintenance and improvements at PIADC.

**Resolution No. 39**

Source: Committee on Import/Export and Committee on Foreign Animal Diseases.

Subject Matter: Importation of Ostriches I/ER #2.

**Resolution**

RESOLVED: that USAHA urge USDA, APHIS to promulgate import
regulations, without delay, for the safe importation of ostriches and other ratites, to assure their freedom from ticks, other ectoparasites and foreign poultry diseases. Such regulations should allow the importation of fertile eggs and chicks from captive managed parents. Ostrich chicks should not exceed a size which can be readily and safely handled for inspection and acaracide treatment (e.g. under 15 pounds in weight and 30 inches in height). Other ratites should similarly be restricted to a size which can be safely handled. The importation of these birds should be allowed through federal and carefully selected private quarantine stations.

Resolution No. 40
Source: Committee on Import/Export.
Subject Matter: State Permit for International Import Shipments I/ER #4

Resolution
RESOLVED that USAHA request that USDA-APHIS obtain a prior permit from state or states of destination for all livestock, poultry, or exotic animal or avian species prior to issuing an international permit to the country of origin for importation.

USAHA strongly urges USDA-APHIS to direct their inspector in charge at all import or quarantine stations to notify the State Veterinarian prior to the release of any such imports and supply said official with the name, address, and phone number of the recipient and a list of those animals or birds which are being released to go to that state.

Resolution No. 41
Source: Committee on Import/Export and Committee on Foreign Animal Diseases.
Subject Matter: Animal Import Inspection I/ER #6.

Resolution
RESOLVED: that APHIS be requested to coordinate animal importation procedures with other involved federal agencies and implement such inspections or certifications on dogs and all other warm-blooded or cold-blooded animals as will effect the interception of, and preclude the introduction into the United States of screwworms and disease vectors which could be catastrophic to the livestock industry and wildlife populations.

Any terrestrial vertebrates imported into the United States should be inspected and/or treated for ectoparasites (this means all birds, mammals and reptiles).

Resolution No. 42
Source: Committee on Transmissible Diseases of Poultry and other Avian Species.
Subject Matter: Mandatory Testing for Salmonella enteritidis.

Resolution

RESOLVED that USAHA is strongly opposed to a premature mandatory testing program of all breeder, multiplier breeder and commercial egg production flocks until the scientific knowledge base is developed to allow construction of a program to permit accomplishing the goal of eliminating highly invasive S.E. from all flocks in the table egg industry.

Resolution No. 43
Source: Committee on Epizootic Attack. Failed to pass approval by General Assembly.

Resolution No. 44
Source: Committee on Aquaculture.
Subject Matter: Veterinary Education in Aquaculture and Aquatic Animal Medicine.

Resolution

RESOLVED that the USAHA strongly encourage and support the inclusion and expansion of aquacultural and aquatic animal medicine and production within the professional and continuing education programs of colleges of veterinary medicine and animal health sciences.

Resolution No. 45
Source: Committee on Aquaculture.
Subject Matter: Importation Controls for Protection of Aquaculture and Natural Resources.

Resolution

RESOLVED that USDA urgently requests that USDA, APHIS and USDI, FWS cooperatively provide appropriate control, through quarantines, testing, and/or certification, of the importation and dissemination of live aquatic animals, their germ plasm, larvae, meats, organs, tissues, cells, blood and other products and substances derived from aquatic animals which are or may be infected, infested or accompanied by pathogenic, parasitic or other organisms harmful to the aquatic or terrestrial agricultural and wildlife resources of the U.S.

Resolution No. 46
Source: Committee on Aquaculture.
Subject Matter: Cooperation between Federal and State's Departments of Agriculture and Natural Resources for the Benefit of the U.S. aquaculture industry.
Resolution

RESOLVED that USAHA urges the USDA (APHIS and other agencies), the U.S. Department of the Interior (Fish and Wildlife Service) and other appropriate Federal agencies to work together and in cooperation with State Departments of Agriculture, Natural Resources, and other appropriate state agencies, to promote and enhance provision of services to and development of the U.S. aquaculture industry.

Resolution No. 47
Source: Committee on Epizootic Attack.
Subject Matter: Utilization of Centers of Excellence.

Resolution

RESOLVED that the Epizootic Attack Committee (USAHA) strongly recommends that federal and state agencies and industry support the diagnostic, epidemiologic and research capabilities available at the existing centers of expertise.

Resolution No. 48
Source: Committee on Professional Oversight.
Subject Matter: NAHMS—Reporting of Program and Foreign Animal Diseases.

Resolution

RESOLVED: Whenever NAHMS surveys reveal evidence of Cooperative Program diseases or foreign animal diseases, they must be reported to proper authorities and handled routinely.

Resolution No. 49
Source: Committee on Epizootic Attack.
Subject Matter: Disposal of Dead Animals.

Resolution

RESOLVED that APHIS conduct an experiment as soon as possible to determine if composting birds infected with highly pathogenic avian influenza, will render the product free of the infectious agent.

Resolution No. 50
Source: Committee on Sheep and Goats.
Subject Matter: Establish a genetic base for sheep milk and cheese production in the United States.

Resolution

RESOLVED: That USAHA supports the decision of USDA to import sheep and goat germ plasm from countries that might be scrapie affected
but are free of foot and mouth disease;

USAHA encourages USDA to assign a high priority to funding projects involving development of production of sheep milk and cheese in this country.

**Resolution No. 51**
Source: Committee on Sheep and Goats.
Subject Matter: Research on Chlamydia of Sheep and Goats.
Resolution
RESOLVED USAHA strongly recommends that USDA, through ARS, give high priority to continued research on Chlamydia in sheep and goats.

**Resolution No. 52**
Source: Committee on Sheep and Goats.
Subject Matter: Bovine Spongiform Encephalopathy (BSE).
Resolution
RESOLVED USAHA recommends research by USDA to determine if U.S. strains of sheep scrapie and transmissible mink encephalopathy are related to BSE in cattle.
USAHA further recommends intensified efforts to develop preclinical diagnostic tests for sheep scrapie and BSE and to determine the value of the SIP test in the control of scrapie in sheep and BSE in cattle.

**Resolution No. 53**
Source: Committee on Import/Export.
Subject Matter: Import Notification I/ER #10.
Resolution
RESOLVED, that USAHA urge governing boards of AATA and IATA to proceed to enact rules requiring airlines to have the Importers' Import Permit in hand at the time of booking the flight for exotic species and they be required to notify veterinary officials in the country of destination at least 24 hours prior to departure from Port of Origin.

**Resolution No. 54**
Source: Committee on Epizootic Attack.
Subject Matter: Realignment of APHIS Funding Priorities.
Resolution
RESOLVED, that USAHA urges the Secretary of Agriculture cooperate with APHIS to identify and provide the necessary resources to realign its priorities to develop and maintain the infrastructure to prevent and respond effectively to foreign disease outbreaks.
The APHIS program should include, but not be limited to:
1. The initiation of an emergency response fund;
2. Education of the general public;
3. A mechanism coordinate APHIS activities with ARS research;
4. Developing a cadre of trained animal health professionals; and
5. Developing effective foreign and domestic surveillance and detection programs.

Resolution No. 55

Source: Committee on Epizootic Attack.
Subject Matter: Recognizing Accomplishments of Mexico in exotic disease preparedness.

Resolution

RESOLVED that before changes in personnel are made in these vital programs that APHIS, USDA evaluate very carefully the loss of investment in the training and knowledge of these dedicated career employees that have helped maintain our freedom from these various exotic diseases and pests.

A copy of this resolution be sent as soon as possible to the Administrator of APHIS, to the Assistant Secretary for Marketing and Inspection Services and to the Secretary of Agriculture.
AMENDMENTS TO UNITED STATES ANIMAL HEALTH ASSOCIATION CONSTITUTION AND BYLAWS

In “Article VIII – Amendments” in the Constitution, add a new paragraph between lines 274 and 275 as follows: “In the event of an extreme financial emergency to the association as determined by the Board of Directors, the dues structure of the organization may be amended immediately, solely by action of the Executive Committee at the next annual meeting, as set forth in Article V – Dues of the Bylaws”.

In “Article V – Dues” in the Bylaws, add a new paragraph after line 363 as follows: “In the event of an extreme financial emergency to the association as determined by the Board of Directors, the dues structure of the organization may be amended immediately, solely by action of the Executive Committee, provided that such contemplated increases in dues have been furnished in writing to each member of the Executive Committee at least ninety (90) days before such action is taken.”

In “Article V – Dues” in the Bylaws, on line 356, strike the words “thirty dollars ($30)” and insert in lieu thereof “forty-five dollars ($45)”; and on line 362, strike the words “two hundred dollars ($200)” and insert in lieu thereof “three hundred dollars ($300)”.

REPORT OF COMMITTEE ON ANIMAL WELFARE

Chairman: Dr. M. S. Silberman, Atlanta, GA

Co-Chairman: Mr. Steve L. Kopperud, Washington, DC

JoAnn M. Alumbaugh, IA; Lovell A. Anderson, IA; Leslie G. Billingsley, CA; D. J. Carr, OH; R. L. Crawford, MD; P. M. Eppele, SD; Bruce H. Ewald, NJ; Joe Finley, TX; Bob Gadd, SD; Ann Gonnerman, MO; C. H. Graham, MO; Les Graham, MT; F. E. Hasenauer, CA; Barbara Heffernan, DC; Del E. Hensel, CO; Pat Hoctor, IN; Michele C. Howard, CA; Richard D. Hull, IL; Tom Hunt, MI; Ralph Jones, SD; A. T. Kimmell, KS; John H. Lang, WI; E. T. Littledike, MD; C. W. S. Lum, HI; David Meeker, IA; W. D. Miller, VA; Raymond L. Morter, IN; Victor F. Nettles, GA; Tomas A. Neuzil, IA; Mabel A. Owen, MA; Ronnie Polen, NJ; Robert Rice, FL; Jim Rich, WA; Grover W. Roberts, CA; D. F. Schwindaman, MD; Stephen K. Scott, IA; Christine Stevens, DC; Nancy Stirling, SD; R. F. Taylor, NJ; Amy Wallop, DC; Norman Willis, CAN.

The Committee on Animal Welfare met on Tuesday, October 31, 1989, at 1:30 p.m. Chaired by Dr. M. S. Silberman. There were 22 Committee members and 28 guests present.

Dr. Silberman announced that any member wishing to present a resolution for action by the Committee, should present it in typed form to the Chairman at least 30 days prior to the meeting. The Chair also requested that members advise him of possible topics for the next meeting.

Dr. Joan Arnoldi, Deputy Administrator of USDA, APHIS gave a brief explanation of the table of organization of Animal Welfare Section of APHIS. She explained the new goals for training Animal Welfare inspectors and expressed a positive outlook for the future. She stated that total inspections were down for the fiscal year due to the reorganization changes, but stated that the quality of the inspections was enhanced.

She also said that the agency was reviewing the possibility of including rats and mice under the Act.

Mr. Tom Cook, Executive Vice President of N.C.A. advised the Committee of the interest his organization had in Animal Welfare issues and of their development of a pro-active program on their part. He also discussed various pending federal and state legislation. It appears at this time that none of the proposed bills are finding significant support.

He also stated that Animal Welfare was one of N.C.A.’s top three priorities.

Mr. Jim Rich of North American Deer Farmers Association, a fairly new organization with major membership concentrated in Oregon, Washington and British Columbia, explained that all Cervidae were not suitable for animal agriculture. The preferred ones being Fallow deer, Sika deer, Elk and Red deer. He described the intensive management required in han-
dling concentrations of deer on pastures. He explained that deer farming was a learning experience. It was brought out that all venison from their production was processed in either state or federal inspected plants and that good deer farming areas were also good dairy production areas.

Mr. John Lang, President of Livestock Conservation Institute, showed the Committee a 20 minute video on swine handling centered on moving and transportation of swine. He stated that the film was available at a small price. He stated that well-trained personnel was more important than the type of facility in successful management of swine.

Ms. Cathy Liss of the Animal Welfare Institute gave an update on the 1989 CITES meeting in Lucerne, Switzerland stating that one of the major accomplishments was of the status of the African elephant from threatened to endangered. She also stated that over 100 countries including the U.S. have banned the sale of ivory.

She also told the committee about a new swine management program in the pilot stage, being carried out on a farm in Minnesota. The project is too early for evaluating on a financial basis at the time of the report. Ms. Liss will forward to the Chairman information as it becomes available for distribution to the committee members.

Ms. Liss also expressed AWI's concern with the use of strychnine for the control of feral skunks. She and her organization would support the use of humane trapping methods. Dr. Calvin Lum, State Veterinarian of Hawaii, reviewed their canine/cat import program. Dr. Lum stated that the program is presently under attack and he felt that there was not wide understanding of the issues involved. He sees the issue as a public health problem.

The Chairman stated that he had made an unannounced visit to the Hawaii quarantine facility and found it to be excellently managed. The facility was old and Dr. Lum told the group that construction of new quarters was already under way. The Hawaii facility quarantines about 3,000 animals a year.

The Chairman adjourned the meeting at 4:45 p.m.
REPORT OF THE COMMITTEE ON HEMOPARASITIC DISEASES

Chairman: Dr. W. G. Nelson, Boise, ID
Vice Chairman: Dr. R. L. Hartin, Oklahoma City, OK

F. J. Alderink, MD; J. L. Alley, AL; R. D. Anderson, NV; J. F. Badger, MO; D. M. Bedell, GA; G. M. Buening, MO; A. A. Cuthbertson, NV; W. C. Davis, WA; C. N. Dobbins, GA; W. B. Fairchild, LA; C. A. Gipson, MD; R. Harrington, TX; T. J. Holt, PR; J. D. Huber, MD; O. James, MT; D. Kimbrell, AR; D. L. Notter, KY; J. O. Pearce, Jr., FL; M. R. Ristic, IL; C. E. Starkey, AR; N. R. Swanson, WY.

The Hemoparasitic Disease Committee met on November 2, 1989 at 1:30 PM. 41 members and guests were in attendance.

Dr. Gene Luther of Louisiana State University reported on the field evaluation of an experimental bovine derived anaplasmosis vaccine on three herds of cattle in Louisiana. In a two year study the experimental vaccine was injected into over 1200 head of bred cattle in their last trimester of pregnancy to rule out the presence of erythrocytic antigens in the vaccine that would cause Neonatal Isoerythrolysis (NI) in their offspring. There have been no cases of NI in calves of vaccinated dams and there have been no deaths in the vaccinated cattle from acute cases of bovine anaplasmosis. This killed vaccine could become a commercial reality within a year or two.

Dr. Richard Hidalgo of Louisiana State University gave a report on the 8th National Veterinary Hemoparasitic Disease Conference in St. Louis, MO. The conference was attended by approximately 100 participants from 12 of the United States and 12 foreign countries. Dr. Richard Hidalgo, Program Chairman, reported that 73 papers were presented by authors from 9 foreign countries and 12 of the United States. Papers were presented on etiology and pathogenesis, vectors and transmission, epidemiology, diagnosis, immunization and chemotherapy of diseases caused by Anaplasmababesia, Theileria, Ehrlichia and Cowdria. Proceedings of the conference were recently published.

Dr. David Stiller of the University of Idaho and USDA-ARS, Animal Disease Research Unit, presented a paper on comparison of DNA probe with complement-fixation (CF) and indirect immunofluorescence (IIF) tests for diagnosing anaplasmosis in suspected carrier cattle from an enzootic area near Spokane, WA. Sixty-five cattle initially were tested by these methods, then retested at one month and two months after treatment with long-acting oxytetracycline. Six (9.2%) of the initial serum samples tested CF-positive. By contract, 60 (92.3%) and 64 (98.5%) of the samples tested positive by IIF and the DNA probe, respectively. The DNA probe hybridized with two samples taken one month after treatment and with a different two samples taken two months after treatment. The mean IIF
titers were reduced at both posttreatment intervals. The results suggested that the drug failed to eliminate infection in all animals but did reduce the levels of infection below the sensitivity of the probe and interrupted stimulation of antibody. The DNA probe and the IIF test thus appear to be much more sensitive than the CF test in detecting carrier infections and should be considered for use in epidemiological studies. Dr. Stiller also reported on the transmission of *A. marginale* by male ticks in studies in Southwestern Idaho. The studies indicate that infected male ticks in the field freely transfer from host to host and in the process, transmit *A. marginale* to susceptible cattle.

Additional studies indicate that *D. andersoni* is a natural vector of *A. ovis* in Idaho and that partially fed *dermacentor* females can transmit anaplasma intrastadially and biologically, thus perhaps acting through delayed interhost transfer as epidemic vectors in nature.

Dr. Stiller’s more complete paper is attached to this report.

Dr. Stiller reported some work on the new world tick *Boophilus microplus* as a vector for *Babesia equi* and studies indicate this is the first evidence that *B. microplus* and indeed any new world tick can transmit *B. equi*.

Dr. David Stiller gave an update on Dr. Kathy Kocans work on the involvement of the tick salivary gland on maturation and transmission of *A. marginale*.

A complete copy of Dr. Kocans paper is attached to this report.

Mr. J. O. Pearce, a cattleman from Florida, spoke on the industries’ role in the control or eradication of anaplasmosis.

Mr. Pearce stressed four points he felt were important in anaplasmosis control:

1) Industry must be assured research is done on the vector that transmits the disease.

2) Industry must cooperate with researchers to obtain financing for research.

3) Researchers must convince industry of success and economic feasibility before initiating any program.

4) Success of this program will be contingent upon educating the industry both through national organizations and local producer contacts.

Dr. Gale Wagner of Texas A & M University gave a report on a DNA probe for *Babesia bovis*. The DNA probe appears to be *B. bovis* specific and without further amplification, at least equal in sensitivity to the best light microscopy. Dr. Wagner reported the serological prevalence of *Babesia odocoilei*, the babesia of white tailed deer, does not overlap with the seroprevalence of bovine *B. bovis* in south Texas. Seropositive deer were found primarily in the post-oak savannah/Gulf slope habitats of eastern and central Texas. *B. odocoilei* was isolated from deer in each of those areas.
HEMOPARASITIC DISEASES

*B. odocoilei* has been cultured in deer erythrocytes; the cultured organism will infect deer but not cattle. Additional studies included a look at the remarkable filtering device in the deer spleen that explains why deer RBC's can be infected in vitro with *B. bovis*, but that the deer itself cannot be infected. The committee discussed the need for a current survey on *A. marginale* prevalence in the U.S. and also the problems encountered when susceptible adult cattle are exposed to anaplasma by repeated use of instruments and needles.

There were no resolutions presented.

Committee adjourned at 4:00 PM.

RECENT DEVELOPMENTS IN ANAPLASMA EPIDEMIOLOGY

There is increasing interest in the hypothesis that the males of many tick species that are vectors of *Anaplasma marginale* may have an important epidemiologic role in transmitting the parasite intrastadially and biologically through transfer from infected to susceptible cattle in the field. This hypothesis, however, has not been tested experimentally. Our objective in the present study was to determine whether, and with what frequency, *A. marginale*-infected males of *Dermacentor andersoni* transferred from calf to calf and transmitted the parasite under semi-natural conditions. During May 1988, 8 susceptible splenectomized Holstein calves were confined for 17 days in a field compound (5,600 ft.²) located at 1300 meters elevation in semi-arid desert habitat in an area of southwestern Idaho that is enzootic for bovine anaplasmosis. Two days before being placed in the compound, 3 of the calves (donors) each received (in a dewlap patch) 450 male ticks that 2 weeks earlier had fed on a calf infected with a Virginia strain (VAM) of *A. marginale*. Of 30 male ticks tested with a DNA probe for *A. marginale*, 29 (97%) gave positive probe signals. Also, one of the donors and each of the 5 remaining calves (recipients) received 50 unfed, normal colony female ticks to provide pheromone. All ticks were coded according to original host, which also distinguished them from possible native ticks in the compound. The 8 calves were placed in the compound and their patches removed. The recipients were examined for transferred male ticks on days 4, 8, 12, and 17; the donors were similarly examined on day 17. On day 8, each recipient received another 50 unfed, normal colony female ticks. Transferred male ticks on the recipients were checked for code, then placed in dewlap patches to resume feeding. All calves were moved to a barn on day 17, where they were monitored for signs of infection. Although only 4.9% of the ticks were recovered, 52.6% of the male ticks recovered had transferred, as had 7.6% of the recovered female ticks. Each of the 5 recipients received at least one transferring male tick; all 3 donors were represented by transferring males. All 8 calves developed clinical anaplasmosis and were confirmed to
be infected with VAM by strain-specific monoclonal antibodies and immunofluorescence. Tick transfer appeared to be of the direct, immediate type, as indicated by a high frequency of physical contact among the calves and by very few ticks being collected from vegetation. Results of a separate experiment in which infected ticks were fed to susceptible calves suggest that transmission in the compound resulted from tick bite, not ingestion of ticks. The use in the present study of an exotic \textit{A. marginale} strain, together with removal of the calves from the compound before they became parasitemic, permits the conclusion that the calves were infected by the transferring males, not by native ticks or biting flies. These findings indicate that infected male ticks in the field freely transfer from host to host and can, in the process, transmit \textit{A. marginale} to susceptible cattle.

In a separate series of experiments, normal colony females of \textit{D. occidentalis} and \textit{D. andersoni} were respectively fed for 5 days on an \textit{A. ovis}-infected sheep and an \textit{A. marginale}-infected calf. The ticks were then held off the host for 2 or 4 weeks before being test-fed on susceptible sheep and calves, respectively. Both parasites were transmitted. Of 19 parallel \textit{D. andersoni} females individually tested with a DNA probe for \textit{A. marginale}, all gave positive probe signals. These data suggest that \textit{D. andersoni} is a natural vector of \textit{A. ovis} in Idaho and that partially fed \textit{Dermacentor} females can transmit \textit{Anaplasma} intrastadially and biologically, thus perhaps acting, through delayed interhost transfer, as epidemic vectors in nature.

Finally, progress will be reported on our efforts to develop and refine a non-radioactive label for use with our DNA probe for \textit{A. marginale}.

\textbf{CURRENT RESEARCH ON THE DEVELOPMENT OF ANAPLASMA MARGINALE IN A TICK VECTOR, \textit{DERMACENTOR SPP.}}

Katherine M. Kocan, Willard L. Goff, David Stiller, S. A. Ewing, Selwyn J. Barron, Jakie A. Hair, Travis C. McGuire

We have continued our research on the development of \textit{Anaplasma marginale} in a tick vector, \textit{Dermacentor} spp. Our initial studies were focused on the development of \textit{A. marginale} from infection of nymphs by feeding on an infected calf through transmission as adults.\textsuperscript{1-15} These studies were based on the belief that most organisms were transmitted transstadially by ticks through infection in one stage (larvae or nymphs) with transmission after molting to the next instar. Subsequently, studies were undertaken by Dr. David Stiller, USDA-ARS Animal Diseases Research Unit, to investigate the role of male ticks in transmission of anaplasmosis. Male \textit{Dermacentor} spp. are intermittent feeders that remain in the environment for long periods with the likelihood of transferring among cattle.\textsuperscript{16} Males proved to become infected with \textit{A. marginale} during short periods of
HEMOPARASITIC DISEASES

feeding on an infected calf because they transmitted the organism when they were transferred to and allowed to feed on susceptible calves. Our current studies on the development of A. marginale in ticks are focused on the parasite cycle in these interhost-transferred male ticks. Our main goal in these studies was to develop a strategy for producing ticks with highly infective salivary glands.

Of all forms studied, the tick salivary gland stage is probably the most important to understand because it is passed from ticks to cattle in the natural cycle. This stage may be especially important in the development of vaccines because it may possess unique antigens that, if neutralized by specific antibodies, may prevent infection of cattle. If unique antigens occur on the tick-transmitted stage it may be important to incorporate them into a subunit vaccine. In addition the salivary gland stage would be a likely candidate to propagate in mammalian cell culture because it is the stage that first invades bovine cells in the natural developmental cycle.

The research on the characterization of the salivary gland stage represents a cooperative effort between our laboratory at Oklahoma State University (OSU) and researchers at Washington State University (WSU) under the direction of Dr. Travis McGuire and the USDA-ARS Animal Diseases Research Unit (ADRU) directed by Dr. John Gorham. Experimental protocols have been developed in cooperation with USDA entomologist, Dr. David Stiller of ADRU and University of Idaho at Moscow. A key to our ability to follow the development of this parasite in tick salivary glands is the use of a DNA probe that has been developed recently at WSU and successfully applied to bovine and tick studies by Dr. Will Goff from USDA-ADRU. The probe is an important tool for developmental studies of A. marginale in any tissue, but it is especially useful in the salivary gland studies because we have not been able to follow parasite development consistently with light and electron microscopy or with specific antibodies. With microscopy, we have only been able to demonstrate colonies of A. marginale in salivary glands by subjecting the ticks to a temperature change. Preliminary studies have shown that the probe is sensitive enough to detect A. marginale DNA in an individual salivary gland from an infected tick. It has been well documented that surface antigens of parasite stages often differ, varying with the host and stage; this variability in surface antigens limits the use of specific antisera as a tool for detecting some parasite stages. Specific bovine antisera have not been useful in identifying salivary glands infected with A. marginale.

The DNA probe along with serology, animal inoculation, light and electron microscopy has been used successfully to identify A. marginale in tick gut and salivary gland tissues and had been used to follow parasite development in ticks. Although we established the role for the salivary gland in development of the transmitted stage in our studies of adult ticks infected as nymphs, numbers of organisms in these salivary glands were not adequate for further studies. However, the development of A.
marginale in interhost-transferred males was found to be more intense, resulting in highly infected salivary glands. We are currently proceeding with studies on the antigenic composition of this stage and will test its infectivity in various cell cultures.

REFERENCES


REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: Mr. Clint Booth, TX
Vice Chairman: Mr. Dan Childs, FL

J. A. Acree, MD; W. L. Adams, GA; J. N. Armstrong, NV; R. G. Burdett, OR; R. B. Caffey, MD; R. A. Carmichael, IA; G. P. Combs, MD; T. M. Cook, DC; D. A. Dunn, IL; P. M. Eppele, SD; W. H. Fales, MO; L. A. Ferguson, MD; R. Fetzner, VA; H. J. Hansen, VT; F. H. Harding, IL; W. C. D. Hare, Canada; W. T. Harrer, MT; R. Harrington, TX; J. B. Healy, NM; D E. Herrick, MD; W. P. Heuschele, CA; M. C. Howard, CA; T. Hunt, MI; R. C. Knowles, DE; N. Konnerup, WA; H. A. Kryder, Jr., MD; D. W. Luchsinger, VA; C. A. Mebus, NY; R. M. Nervig, IA; J. Oden, TX; W. D. Prichard, OR; G. B. Rea, OR; W. H. Ritchie, MD; J. D. Roswurm, CA; J. R. Stafford, VT; P. J. Taylor, MT; S. V. Timberlake, NY; C. D. Vail, CO; W. H. Waldo, NE; J. S. Walker, DC; A. Wallop, DC; H. A. Waters, VA; C. R. Weston, NH; R. D. Whiting, MD; G. O. Winegar, MD; R. J. Yedloutschnig, NY.

The Committee on Import-Export met at 1:30 pm on Wednesday, November 1, 1989, during the annual meeting of the USAHA, held at the Riviera Hotel Convention Center in Las Vegas, Nevada. The chairman called the meeting to order with 40 of the 48 members present and with a total attendance of over 90 people.

The following report from the Import/Export Animals Staff was given by Drs. D. E. Herrick, George Winegar, S. S. Richeson and Mr. Wade Ritchie.

IMPORT ANIMALS

Three shiploads of approximately 27,000 wether lambs each, a total of 79,484 lambs, were released from New Zealand during Fiscal Year 1989. The lambs were quarantined for 30 days at the U.S. Department of Agriculture (USDA) supervised, privately owned quarantine facility in Portland, Oregon. There were no major disease occurrences in quarantine. The death loss in quarantine was near .6 percent, death loss on the ship while the lambs were en route to the United States was less than 2 percent. The importer and facility operator plan to increase shipment size to 40,000 to help reduce their costs.

There were 472 llamas and alpacas from South America released from a 90-day quarantine at the Harry S Truman Animal Center (HSTAIC), Fleming Key, Florida, in December 1988. The animals met all USDA health requirements and negative tests for foot-and-mouth disease (FMD), Trypanosoma vivax, brucellosis, tuberculosis, blue tongue and other exotic disease.

There were 140 Meishan, Fenjiang, and Meng breeding pigs from China released from a 120-day quarantine at HSTAIC in July 1989. USDA imported the swine in cooperation with Iowa State University and the University of Illinois for research purposes.
There were 1200 doses of swine semen imported from China in July 1989 after meeting USDA health requirements. There were 13,771 doses of bovine semen imported from Brazil in October 1989.

The above importations from China and South America, where foot-and-mouth disease (FMD) and other serious exotic diseases exist, were directly supervised in the country of origin by USDA, Animal and Plant Health Inspection Service (APHIS), Veterinary Service (VS), foreign animal disease trained veterinarians and test specimens tested prior to release at USDA's Foreign Animal Disease Diagnostic Laboratory (FADDL) on Plum Island, New York.

Ticks capable of transmitting East Coast Fever and other livestock disease, were found on one animal of a shipment of 10 black rhinoceroses imported from South Africa in July 1989. The animals were inspected on arrival in Dallas, Texas, by USDA, APHIS, VS, veterinary and animal health technicians, sprayed several times to eliminate the ticks, and then released to the importer for entry into the United States.

Screwworms were found during quarantine inspection by a USDA, APHIS, VS, veterinary inspector on a horse imported from South America. The horse was quarantined for entry in a USDA approved temporary quarantine facility to handle returning exhibition horses San Juan, Puerto Rico. The horse was treated for the larva and sterile flies were air dropped over the area at risk for the period of time deemed necessary to prevent the establishment of screwworms in Puerto Rico.

Czechoslovakia reported an outbreak of contagious equine meteritis (CEM) and was placed on the list of countries considered CEM-affected by USDA in the Department's import regulations.

A regulation has been published which allows horses to be imported for no more than 60 days to participate in special events without meeting CEM import requirements. They then must return to their country of origin. Only certain racing thoroughbreds were eligible to enter the United States without meeting all CEM requirements prior to this regulation.

Several hundred goat embryos were imported from New Zealand and increasing numbers of cattle embryos are being imported from Great Britain, Canada, and Australia. Bovine embryos were imported from Costa Rica.

USDA, APHIS suspended the importation of cattle and other ruminants from the United Kingdom because of the outbreak of bovine spongiform encephalopathy (BSE). There were approximately 3,500 infected herds and reports of 100 to 150 infected herds per week according to veterinary officials in the United Kingdom when contacted in July 1989. Bovine embryos and semen may be imported with special certification from the Ministry of Agriculture, Fisheries and Food of the United Kingdom, since it has been determined these items may be imported without risk of introducing BSE.
There are requests to import large numbers of Red deer from New Zealand. USDA is reviewing health and quarantine requirements including *Elaphostrongylus cervi*, the tissue worm of deer. U.S. wildlife agencies are concerned because the worm can cause hind limb paralysis and pneumonia in deer. It is believed to be exotic to the United States.

There has been considerable interest during Fiscal Year 1989 to import llamas and alpacas born in South America where foot and mouth disease exists, through another country (third country) that is free of FMD. If Chile is designated free of FMD, as proposed in the Federal Register, a separate regulation was proposed which provides less restrictive quarantine and health requirements for import.

**AVIAN IMPORT ACTIVITIES**

A proposed rule to change the regulations allowing for the offspring of birds that originate in the United States without quarantine was published in December 6, 1988, with a 90-day comment period. There were approximately 700 comments opposing the rule change, and three comments in favor of the proposal. No final action has been taken at this time on the proposal.

A. Poultry and Hatching Eggs

There were 6,976,997 poultry including day old chicks and 22,321,412 hatching eggs imported into the United States during FY 1989. Most of these were from Canada except approximately 2,000,000 hatching eggs.

Approximately 250,000 turkey hatching eggs were imported from the United States, quarantined for 8 weeks post hatch with serological testing to determine their status for viral turkey rhinotracheitis prior to release. Quarantines were conducted in California and West Virginia.

Currently USDA is requiring serologic negative certification for adenovirus 127 (egg drop syndrome), *S. enteritidis*, and the causative agent of viral turkey rhinotracheitis (swollen head syndrome in chickens), in addition to the required health certification CFR Part 92.5 for all chickens and turkey hatching eggs.

A poster session was presented at the AVMA convention in Orlando, FL, in July, 1989, explaining the USDA rules and regulations for the importation of poultry and their hatching eggs.

B. Commercial Birds

Importation of commercial birds continued at the FY 1988 level. There were 447 lots of 36,8098 birds offered for importation with 4 lots of 3202 birds being refused entry due to VVND. Those birds were from Honduras and Indonesia.

In response to a request by the Association of Avian Veterinarians, the USDA promulgated regulations to permit USDA license vaccines except vaccines for Newcastle disease, avian influenza or other hemagglutinating
import-export

virus of poultry to be administered to birds while in quarantine. As a result, two killed virus products, Pacheco's disease and psittacine pox vaccines, have been given conditional license for use. A third vaccine, psittacine reovirus, is currently being used in field trials prior to conditional licensing. These products are available for use with birds quarantined in USDA approved stations, and for use within the domestic population with state official approval.

C. Pet Bird Program

Pet birds are currently being imported and quarantined at New York, Miami, Los Angeles, and Honolulu. There were 2,811 birds imported and quarantined during FY89. All pet birds were tested for VVND, and no Newcastle was isolated.

D. Smuggled Birds

Birds that illegally entered into the United States and are seized were quarantined at the USDA facilities designed for smuggled birds located at Mission, Texas and Otay Mesa, California.

There were 1,095 birds quarantined and auctioned to the public as permitted by a Memorandum of Understanding between the Departments of Agriculture, Interior, Justice, and Treasury.

A campaign to alert the public of the risk of dealing with smuggled birds was carried out through news releases, a poster display, and a radio spot presentation for national distribution. There were 2 outbreaks of VVND in the United States during FY 89, and the outbreaks were believed to occur from exposure of some young amazon parrots which may have entered the United States illegally.

There were only three lots of smuggled birds positive for VVND during FY89. One lot consisted of 115 birds, with numerous endangered species involved. These birds were part of a Fish and Wildlife Service undercover operation in Southern California in which 12 people have been charged with smuggling.

E. Ostrich Imports

Ostriches were imported into the United States under the USDA Agriculture regulations which pertain to commercial bird importation. These regulations require a USDA import permit, a minimum 30-day quarantine in USDA facility or any approved private facility, and a health certificate issued by a full-time salaried National government Veterinarian from the exporting country. The health certificate for the birds, including ostriches, must state that all the birds covered by the certificate have been inspected by the foreign government veterinarians and no evidence of Newcastle disease, ornithosis, or other communicable disease of poultry was found. In addition, insofar, as has been possible to determine, they were
not exposed to any such disease during the 90 days immediately preceding their exportation.

Ostrich imports have been a recent occurrence in the United States. There were no ostriches offered for importation during 1986. In 1987, one group of 150 chicks arrived in New York, and one group of 198 chicks arrived at the port of Brownsville, Texas. In 1988 537 birds arrived in New York; 73 were adults, and the remainder chicks. In 1989, there have been 345 ostriches imported into the New York Port, 380 into the Brownsville Port, and 100 imported into the Chicago Port of entry consisting of chicks and adults.

Ostriches have been imported from the following countries: Angola, Belgium Botswana, Dominican Republic, Israel, Portugal, Switzerland, and Tanzania (over 50%).

To date a grand total of 1710 ostriches have been imported since 1987. We are facing a challenge to develop the proper means of handling a species where we have had very limited experience. This is especially true regarding the examination and treatment of imported birds for what they may harbor in the way of exotic diseases including ecto and endoparasites.

No shipments of ostriches have been refused entry by the USDA for communicable infectious disease. All of the birds have been tested at our National Animal Disease Laboratory in Ames, Iowa. This testing has been limited to viral diseases that are communicable to poultry.

The New York Animal Import Center has collected ticks and parasite eggs from the fecal samples of quarantined ostriches to submit to the Ames Laboratory for identification. The birds were also treated with the recommended acaricide.

On June 22, 1989, the Deputy Administrator of the Animal and Plant Health Inspection Service placed a ban on the issuing of permits for the importation of ostriches. On August 21, 1989, an interim rule was published which bans the importation of all ratites (ostriches, rheas, emus, and cassowaries). This rule was published as a result of the exotic ticks that were found on imported ostriches which had been located in Texas and Ohio. It is not the intent of the USDA to permanently ban the importation of all ratites. Rather, USDA feels they should be prohibited until methods can be developed, and regulations published, to permit their safe entry into the United States without a risk to our domestic animal population. These exotic ticks are known to be vectors of heartwater and East coast fever of cattle.

A public meeting was held on August 17, 1989, with the Secretary Foreign Disease Advisory Committee, to gather information on ostrich imports. When a method can be identified that would allow for the safe importation of ostriches, regulations will be promulgated.
IMPORT-EXPORT

ANIMALS-BIR.DS/POULTRY IMPORTED

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CATTLE

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SWINE

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(ESTIMATE)

AVIAN

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*The “Other” category is for noncattle ruminants such as wild ruminants, llamas, alpacas, tapirs, rhinoceroses, and hippopotami.

EXPORT ANIMALS

During Fiscal Year 1989, two export training seminars were held for Area Veterinarians in Charge and export clerks. A total of 54 people attended the seminars. An exporter and an embryo producer participated in the seminars, highlighting problems they encounter in the preparation of export shipments. In addition, a similar course was conducted for 20 entry level veterinarians (PVPC groups.)

Reviews of the export programs in Kentucky and Nebraska were conducted. Only minor problems needing revision were detected during the reviews. Four scheduled reviews were cancelled due to lack of staff personnel and budget reductions.
REPORT OF THE COMMITTEE

Importation of swine was halted by Mexico April 1, 1989, due to hog cholera outbreaks in Mexico in imported U.S. swine. Mexican authorities advised that animals were being diverted from slaughter channels to farms and, with subsequent exposure, hog cholera outbreaks were occurring. The United States could not vaccinate export swine against hog cholera, as requested, due to our free status.

Breeding swine exports were resumed in August. After arrival, the animals were vaccinated for hog cholera by the importers. Imports of slaughter barrows were allowed beginning August 1, 1989. On October 1, 1989, all classes of swine were allowed to be imported for slaughter.

Mexico stopped importations of everything for slaughter but castrated male cattle, sheep, goats, and horses on August 1, 1989. The importations of steers were allowed only if the animals were branded with a letter "U" and tuberculin tested. Through negotiation, slaughter horses were allowed after August 1, 1989 without the "U" brand.

As of October 1, 1989, the following rules apply for slaughter animals:

1. All classes of slaughter cattle and horses are allowed without testing if consigned to Mexican federally inspected abattoirs and transported in sealed trucks.

2. Steers are allowed to non-federally inspected Mexican abattoirs but they must be tested for TB.

3. All classes of swine, sheep, and goats are allowed for slaughter to any Mexican abattoir.

All animals are to be inspected in the United States by Mexican veterinarians prior to crossing the border. Crossing points allowed, to date, are: Brownsville, Del Rio, Eagle Pass, and Laredo, Texas; San Luis, Douglas, and Nogales, Arizona; and Calexico, California. Crossing is to be permitted in the near future at Presidio, Texas, and Columbus, New Mexico. Mexico removed the final restriction on poultry and hatching eggs from certain states due to avian influenza.

Negotiations between VS Export Staff veterinarians and animal health officials of the following countries occurred during Fiscal Year 1989: Argentina, Australia, Brazil, Canada, Chile, Columbia, India, Israel, Mexico, People's Republic of China, Spain, Switzerland, Taiwan, and Tunisia.

Animal officials from other countries were brought to the United States at Foreign Agriculture Services' cooperator expense. They had tours where they visited artificial insemination and embryo transfer centers before visiting VS offices in Hyattsville, Maryland, to negotiate changes in their import protocols. This procedure has been very useful in acquainting the foreign officials with U.S. production systems for animal germplasm, and the effect that their regulations could have on importations from the United States.
The first shipment of porcine semen to Japan occurred in 1989. The first shipment of cattle to Russia was made this year.

During three/fourths of the fiscal year, there were only two Staff veterinarians on the Export Staff due to a transfer of one Staff member. In early September 1989, the third position was filled, and should assist in reducing Staff response time to field questions and requests.

There were a large number of cattle exported in 1987-1988 on the Export Enhancement Program (EEP) contracts. The program was not renewed in FY '89. Discussions about renewal of the program are in progress at the present time, but no decision has been made public.

During the year, three new permanent facilities were approved for inspecting animals prior to embarkation. The facilities are: Steve Doring Services of America, at Seattle, Washington; and Kneif Quarantine Facility, at Burlington, Illinois; and Port of Olympia Facility, at Olympia, Washington. These three facilities are now listed in Title 9, Code of Federal Regulations, Part 91.14. Olympia, Washington was approved as a port of embarkation and is now listed in Part 91.14.

Exportation of horses to New Zealand was resumed in this fiscal year. New Zealand had placed a hold on importing U.S. horses due to our exportation of horses with stable EVA titers that were higher than they had wanted, although this had never been specified. The latest requirement is for two EVA tests on the premises of origin, and two tests during the 30-day preembarkation quarantine with negative or stable titers no greater than positive at a 1:16 dilution.

In FY '89, there were instances, that we are aware of, where mechanical failures in ventilation systems caused serious losses in export shipments. One was a plane load of cattle destined to Lima, Peru, that could not land due to fog. The airport where the plane landed did not have external ventilation equipment, and a large number of the animals on board died. The second incident involved a plane load of cattle destined to Colombia. Problems developed in the ventilation equipment before arrival at the destination, and a large percentage of the animals had expired by the time the plane doors were opened. Columbia officials refused entry of the remaining live animals, and the entire load was returned to the United States. Almost 100 percent of the animals had expired on arrival in Miami, Florida. The entire load was disposed of by rendering under VS supervision.

One shipment of bovine semen to Brazil was refused entry, as the owner tried to enter it without any health documentation whatsoever. The importer had purchased the semen from farmers who had excess semen from a few high priced bulls. The donor sires were eligible for producing semen for Brazil, but it was too late to obtain health certificates.

One shipment of bovine embryos to Great Britain contained a few embryos that did not meet the import requirements of Great Britain. The semen used to produce the embryos did not meet Great Britain require-
ments. The embryos not meeting the requirements were destroyed, and the remaining ones were permitted entry.

A shipment of cattle went from the isolation facility at Grays Lake, Illinois, directly to mainland Australia in April 1989 without having to pass through the Cocos Island quarantine station. All previous shipments had to pass through an extended quarantine on the Cocos Island facility before being returned to the mainland. Two shipments are planned for the winter/spring '89-'90 season.

A new bovine protocol for Australia was developed in FY '89. Some embryos were produced and exported to Australia this year. Negotiations on a new semen and embryo protocol are in progress, with some reduction of the requirements expected.

A program is being developed to collect data from endorsed export certificates and from export health inspection certificates. In the past, we have relied on data from the Department of Commerce for numbers of animals exported. From this data, we were unable to determine state of origin, breed, importers, etc. Frequently, there are requests from the Administration, Congress, breed associations, and others which we cannot answer. With the development of a computerized data collection system, data could be collected that is current to the day. The system would also allow Area Offices to supply their State counterparts, on a monthly basis, with data on exports from their State.

**LIVESTOCK EXPORTS**

<table>
<thead>
<tr>
<th></th>
<th>FY 1988</th>
<th>FY 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10/88-7/89</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>252,771</td>
<td>232,146</td>
</tr>
<tr>
<td>Horses</td>
<td>26,506</td>
<td>31,011</td>
</tr>
<tr>
<td>Sheep</td>
<td>115,938</td>
<td>285,589</td>
</tr>
<tr>
<td>Swine</td>
<td>27,020</td>
<td>141,002</td>
</tr>
<tr>
<td>Total Livestock</td>
<td>422,235</td>
<td>689,748</td>
</tr>
<tr>
<td>Baby Chicks (Breeding)</td>
<td>22,321,290</td>
<td>18,045,532</td>
</tr>
<tr>
<td>Baby Chicks (Not Breeding)</td>
<td>17,584,664</td>
<td>12,378,559</td>
</tr>
<tr>
<td>Turkey Poults</td>
<td>1,090,088</td>
<td>2,200,408</td>
</tr>
<tr>
<td>Other Day-old Poultry</td>
<td>412,808</td>
<td>115,735</td>
</tr>
<tr>
<td>Live Poultry (Not Day-old)</td>
<td>2,883,184</td>
<td>3,456,131</td>
</tr>
<tr>
<td>Total Live Poultry</td>
<td>44,292,034</td>
<td>36,196,365</td>
</tr>
<tr>
<td>Hatching Eggs (Dz)</td>
<td>23,555,732</td>
<td>20,369,564</td>
</tr>
<tr>
<td>(Value in $)</td>
<td>$43,850,268.00</td>
<td>$38,035,862.00</td>
</tr>
<tr>
<td>Bovine Semen</td>
<td>$41,167,096.00</td>
<td>$40,166,334.00</td>
</tr>
</tbody>
</table>
IMPORT-EXPORT

Bovine Embryos
(Value in $)                     \$5,189,875   \$8,820,229
Total Germ Plasm                \$90,207,239  \$87,022,425

The following report from the Import/Export Products Staff was given by Dr. R. D. Whiting.

REQUESTS BY FOREIGN GOVERNMENTS TO BE RECOGNIZED AS FREE OF SPECIFIED DISEASE.

During FY 1988, USDA formally recognized Great Britain as being free of hog cholera, Argentina free of Venezuelan equine encephalomyelitis, and Papua-New Guinea free of FMD. A request by Ireland to be considered free of contagious equine metritis was denied. The request by Morocco to be considered free of African horsesickness (AHS) was withdrawn when an outbreak of AHS in October 1989 was reported in that country. Currently under consideration are requests by Romania and Poland to be considered free of FMD and a proposal by Chile to be recognized free of viscerotropic velogenic Newcastle disease. Because of reported outbreaks of AHS in Portugal and The Republic of Yemen in FY 1989, USDA has initiated action to place in these two countries on the list of nations where AHS is considered to exist.

RESTRICTIONS ON THE IMPORTATION OF EGGS OTHER THAN HATCHING EGGS

On April 13, 1989, an interim rule was published in the Federal Register which placed restrictions on the importation into the United States of eggs (other than hatching eggs) from all countries where Salmonella enteritidis, phage-type 4, is considered to exist. The restrictions went into effect on April 12, 1989. At the time of publication, Canada was the only country outside of the United States considered to be free of this pathogen. The final rule of affirming the interim rule was published in the Federal Register on July 31, 1989.

TESTING SPANISH HAMS FOR THE VIRUCIDAL EFFECT OF CURING

The production of virus-free Parma ham through controlled dry curing of hams obtained from pigs infected with the viruses of African swine fever, FMD, hog cholera, and swine vesicular disease, respectively, is the basis for the examination of the virucidal effect of commercial processing methods for the production of Spanish-type pork products, including Serrano hams. The Animal and Plant Health Inspection Service has signed an agreement with the Ministry of Agriculture, Spain, to examine the stability of these viruses in specialty products such as Spanish cured hams, Iberian cured hams and Iberian cured loins. The study will be conducted at the Plum Island Animal Disease Center over a period of 18 months beginning in 1990 and all costs associated with the project will be paid for by the Spanish Ministry of Agriculture.
REPORT OF THE COMMITTEE

ANIMAL PRODUCTS AND BYPRODUCTS

During FY 1989, 1,247 permits were issued authorizing the importation of restricted animal products/byproducts and controlled materials for evaluation, testing, or commercial distribution. During the same period of time, an additional 2,560 permits were issued for controlled biological materials other than veterinary biologics for research, evaluation, and diagnostic purposes. These permits involved authorization for either the interstate or international movement of animal disease organisms or vectors. Due to new technological developments and the demand for such materials by the biomedical research community, the trend for increased requests for such permits is expected to continue over the next decade.

AMENDED REGULATIONS FOR THE IMPORTATION OF ANIMALS THROUGH THE HARRY S TRUMAN ANIMAL IMPORT CENTER (HSTAIC)

A proposed rule to amend the regulations concerning the HSTAIC was published in the Federal Register on September 5, 1989. The proposal includes procedures which will more efficiently allocate space at that facility and establish priorities for use of HSTAIC. The proposal contains provisions which would prevent any one importer from using the facility more than once during a 2 year period if other importers want to use HSTAIC. The proposal also contains amendments for the criteria for approving embarkation quarantine facilities in the country of origin of the animals to be exported to the United States. The public comment period for the proposal ended on October 5, 1989, and Veterinary Services is now in the process of evaluating the comments which were submitted prior to the closing of the comment period.

CHANGE IN DISEASE STATUS OF CHILE BECAUSE OF FOOT-AND-MOUTH DISEASE (FMD)

A proposed regulation to recognize Chile as being free of FMD was published in the Federal Register on August 17, 1989. The original 60-day comment period was extended to November 15, 1989, in order to allow sufficient time for all interested parties to comment on the proposal and to have the proposal discussed at the 1989 U.S. Animal Health Association meeting. A companion proposal containing the certification, testing and quarantine requirements for the importation of llamas and alpacas from Chile was published in the Federal Register on September 28, 1989. The comment period for this proposal will end on November 27, 1989.

STANDARDS FOR PRIVATE EQUINE QUARANTINE FACILITIES

A proposed regulation containing standards for permanent, privately operated equine quarantine facilities was published in the Federal Register on September 6, 1989. The proposal also contains certain amendments for private temporary equine quarantine facilities and procedures for collection of fees for services the government provides at such facilities. The comment
period for this proposal ends on November 6, 1989.

The following report from Plant Protection and Quarantine was given by Dr. R. B. Caffey.

**X-RAY BAGGAGE INSPECTION**

Plant Protection and Quarantine (PPQ) continues to expand the use of X-ray equipment to screen baggage of air passengers arriving at International airports. Fourteen systems have been delivered. The major airports include Miami, Honolulu, Los Angeles, San Francisco, Chicago, Houston, San Juan, and Boston. All units are scheduled to be in place prior to the end of Fiscal Year (FY) 90.

A 6-month trial project has been completed on the Mexican border at San Ysidro, California, testing the screening of hand-carried baggage of pedestrian traffic. Data indicates that the X-ray was both effective and efficient. Additional units are planned for other large land border stations.

**DETECTOR DOG PROGRAM**

Ten trained teams are currently in place at Miami, John F. Kennedy International Airport in New York, Los Angeles, San Francisco, Chicago, Boston, and Seattle. Recruiting is underway for seven additional teams including one in Houston.

**AUTOMATED COMMERCIAL SYSTEM (ACS)**

The installation of the ACS at 46 PPQ Work Units marked the beginning of an automated system to query air and maritime cargo manifests. Although the U.S. Customs Service (Customs) has not become fully automated as yet, a paperless system is a reality. This will permit our officers to place holds and releases while querying the manifests in the offices. Participation by shipping lines is presently on an elective basis and has been progressing slowly.

**OTHER COMPUTERIZED SYSTEMS**

Work continues on several systems within APHIS to incorporate computerization. These other systems include EXCERPT: A project of Export Summaries; PINET: An information system concerned with pest controls; NAPIS: The National Agricultural Pest Information System, primarily concerned with pest detection and data systems.

**REGULATED GARBAGE, MARPOL ANNEX V**

The U.S. Coast Guard is the lead or enforcement agency for Annex V of the International Convention to Prevent Pollution of the Seas (MARPOL 73/78).

This Annex prohibits discharge into the sea of "all plastics including, but not limited to, synthetic ropes, fishing nets, and plastic garbage bags." It also prohibits discharge of food wastes and other floating materials within specified distances of land. These regulations became effective December 31, 1988. USDA regulated garbage handling requirements have not changed. All food, or food contaminated plastics or other materials, must be retained.
REPORT OF THE COMMITTEE

aboard the vessel in covered, leakproof containers or, if offloaded, must be incinerated or heated to an internal temperature of 212°F for 30 minutes. The number of APHIS approved regulated garbage handling/disposal facilities for maritime ports has increased dramatically as a result of Annex V.

AIRPORT 1990'S

Processing the increasing number of international travelers presents many challenges to all Federal clearance agencies. Customs has adopted a new plan called "Airport 1990's." This plan calls for Customs to be more selective and examine reduced numbers of passengers and bags. In response APHIS will have to be more aggressive in its passenger inspection techniques. Many passengers that customs would have inspected will now be sent to APHIS secondary. APHIS will try to handle these increases in passengers with selectivity and technology, but future staff increases may be required at many airports. Currently, four airports, Los Angeles, San Francisco, Chicago, and Miami, are in various stages of implementation of the new procedure.

FISCAL YEAR 1989 REPORT OF ANIMAL PRODUCTS IMPORTED/EXPORTED

VEssel and AIRcraft ARRIVALS

<table>
<thead>
<tr>
<th>Arrivals</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels arrived</td>
<td>74,400</td>
</tr>
<tr>
<td>Vessels boarded</td>
<td>46,397</td>
</tr>
<tr>
<td>Vessels monitored for garbage violations</td>
<td>12,738</td>
</tr>
<tr>
<td>Lots consisting of garbage removed from vessels</td>
<td>4,800</td>
</tr>
<tr>
<td>Lots consisting of garbage removed from aircraft</td>
<td>611</td>
</tr>
<tr>
<td>Lots consisting of garbage removed from aircraft</td>
<td>348,375</td>
</tr>
<tr>
<td>Kilograms of garbage removed from aircraft</td>
<td>12,620,367</td>
</tr>
</tbody>
</table>

MEAT AND OTHER ANIMAL PRODUCTS CONFISCATED/REFUSED ENTRY

<table>
<thead>
<tr>
<th>Baggage Type</th>
<th>Lots</th>
<th>Kilograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ship passenger baggage</td>
<td>581</td>
<td>12,318</td>
</tr>
<tr>
<td>Aircraft passenger baggage</td>
<td>131,769</td>
<td>328,028</td>
</tr>
<tr>
<td>Border crossing</td>
<td>31,330</td>
<td>56,258</td>
</tr>
<tr>
<td>Post office</td>
<td>8,679</td>
<td>11,989</td>
</tr>
</tbody>
</table>

FOOTWEAR CLEANED AND DISINFECTED

<table>
<thead>
<tr>
<th>Footwear Type</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOOTWEAR</td>
<td>8,545Pair</td>
</tr>
</tbody>
</table>

MARITIME GARBAGE CIVIL PENALTIES

<table>
<thead>
<tr>
<th>Penalties Type</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PENALTIES</td>
<td>$93,550</td>
</tr>
</tbody>
</table>

BAGGAGE CIVIL PENALTIES

<table>
<thead>
<tr>
<th>Penalties Type</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PENALTIES</td>
<td>$631,640</td>
</tr>
</tbody>
</table>
The meeting was called to order shortly after 1:30 pm by Chairman Shelby Timberlake. There were 34 in attendance including the entire subcommittee consisting of 13 members.

**Dr. Michel Thibier**, Director of the Laboratory for the Control of Reproduction, Maison Alfort, France, presented information from the EEC and O.I.E. He described the magnitude of ET activity in 13 European countries (150,000 collected, 280,000 transferred). He addressed the French Sanitary restrictions (in effect for 3 years) which emphasize on control of people and procedures and specific emphasis on control of “in vitro” phases of embryo transfer. He stated that the O.I.E. has enacted appendix 5.2.3.1 for handling of Bovine embryos – (Produced in 3 languages and circulated to members). He outlined directives for intracommunity trade and importation from 3rd countries of ZP intact bovine embryos for European economic community to be enacted January 1, 1991. The basic concept of directive: *Control of Embryo Collection Team.* In general, to export to EEC: countries must be “listed” and collection Teams under the supervision of official veterinarians in these countries must be “approved.” Part of condition for approval of Teams will be willingness to be subjected to quality control.

**Dr. Richard Ford** – Veterinary Attache, Australian Embassy, Washington, DC

- statement of change in policy – in recent years, movement from a policy of total exclusion of risk to one of risk assessment based on current scientific information as relates to movement of embryos.
- for future – believes should focus on a mutual import-export package of regulations involving movement of embryos between USA and Australia.

**Dr. George Winegar**, Chief Staff Veterinarian, USDA, APHIS. – APHIS Update

- indicated a hard job ahead – to gear up for meeting requirement for exporting to EEC – especially a mechanism for approval of collection teams.
- export embryos from U.S. to Mexico – requirements negotiated must collect embryos from clinically normal cows and washed 10 times.
- plan a computer program for collecting data on approvals to export embryos so animal information can be reported annually.
- APHIS received request to modify part 98 on importing embryos from certain countries. To negate need for testing embryo donors, are examining request but don’t anticipate changes in near future.
REPORT OF THE COMMITTEE

- Ref. Resolution #24 – only known country keeping accords is France – Dr. Thibier indicated no reported diseases in recipients and current consistent pregnancy rate.
- Ref. Resolution #25 – Utah State research continues and currently involved in joint research proposal with Argentina to investigate FMD transmission hazards with movement of small ruminant embryos.

- 48 donors (exposed/infected).
- Embryos collected from 37.
- Embryos sent to PIADC equals 171 (all negative to virus isolation).
- Embryos retained in Argentina equals 92.
75 transferred (February & July)
11 of February recipients pregnant.

Dr. Mebus – Report on ovine embryo – FAD Project at PIADC Results: FMD Virus adheres to ovine embryo after in vitro exposure but could not isolate from embryos collected from infected ewes. Conclusion: Sheep embryos are not small cow embryos.

Dr. Heuschle – Research Director, San Diego Zoo – Addressed concerns of importation of ostrich from Australia that might be Tick infested – suggested a limitation on import of chicks that are more easily handled, quarantined, treated, etc.

Dr. Richard Carmichael – Maplehurst Ova Transplants
- Report on AETA meeting in Nashville – commented that AETA has been interested in quality control of industry since its inception.
- AETA has become a foreign Agricultural service cooperator – first trip to Australia, New Zealand, Japan just completed.
- AETA continues to work with APHIS Import people to alter Part 98 to facilitate importation of embryos so that other countries will be more cooperative in allowing importation of American embryos.
- Hope that AETA will be allowed to work with APHIS on approval protocols for collection teams for collecting embryos for export to EEC.

Dr. Acree – Indicated Bluetongue project results will be written up and HF will formally submit for publication, hopefully by end of 1989.

Dr. Nels Konnerup – Brief comments indicating that Dr. Thibier had answered his questions.

Dr. Howard Hanson – IETS committees have met (Oct. 28, 29)

Research committee – have initiated study on INF procedures, looking at appendices for small ruminants and swine – addressed form changes to handle embryos resulting from nuclear transplantation – Committees will meet again in January. Also: manual is in process of updating and will expect completion by mid 1990.
Dr. Michael Gilsdorf – APHIS Embryo Disease Committee – Developed Recommendations – See Handout

Several Comments from the floor indicated displeasure that we have been procrastinating in the U.S. on reducing the stringency of embryo import requirements accordingly with the current level of scientific knowledge.

Dr. Paul Sutmoller – Uncontrolled movement of genetic material in Latin America

(A) From India to South America (Rinderpest and FMD threat)
(B) From South America (Brazil) to Panama and other countries north (including Mexico)

Includes semen and embryos (mostly semen)
- Has been stimulated by lack of mechanism for facilitating legal entry of Germplasm
- PAHO has developed preliminary protocols for this end.

Dr. W. C. D. Hare – Update IETS research subcommittee IETS Disease Categorization from 1988 Recommendations

(A) Moved FMD and B. abortus in cattle to category 1

Note on how: Decisions made by committee – use data from published, peer reviewed reports.

Refer to OIE publication for other diseases and categorizations.
- Comment that organisms other than viruses (i.e. some bacteria mycoplasma) may create some problems.
- Initiated study of sterility of biologicals used in ET procedures.

Dr. Carmichael – Regulatory committee IETS
- Comments – AETA & IETS should have input with APHIS on approval procedures to meet new EEC requirements.

Dr. Jerry Walker – OICD
- Sheep embryo work in Europe.

- hope to set up quarantine Island in Adriatic sea (for import of sheep and goat genetics)
- Yugoslavia – bovine embryo project just underway to open up market.
- Yugoslavia – embryo disinfecting project to begin in next year.
- try to initiate project where expertise exists to open up markets for U.S. embryos in cooperating countries.

Dr. Herrick – Response to question about shortages of personnel in USDA/APHIS import/export staff. Hopeful that more people will be hired.

As there was no more old business or new business to discuss, the meeting was adjourned at 4:30 pm.
REPORT OF THE COMMITTEE

Dr. Roger Breeze, Center Director of Plum Island Animal Disease Center reported on the mission of PIADC, the current activities and the future plans and needs. He told of the need to keep the Center on Plum Island, as opposed to moving it to a mainland location, and the progress on the consolidation plan of ARS/APHIS. He showed slides of PIADC and the new high speed ferry and told of the advantages it was offering: an expanded population base from which to draw employees and interchange with the cooperative projects ongoing with several universities in Connecticut. Dr. Breeze discussed their efforts to prioritize projects and diseases and concentrate their funding on the major problems: FMD, African Swine Fever and Hog Cholera. He discussed the need for improved facilities and their maintenance, and the funding that would be needed.

Mr. Tom Mantzel, Texas Ostrich Company told the committee about the ostrich farming industry and the need for imports to increase the population for a viable industry and outlined proposals for the safe importation of ostriches:

1. No adult ostrich imports.
2. Imported chicks should be based on size not age (i.e. not exceeding 15 pounds or 30 inches in height. They should be pen raised not wild.
3. The chicks should be quarantined for 30 days in a federal or special selected approved private facility.
4. Chicks should be examined and sprayed with a safe and effective acaricide before shipment and in postentry quarantine as well as being tested for avian influenza and HVND.
5. Eggs should be allowed to be imported. They should be incubated to hatching in a confined facility. Hatched chicks should be tested for avian influenza upon hatching.

A proposed regulation (using standard APHIS protocol) to recognize Chile as being free of FMD was published August 17, 1989 and the comment period ends November 15. Another regulation for the certification, testing and quarantine requirements for the importation of llamas and alpacas from Chile was published September 27, 1989 and the comment period ends November 27, 1989. Dr. J. J. Callis spoke to the safety of the protocol described in the APHIS proposed regulation of llamas and alpacas from Chile through Harry S Truman Animal Import Center.

Dr. Robert L. Hartin discussed the reasons for the need of a state permit for international import shipments, and notification of the state veterinarian in the receiving state by officials at the quarantine station prior to the release of a shipment.

Mr. Tom Schooler, Animal Port Houston, discussed the reasons for notification of APHIS by International Air Transport Association, Live Animals Board of bookings for import shipments of exotic animals and birds.
Dr. Richard Ford, Veterinary Attache, Australian Embassy, Washington, DC gave a talk on the possible trade between Australia and the United States in Bovine Genetic Material.

Dr. Tom Cook, National Cattlemen’s Association, Director of Industry Affairs, Washington, DC gave the committee an update on the EC hormone ban and its affect on U.S. exports to EC. A few weeks ago, Tom and NCA President Mr. Bob Joserand spent a week in Europe talking to EC officials and other interested parties about the ban on U.S. beef. This is a non-tariff trade barrier and not an animal health issue, and no change in position is likely any time soon.

Dr. Alex Thiermann, Deputy Administrator, International Services, APHIS, USDA told the committee of the role and workings of International Services around the world.

Mr. James Innes and Mr. Peter Floyd presented the following information on deer farming and the possible importation of red deer from New Zealand into the United States.

Presently, there are 1.2 million deer intensively farmed throughout the world of which 750,000 are in New Zealand and about 25,000 in the USA.

Since New Zealand pioneered the intensive management of deer, expanding markets have been developed throughout the world from the various superior quality products that are derived from these animals.

The interest in farming deer has snowballed throughout the world. The limiting factor in most countries being the lack of suitable species that adapt well to intensive farming practices.

The USA represents one of the biggest potential developing markets for both venison and velvet products. Demand for venison is growing at a rate in excess of 300% per year. Last 84% of the commercial venison sold in the USA was imported. In the face of this explosive demand and the lack of available brood stock to fuel this fledgling industry, the domestic share of this commodity market will diminish even further.

The controlled importation of brood stock from New Zealand is necessary if the domestic deer farming industry is to become an authentic part of the agricultural community.

Canada recognized this and established technically sound protocols to allow the controlled importation of deer from New Zealand and other countries through privately funded government supervised quarantine facilities. In the last 12 months several thousand herd were successfully imported from New Zealand.

Deer are routinely imported in small numbers into the USDA through Federally owned and operated facilities. To enable large numbers to be imported to establish an economically viable industry, larger quarantine facilities are going to need to be established. The precedent has already
been set to enable the large scale importation of sheep from New Zealand in an orderly fashion through privately owned, Federally supervised quarantine facilities.

New Zealand interests propose to import red deer, the most widely farmed and perhaps most efficient species to husband to the U.S. The major issues to be resolved are:

Protocol. Protocol for the import of sheep, similar in biology to deer, is well established between New Zealand and the U.S. Technical issues such as the parasite *Elaphostrangulus cervi* will always arise. They need to be addressed and resolved. *E. cervi* can be excluded by an appropriate quarantine period and insurance that anthelmintics are not used to mask the detectable larval stage.

Demand. In the face of economic potential for the industry risk exists that attempts will be made to farm undesirable species with poor results or to import stock through third countries. A well ordered expansion of brood stock through foreign shore acquisition is necessary to fuel this fledging industry to reduce U.S. import of processed venison.

Hybridization. There is a potential risk that red deer can hybridize with native elk. This risk exists today from numerous backyard hobbyist and roadside exhibitor. The threat from the serious farmer practicing sound, intense management of this very expensive stock is minimal in comparison to that currently existing.

Schedule. The reproductive cycle of deer is solely controlled by the photo period centered on June 22 as the longest day in the northern hemisphere. To minimize stress and ensure optimum reproductive performance, deer must be moved from the southern to northern hemisphere by April. Experience dictates that unbred yearling females are most suitable to undergo the hemispheric shift. Due to the limited amount of stock worldwide, the economics of the situation dictate the window of import opportunity is limited to a couple of years.

The committee considered and passed the following resolutions:

1. Plum Island Animal Disease Center
2. Importation of Ostriches
3. State Permit for International Import Shipments
4. Bont Tick – Heartwater Project in Antigua
5. Animal Import Inspection
6. Fraudulent Certification of Export Shipments
7. Illegal Movement of Germ Plasm to Central America, Panama and Mexico
8. Import Notification
IMPORT-EXPORT

The committee considered and endorsed the following resolutions from the Committee on Infectious Diseases of Horses.

1. Importation of Ringling Brothers Barnum and Bailey Show Stallions
2. Establishment of Equine Staff Veterinarian Position in USDA

The committee considered and did not pass the following resolution.

1. Importation of llamas and Alpacas from Chile through Harry S Truman Animal Import Center

The committee adjourned at 5:45 p.m.
Chairman: Mr. L. Graham, Helena, MT
Vice Chairman: Dr. E. R. Hinshaw, Phoenix, AZ

J. M. Alumbaugh, IA; J. B. Ashcraft, CO; D. R. Bridgewater, CO; J. S. Cargile, TX; D. Casey, MO; J. P. Davis, MD; F. Dickinson, OH; H. F. Embry, IL; M. A. Essey, MD; T. B. Falls, VA; R. Gadd, SD; B. Gallagher, SD; T. V. Haas, KY; J. N. Huff, CO; C. N. Jewett, SC; R. D. Jones, SD; D. Likes, KS; R. E. Nelson, VT; T. A. Neuzil, IA; A. B. Park, MD; N. F. Powers, Jr., NY; G. W. Roberts, CA; E. C. Roukema, VA; R. S. Schnell, ND; K. W. Scritchlow, IL; G. L. Seawright, NM; J. Selz, WI; S. L. Spahr, IL; W. E. Stemler, IL; J. E. Thomas, ID; C. R. Watson, ID.

The Committee met on November 1, 1989, with 41 in attendance including 19 members.

Dr. Janet Payeur of the National Veterinary Services Laboratories reported on a study of microchip Implants for Cattle Identification at Slaughter.

Objective

To evaluate the effectiveness of implanted passive transponder microchips for the identification (ID) of cattle at slaughter.

Introduction

The primary method used to detect Mycobacterium bovis-infected cattle herds is epidemiologic tracing of lesioned, tuberculous cattle detected during inspection by the Food Safety and Inspection Service (FSIS) personnel. Permanent individual identification is crucial for successfully tracing an infected, lesioned animal detected at slaughter to its herd of origin. During the last 3 fiscal years, 55% (11 of 20) of properly identified lesioned cattle have been traced to their source herd while only 2% (6 of 292) of lesioned cattle without adequate identification have been successfully traced.

Identification problems are especially severe in "rapid kill" establishments that specialize in steer slaughter. In these plants, the hide and all identification devices such as ear tags have been removed and discarded before inspection by the FSIS personnel. Passive transponders, encoded with unique alphanumeric identification, could be implanted in tissues still present at slaughter inspection. These identification devices would facilitate epidemiologic tracing of tuberculous cattle to the infected herd of origin.

The transponders (electronic identity tags) are tiny (0.083" X 0.400") cylinders which contain an electromagnetic coil and microchip sealed in a glass envelope. Each one weighs only 54 milligrams. The chip is pre-
LIVESTOCK IDENTIFICATION

programmed with a unique ID code (10 alphanumeric characters) that cannot be altered and over 34 billion individual code numbers are available. When the transponder is activated by a low frequency radio signal, it transmits the ID code.¹

The implanting instrument (implanter) is a spring-loaded syringe with a retractable 12-gauge needle for safe, sterile implantation. The syringe includes 1-1/4" and 1/2" depth limiters so that the transponders can be implanted in large or small animals. Disposable needles come in a sterile pack that includes a transponder and a polypropylene retention sheath.¹

The handwand or scanner generates a low frequency radio signal as it scans the transponder site. The signal activates the transponder which in turn transmits the encoded ID to the reader which displays the code in simple "read only mode," and then stores the tag numbers in memory or transmits the numbers to an external computer, printer, or modem. The portable reader is lightweight and durable for use in the field or the laboratory. It can store 5000 transponder codes for later retrieval. The reader is battery-operated and can be recharged or operated directly from any standard 110 VAC outlet by using the battery charger included with the unit.¹

Materials and Methods

Ten calves were implanted with three or four transponders each randomized between the following sites:

1. Anterior muzzle, midway between the external nares, approximately 5 mm deep.
2. The area of reflection between the inner upper lip and the dental pad, on the midline.
3. Between the eyes, on the midline.
4. The lower half of the ear.
5. Ventral periorbital area, right eye.
6. Between the base of the ear and the horn bud, superficial to the posterior auricular muscles, right side.
7. Supraorbital depression, right eye.

The transponders were scanned immediately following implantation and at 2 weeks, 4 weeks, 2 months, 5 months, and 10 months after implantation. Ten months after implantation of the transponders, all calves were necropsied; and the transponders and associated tissues were collected and frozen at -20C.

¹Destron/IDI, Boulder, CO
REPORT OF THE COMMITTEE

Results

Of 38 implants placed in 10 animals, 26 (68%) were still working at the time of necropsy. Implanted transponders were detected at various sites and times as described in Table 1.

Table 1. Number of Transponders Still Working at Various Days

<table>
<thead>
<tr>
<th>Site</th>
<th>0 Days</th>
<th>14 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>150 Days</th>
<th>280 Days</th>
<th>300 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Muzzle</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2. Upper lip</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3. Between eyes</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4. Lower ear</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5. Below eye</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6. Horn bud</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>7. Supraorbital depression</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>38</strong></td>
<td><strong>37</strong></td>
<td><strong>37</strong></td>
<td><strong>30</strong></td>
<td><strong>28</strong></td>
<td><strong>26</strong></td>
<td></td>
</tr>
</tbody>
</table>

At necropsy, it was found that transponders implanted at sites 1 through 6 could be damaged or removed and discarded during routine slaughter procedures such as skinning. One of the transponders implanted at site 6 had migrated about 4 cm to a site above the eye.

Ten transponders were not found at necropsy at the following sites: muzzle (4), upper lip (2), between the eyes (3), and under the eye (1). Two transponders were found broken at site 3 (Table 2).

Transponders at site 7 were enclosed in an accumulation of fatty tissue in the supraorbital depression. These transponders were not removed when the head was skinned, but they could be easily recovered by scooping or spooning the entire fat pad out of the supraorbital depression.

No inflammatory or adverse reactions to intact transponders were noted. A focal subcutaneous abscess was associated with one transponder that had been broken in situ at site 3.

The transponders and associated tissue were frozen at -20C. All of the transponders that worked at the time of slaughter were still operating 10 months later, for a total of 20 months from implantation.

Discussion and Conclusions

The implant sites had to meet the following criteria: (1) afford good survival of transponders, (2) remain with head after skinning, (3) not involve edible parts, (4) not cause physical damage or pain, and (5) allow easy implantation and recovery of transponders.
LIVESTOCK IDENTIFICATION

Table 2. Effectiveness of Transponders at Various Sites

<table>
<thead>
<tr>
<th>Sites</th>
<th>Number Implanted</th>
<th>Working at Necropsy</th>
<th>Removed with Skinning</th>
<th>Remained with Head</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Muzzle</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2. Upper lip</td>
<td>7</td>
<td>5</td>
<td>71</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3. Between eyes</td>
<td>8</td>
<td>3</td>
<td>37</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4. Lower ear</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5. Below eye</td>
<td>4</td>
<td>3</td>
<td>75</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6. Horn bud</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>7. Supraorbital depression</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>26</td>
<td>24</td>
<td>9</td>
<td>100</td>
</tr>
</tbody>
</table>

Information in Table 2 indicates that the supraorbital depression appears to be the most promising site for transponder implantation. There were no transponder failures at this site, and they were not removed during routine slaughter procedures. There was no evidence of migration in the tissues or tissue reaction to the transponders at this site. In addition, this site would allow easy implantation and removal of transponders after slaughter inspection.

The purpose of this project was to explore alternatives for the identification of cattle that would still be present at slaughter. Implant identification is not externally visible. The external invisibility could also be an advantage since it would decrease the likelihood of the identification device being lost or tampered with. This system interfaces with a computer every easily, thus increasing the accuracy and speed of information retrieval. This form of identification also has the potential of giving traceback information on cattle with other diseases detectable at slaughter.

The present cost per single implant is $7 although cost estimates are as low as $2 when bought in large quantities. Each implant comes with its own needle and is ready for use. The implanter costs $12.

The electronic reading device is relatively inexpensive ($900), and any establishment submitting substantial numbers of VS Form 6-35 reports could be equipped with one. There are about 25 such establishments in the United States at this time. Even without electronic readers, supraorbital adipose tissue is easily extracted in a single operation with a very high probability of recovery of the transponder.

REFERENCES

REPORT OF THE COMMITTEE

The use of a particular manufacturer's product does not constitute an endorsement on behalf of the USDA.

Dr. Richard Nelson reported on the meeting of the National Electronic Identification Board. The meeting was held on November 1, 1989 with interested livestock groups attending including representatives from Canada. The Board concluded that we need to develop a system compatible with other international livestock producing countries. The type of system is not identifiable. The evaluation is continuing of programs into unique numbering systems to individually identify purebred and commercial livestock.

Dr. Gary Couman reported on traceback studies conducted by the National Cattlemen's Association. Contracts for the study were awarded to South Dakota State University and to Texas A&M University. The final report from these studies will be published by the National Cattlemen's Association in December 1989. Preliminary information from South Dakota indicates that from six slaughter plants in five states shows that 76.6% of the cattle slaughtered were traceable to the point of origin. Approximately 10% had lost their man-made identification. Texas A&M has not submitted data to date, but have found traceability to feedlots to be exceptional based upon feeding records.

Dr. James P. Davis, Staff Veterinarian of Cattle Diseases and Surveillance Staff of USDA, APHIS, submitted the following report on swine identification.

REPORT TO LIVESTOCK IDENTIFICATION COMMITTEE
UNITED STATES ANIMAL HEALTH ASSOCIATION
LAS VEGAS, NEVADA
NOVEMBER 1, 1989

James P. Davis
Staff Veterinarian
Cattle Diseases and Surveillance Staff

SUMMARY

A proposal to provide for the identification of swine in interstate commerce at the first point of concentration was published October 20, 1989, the comment period ends December 19, 1989. This proposal is consistent with industry's need for the animals to be identified within normal marketing channels. The present approved methods of swine identification need to be improved to provide for better animal identification.
LIVESTOCK IDENTIFICATION

SWINE IDENTIFICATION UPDATE

Industry and the Animal and Plant Health Inspection Service (APHIS) should work together in developing an acceptable form of animal identification that meets the needs of industry as well as APHIS. The present swine identification regulation Title 9, Code of Federal Regulations (9 CFR), Part 71.19, became effective November 14, 1988. This regulation mandates that all swine in interstate commerce be identified by means of identification approved by the Administrator of the Animal and Plant Health Inspection Service (APHIS) and records kept of this identification. All swine in interstate commerce are required to be identified with approved identification prior to being sold, offered for sale, or being transported.

There are two basic reasons why swine need to be identified back through marketing channels to their premises of origin: Either the meat from the animal is adulterated or the animal is diseased.

The types of official identification used to identify swine in interstate commerce are listed in 9 CFR, Part 71.19(b). It is essential that all forms of official identification be collected at slaughter to ensure successful traceback through the market system to the premises of origin. The types of approved identification most often used are:

(a) Official eartags—an identification eartag approved by Veterinary Services (VS), as being tamper-resistant and conforming to the nine-character alpha-numeric National Uniform Eartagging System, which provides unique identification for each animal. These eartags may be used on any class of swine. The eartags, usually metal, are approximately 3/8 inches wide by 1-1/2 inches long with nine characters on the top surface. Example, 42 CPP 2549, the first two characters designate the State, the remaining seven provide individual animal identification. The official eartags are not problem free. They are inconvenient to use by the producer, hard to read after being applied, may cause ear damage, and are hard to retrieve at slaughter.

(b) USDA swine backtags, which may be used on swine moving to slaughter. This tag is 1-1/2 inches in diameter, is issued by VS, conforms to the eight-character-alpha-numeric National Backtagging System and uniquely identifies each individual swine. This code identifies the state (2 characters) market (2 characters), and individual identification numbers (4 characters). At present, the backtag is not fully meeting the need for animal identification traceable to the premises of origin. Additional studies need to be conducted to improve this type of identification for swine. APHIS recognizes the need for better swine identification and is currently conducting tests in two States comparing the effectiveness of swine backtags with bangle tags.

(c) VS approved tattoos may be used on swine moving to slaughter, except sows and boars destined for skinning plants. This tattoo conforms to the six character alpha-numeric tattoo system and provides unique identification.
REPORT OF THE COMMITTEE

for each lot of swine. It consists of three characters for State and market identification (17,576 possible combinations) and three characters for lot numbers.

Example — ABC (State and market)

123 (lot number)

(d) Four-character tattoos as used by industry. These tattoos indicate the buying station and lot number for a packer marketing system. This system works well as long as the animals do not change marketing systems. Tattoos will meet part of the need for animal identification but several issues complicate their use for APHIS program identification. These issues are: multiple tattoos confuse the true source of the animal and the inability to read and record the tattoo at the time of sample collection.

(e) Ear notching or ear tattoos may be used when they have been recorded in the book of record of a purebred registry association. This system does not lend itself well to slaughter surveillance.

The above examples show that all current methods of swine identification have deficiencies and demonstrates the need for a single effective and practical type of swine identification for official use. This identification will need to be inexpensive, easy to apply, easy to retrieve at slaughter, easily visible and have a high retention rate. Industry, Food and Safety Inspection Service, and APHIS need to work together to develop this needed form of swine identification. An inexpensive bangle tag that could be applied at either the market or premises of origin is one possible solution.

A new method of identification that could also be considered is a readable (and edible) implant whose recovery during slaughter provides carcass identification. This method would provide accurate traceback information about the previous owner and would only have to be collected if the carcass is inspected for residues or other concerns. However, this would not be an effective means of identification for the brucellosis and pseudorabies swine diseases programs. These programs have a need for visible external removal identification. This identification needs to be inexpensive, easy to apply and retrieve, and stay on the animal.

It is vital that all identification devices, whether official or not, be collected to help ensure efficient tracebacks regardless of whether the tracing is for a disease or residue problems.

Another major industry concern with the present swine identification regulation is the necessity for the identification to be applied by the producer prior to being moved or sold in interstate commerce. At present, this regulation mandates that over 300,000 individual swine producers have to identify their animals before moving them interstate. Industry believes this is impractical and has requested that the regulation be amended to allow for the identification of swine in interstate commerce at the first point of concentration.
A proposal to amend the swine identification regulations to allow for the movement of swine to the first point of concentration was published in the Federal Register, October 20, 1989, with the comment period ending December 19, 1989. Future identification requirements will be influenced by these comments.

Mr. Mike Haas gave a brief report on Retention of Eartags in Livestock Identification. Metal tags have a retention of 98% if properly applied; plastic tags approximately 80% if properly applied. Mr. Haas stressed that proper application is the key to retention. Efficiency of the mechanical application is the key to proper application.

Following a discussion of the publication of the Proceedings of the Livestock Conservation Institute's International Identification Symposium held in St. Louis, Missouri, on December 7 and 8, 1988. The meeting was adjourned.
REPORT OF THE COMMITTEE ON PROFESSIONAL OVERSIGHT

Chairman: Dr. J. R. Ragan, Nashville, TN
Vice Chairman: Dr. D. U. Walker, Montpelier, VT
C. L. Campbell, FL; J. A. Cobb, GA; L. D. Konyha, FL; N.W. Kruse, NE; J. O. Pearce, Jr., FL; G. B. Rea, OR; P. L. Smith, CA; S. T. Wilson, Jr., MD

The Professional Oversight Committee met on Thursday, November 2, 1989, with seven members and a number of interested persons present.

It was determined that prior years' resolutions and proposals had been considered and responded to by appropriate parties.

The administration of the veterinary accreditation program was discussed at length. We recommend a comprehensive review of the program by a task force which includes APHIS representatives from the area (field) level, state animal health authorities, and practicing accredited veterinarians. This review should recognize the limited scope of accredited veterinarians-involvement in animal health programs and therefore seek to simplify and enhance specific training and the content of the National Accreditation Examination. In addition, it should consider the proper role of state authorities in granting accredited status and carrying out disciplinary actions. Further, we recommend that it consider whether all practicing veterinarians have need of accreditation, or only those involved in livestock program activity.

Several concerns were expressed regarding the National Animal Health Monitoring System (NAHMS). The Committee feels that whenever NAHMS surveys reveal cooperative program diseases or foreign animal diseases, they must be reported to proper authorities and handled routinely.

We further feel that the scope of surveys should be limited to areas which lend direct support to state or federal disease control programs or efforts.

We reiterate the concern expressed by the State-Federal Relations Committee the NAHMS be operated in a cost effective manner and that its funding not impair funding of disease control and eradication programs.

In this regard, we request that USAHA be provided with information on the total annual cost of the NAHMS program, including administration, staff, field, laboratory, and equipment costs.

One resolution was voted approved by the Committee. It was brought to the Committee's attention that on occasion resolutions have been proposed to standing committees of USAHA by persons who may be association members, but are not members of the Committee of reference. We wish to make the Executive Committee, and especially Committee Chairman, aware of member concerns that all such resolutions and motions should be properly considered and moved through the committee system.
REPORT OF THE COMMITTEE ON RABIES

Chairman: Dr. L. H. Russell, College Station, TX
Vice Chairman: Dr. W. R. Miller, Summerdale, AL

R. R. Brown, AR; H. M. Chaddock, MI; H. Draayer, IL; T. J. Galvin, MD; E. P. J. Gibbs, FL; B. B. Hancock, IA; S. K. Harris, IA; O. James, MT; R. B. Miller, MD; J. C. New, TN; R. L. Sharpee, NE; L. J. Swango, AL; T. H. Woods, AR; J. C. Wright, AL.

The Committee met at 1:30 pm on Monday, October 30, with 30 members and guests present.

Old Business

Issues regarding labelling of rabies vaccine and USDA policy restricting the slaughter of food animals exposed to rabies were discussed.

No actions were taken.

New Business

Changes in the Compendium of Animal Rabies Control, 1990, were discussed including flexibility to add licensed vaccines for exotic and wild animals, the keeping of ferrets as pets and exposure of animals to rabid animals.

The need for coordination between federal and state agencies when licensed rabies vaccine for wildlife becomes available was discussed.

Motion: That the Chairman of the Rabies Committee meet with the Wildlife Disease Committee to discuss the development of a resolution regarding a national wildlife rabies vaccination policy based on a USDA licensed rabies vaccine. Motion passed unanimously.

Papers

1:30–2:00 Rabies Vaccination of Raccoons: Proposed Field Trial — Update
Dr. Robert B. Miller, USDA, APHIS
Gaithersburg, MD

2:00–2:30 Wildlife Rabies Vaccination in Canada
Dr. Douglas C. Alexander, Agriculture Canada
Ontario, Canada

2:30–3:00 Skunk Rabies: Determination of Annual Risk
Dr. Foy McCasland, Texas Department of Health
Austin, TX
REPORT OF THE COMMITTEE

3:00–3:30 Break

3:30–4:00 Rabies Surveillance, United States, 1988
   Dr. Leon H. Russell, Texas A&M University
   College Station, TX

4:00–4:30 Rabies, Wolves & Wolf-Hybrids — An Update
   Dottie Prendergast, Wolf-Hybrid Times
   Gallup, NM

4:30–5:00 Importance of Titers & The Vaccination of Wildlife Pets
   Dr. Larry Swango, Auburn University
   Auburn, AL

The meeting was adjourned at 6:00 p.m.
REPORT OF THE COMMITTEE ON
STATE-FEDERAL RELATIONS

Chairman: Dr. M. A. Van Buskirk, Harrisburg, PA

J. L. Alley, AL; P. E. Bradshaw, IL; J. A. Cobb, GA; T. W. Freas, IN;
T. J. Hagerty, MN; B. R. Hillman, ID; S. R. Nusbaum, NJ; J. R. Ragan,
TN; J. C. Shook, PA; P. L. Smith, CA.

The State-Federal Relations Committee met in College Park, Maryland,
March 6-9, 1989. This Committee met with representatives of the Food and
Drug Administration—Center for Veterinary Medicine (FDA-CVM) and
the United States Department of Agriculture (USDA) who reviewed their
animal health programs and responded to issues raised by the Committee.
The Committee received written responses to the 1988 USAHA resolutions.

USDA staff presentors gave detailed explanations of the reorganization
of APHIS and responded to Committee inquiries regarding implementa-
tion of the reorganization and its possible impact upon delivery of federal
and state animal health services.

The Committee commended USDA and FDA for the excellent coopera-
tion extended to USAHA in the planning of animal health programs and
expressed to USDA its ongoing interest in timely and meaningful input into
budgeting of animal health resources.

The Committee was especially appreciative of the opportunity to meet
with Assistant Secretary of Agriculture, Dr. Kenneth Gilles, ARS Admin-
istrator, Dr. R. D. Plowman and APHIS Administrator, Dr. James Glosser.

This report will make note only of those matters of special interest or
concern to the Committee.

Food and Drug Administration, Center for Veterinary Medicine

The Committee urges FDA and USDA to improve communications
regarding efforts to protect the public health from zoonotic diseases such as
egg-associated Salmonella enteritidis.

Policy and Program Development

The Committee supports USDA efforts to improve APHIS decision-
makeing processes. The Committee is, however, concerned that the policy
and program development staff may have been expanded at the expense of
program delivery and that adequate consideration may not have been given
to input from program staff.

Animal Health Information

The Committee appreciates the NAHMS concept but recommends that
funding and development of the program be tempered in terms of its cost
effectiveness. The Committee is concerned that the cost may exceed the
demonstrated value of the product produced.
REPORT OF THE COMMITTEE

Regulatory Enforcement and Animal Care

Although the Committee does not support the removal of the compliance function from Veterinary Services, it endorses the coordination of regulatory enforcement with Veterinary Services. Review of compliance cases at the state veterinarian, AVIC and staff level is essential if the regulatory enforcement program is to function effectively. A memorandum of understanding between VS and REAC that outlines the function of Regulatory Enforcement as a service agency to VS could help to insure the proper function of regulatory enforcement.

We commend staff for their efforts to improve the ability of REAC to respond to animal welfare concerns. We agree that strategic long range planning involving state animal health officials, industry and other interested groups is essential to effectively deal with the problems that face the food animal industry in the area of animal rights.

Veterinary Services

It is apparent that the five year brucellosis eradication plan is not going to accomplish its goals and that appropriate alternatives are not being developed. It appears that collapse of the brucellosis eradication program is imminent in several states. This situation demands immediate review by APHIS and appropriate consultation with industry and state regulatory agencies.

The Committee requests that opportunity be given USAHA to comment on proposals resulting from the Scrapie Negotiated Rule-Making session until December 1, 1989.

Momentum of individual states to control and eliminate pseudorabies has progressed to a critical point with respect to APHIS assistance. It is essential that APHIS continue and expand support in order to advance these programs. Swine identification regulations should be revised to make them more compatible with modern marketing systems.

The importation of Mexican cattle constantly threatens to introduce tuberculosis into the U.S. Evaluation of the danger and contingency plans to modify importation requirements is essential.

The Committee recognizes the outstanding achievements of the Import-Export program in preventing the introduction of disease into the U.S. and in protecting the reputation of our exported animals and animal products. The Committee strongly supports the 12 month delay in recognizing FMD free status of foreign countries. Further the committee recommends careful analysis of Chile’s FMD status because of two recent failures to remain disease free after their application for free status. The Committee believes that it is appropriate for Import-Export personnel to act in an advocacy role in promoting development of reasonable requirements for export of American livestock. Import-Export staff should not be unduly influenced by economic and/or political factors.
The Committee urges USDA to coordinate development of electronic data processing systems with the needs of individual states.

The Committee emphasizes the need for APHIS to offer more indepth education and training opportunities for qualified individuals in epidemiology, biostatistics and agricultural economics at colleges and schools of veterinary medicine.

The Committee supports APHIS recruitment and training goals. A close working relationship with colleges of veterinary medicine and veterinary organizations will insure the recruitment of capable people. The Committee feels development of new employees and training of all employees is very important in our fast changing society.

The Committee is concerned that insufficient trained professionals are available and ready to contain and eradicate an outbreak of highly pathogenic disease. We propose consideration of a reserve system of retired persons similar to the military program. This Committee also advocates that training exercises be conducted to improve emergency preparedness and that appropriate funds be allocated for that purpose.

The Committee urges Veterinary Services to adopt the recommendations of the American Veterinary Medical Association regarding the duties of state-federal accredited veterinarians. An efficient mechanism should be developed to communicate regularly with accredited veterinarians and a system should be developed for continuing education of accredited veterinarians who participate in official program work.

**Food Safety Inspection Service**

The Committee supports the new program to evaluate state meat inspection systems. We commend FSIS for the increased efforts to improve the quality of meats and meat food products through increased microbiological monitoring, the establishment of new residue detection methods and conduct of research into methods for residue prevention. The Committee is concerned about the lack of adequate inspection of exempt plants to assure the continuous production of wholesome and quality products.

**Biotechnology, Biologics and Environmental Technology**

The Committee continues its support for uniform implementation of the Virus, Serum and Toxin Act as amended. We urge that sufficient resources be made available to Veterinary Biologics to deal with the impending termination of the exemption period for unlicensed intrastate products. The Committee supports the protocol which has been developed for evaluation and licensing of biologic products produced through new biotechnology. We urge that USDA review and evaluate these new products in such a way as to make them available for use as expeditiously as is consistent with safety assurance.

**Science and Technology**

Inasmuch as science and technology are some of the most critical
activities of APHIS, we urge top priority be given adequate allocation of funds to these activities. Funding should not be dependent upon legally and politically ill-defined mechanisms such as user fees. We recommend that the APHIS facilities at NVSL and Plum Island be given the highest priority for funding. Emphasis should be placed on assistance to state laboratories, to preparation and distribution of diagnostic antigens and reagents not otherwise available and to technical training of federal, state, and other personnel.

Agricultural Research Service

We commend ARS for its continuing research efforts in support of animal agriculture and animal health programs. We urge that ARS promptly move forward to redefine and refocus their relationship and liaison with APHIS. We agree with their determination to maintain a heavy emphasis on food safety issues such as aflatoxins and bacterial contamination. We feel that a high priority must be maintained for development of diagnostic capabilities to support existing disease program efforts and for characterization of new and emerging disease agents such as Bovine Spongiform Encephalopathy.

The Committee feels that it is important that adequate formula funding be maintained to support CSRS broad agenda of research across the nation. We support competitive grants only as a supplement to long term funding.
REPORT OF THE COMMITTEE ON ZOOLOGICAL ANIMALS

Chairman: Dr. W. B. Amand, Philadelphia, PA
Vice Chairman: Dr. R. L. Crawford, Hyattsville, MD

W. B. Amand, PA; L. H. Cornell, CA; P. M. Eppele, SD; A. Gonnerman, MO; D. E. Herrick, MD; W. P. Heuschele, CA; P. D. Hoctor, IN; D. J. Kelley, NC; J. Lubroth, TX; C. W. S. Lum, HI; C. J. Mikel, OK; G. P. Pierson, MD; S. S. Richeson, MD; C. O. Thoen, IA; E. T. Thorne, WY; H. A. Waters, VA; E. Y. Williams, CO; R. J. Yedloutschnig, NY.

Committee Members Present:

W. B. Amand; R. L. Crawford; P. M. Eppele; W. P. Heuschele; P. D. Hoctor; C. W. S. Lum; J. Lubroth; C. J. Mikel; S. S. Richeson; M. S. Silberman; E. T. Thorne; D. Whittlesey

The meeting was called to order at 1:30 p.m. on Wednesday, November 1, 1989 by Chairman Amand. Introductions were made and a review was given on the action taken on the following 1988 resolutions:

- a resolution recommending closer working relationships between APHIS, AAZPA and AAZV has been implemented and several meetings have occurred between the groups in the past year and will, hopefully, continue.

- a resolution recommending that APHIS inspectors be better trained in zoo and wild animal husbandry has been acted on by APHIS, REAC, and will be further implemented by the establishment of short term internships in the zoo animal and laboratory animal fields.

The Chairman called for resolutions to be presented to the committee and none were offered.

Dr. Vic Nettles presented an update and status report on the Model State Regulations for wild and exotic animals developed in accordance with a 1985 resolution concerning disease control and interstate movement of exotic hoof stock. Dr. Nettles advised that he had presented the third and final draft to USAHA in 1988 and had received no input or response since that time. He stated that the model law is being used as guidelines by various groups and States.

The subject was discussed at length by the Committee and guests. The Committee commended Dr. Nettles for his efforts in behalf of Resolution No. 9 of the 1985 meeting and recommends that USAHA make the draft model regulations available to interested parties.

Dr. Dave Wilson gave a presentation on the USDA experience with exotic ticks that had been imported on wildlife in recent years. He further reviewed the facts leading to the total ban on importation of Ostriches and other ratites. After a period of discussion, Dr. Werner Heuschele requested that the Committee provide endorsement of a resolution regarding impor-
tation of Ostriches which is being brought before the Import/Export Committee. After deletion of that portion of the resolution dealing with weight or size restrictions, the Committee voted in favor of the endorsement by a vote of 9 to 0. A further discussion ensued referable to a separate resolution also being placed before Import/Export Committee regarding animal import inspection. In this later resolution, there is a call for inspection and/or treatment for ectoparasites on any and all terrestrial vertebrates being imported into the United States. Although discussed, no Committee action was taken on the resolution.

Mr. Fred Bauer, President of the International Llama Association, provided a review and update on the issue of importing llamas and alpacas from Chile as set forth in the proposed rule by USDA, APHIS and published in FR. Vol 55, No 187, on Thursday, September 18, 1989. Despite the importation guidelines outlined in the above proposed rule, the International Llama Association expressed concern that importation from countries, such as Chile, presents certain unacceptable risks to the llama industry and they urge the continued use of the Harry S Truman quarantine facility for future llama/alpaca importations. Considerable discussion followed Mr. Bauer's presentation. The Committee, however, took no action relative to a resolution being presented to the Import/Export Committee.

Dr. Sam Richeson presented a review of activity referable to experience with the importation of elephants, hippopotomi, rhinoceroses and tapirs as it related to control of ectoparasites on the above named species (9CFR Parts 93 & 99). Of particular note is the difficulty being experienced at sites of importation with respect to adequate examination/handling and adequate spraying. After some discussion, the Committee, in a vote of 8 to 0, recommended that adequate funds be made available to USDA in order to establish and maintain proper facilities at sites of importation which would allow for proper examination and spraying for ectoparasites.

Dr. R. L. Crawford presented an update and review of APHIS, REAC reorganization and activities for fiscal year 1989, including training of REAC inspectors. A report was also given on the status of the Animal Welfare regulations and standards, 9 CFR, Parts 1, 2, 3.

There being no further business from the floor, the Chairman adjourned the Committee at 4:10 p.m.
Initial Concept & Requirements
The initial thrust of the above project was to investigate methods for storing, examining and retrieving geographic elements of epidemiological data on microcomputers. This quickly expanded into an investigation of the general use of graphics-oriented data bases with hypertext capabilities for a wide range of animal health-related data. Several basic requirements were established:

1) The system must be able to handle both text and graphics and have an integrated command language for programming special features.

2) The system must make maximum use of the graphical interface to minimize the training time for new users. Graphical pointing and activating devices such as a mouse, trackball, or digital tablet is required.
3) The design of the working database must be intuitive and flexible. The user should be able to browse through the database with minimum use of keyboard-entered commands. Icons, buttons, and other selection devices must be provided.

4) Additions and/or changes to the database must be able to be made by personnel with a minimum of formal computer training. This will require the presence of an extensive array of graphical drawing tools and text handling capabilities.

5) Capabilities must be present for both importing and exporting graphics and/or text to other software. Procedures should not require exiting one software package before opening the next.

6) All of the above elements must be present in one software package to maximize the efficiency of the operation.

7) On-site printing capabilities must be available for any graphic image and/or text material.

8) The entire system must be portable enough to be transported by air or car to a remote site and set up by personnel with a minimum of computer experience.

Pilot Project

The pilot project focused on the projected field needs of an emergency task force to combat diseases in domestic animals. In the process, management personnel pointed out that the same database would be very useful in the day-to-day management activities of a State or Region.

The present project is utilizing two software packages available for the Apple Macintosh (Hypercard & MacDraw II). Hypercard is furnished free with the purchase of the Macintosh. MacDraw can be purchased from several vendors for about $300. Hypercard does meet all of the basic requirements. MacDraw II adds several enhancements.

The system has been operated on both a Macintosh SE and a Mac II with from 2.5 to 4 megabytes of memory.

State Outline Maps

Upon opening the Hypercard stack, the user is presented with a map of the Western Region of the United States. Transparent sensitive areas or buttons are located within the outline of each state. To display a county outline map of any state, the mouse or other pointing device is used to move the pointer near to the abbreviated name of the state. A button on the mouse is then quickly depressed and released. The clicking action starts a small modular program connected to the sensitive area on the computer screen that tells the system where to find the state map and how to display it.
The above picture shows a cropped view of the image displayed on the MacIntosh. The left and right facing arrows are provided to move to the next or previous state in alphabetical sequence. Clicking on the button labeled “Colorado” displays an organizational chart of the APHIS-VS organization in the state. A home icon is provided to return the user to the master or home card.

County Outline Maps

Transparent buttons are located over each county name. Clicking on a county name displays an outline map of the county and a text field for information relating to that county. The sample to the right shows Douglas County, Colorado. The sample on the adjoining page shows Hawaii County, Hawaii. The text field can be displayed at all times or hidden and displayed only when an information button is clicked. It is possible to have several overlapping information fields covering different topics. Each field would be displayed by clicking on a labeled button.
state map or return to the Western Region map.

Request county map. Additional buttons are provided to move back to the labeled County map or near the county name moves directly to the map.

On the Colorado map, each of the adjoining counties on the map are labeled. Clicking on one of the adjoining counties on the map are.

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HAWAII COUNTY

Table: 1. Sheep Flocks: 13,300 head
       2. Ecuador Pigs: 5,500 head
       3. Hogs: 3,700 head
       4. Dairy Herds: 2,000 head

Features displayed on different maps.

Valuable information.

In the picture below, detailed information on roads, ranches, and game.

Each county outline map can be used to display other features important.
**Broad Detail Maps**

Clicking on any point within the boundaries of the county outline should bring up a map of that section of the county. In the pilot project, only maps relating to Douglas County, Colorado were drawn. In a full-blown project, maps in the detail shown above would be available for the entire United States. In the near future, it should be possible to obtain computerized versions of such maps through commercial vendors.

The map shown is approximately 1:300,000 scale. The small crosses are located at the corners of townships. The area of the map covers approximately 25 miles by 30 miles. The details on the map were selected to display the major geographical features within Douglas County. Hypercard drawing tools can be used to add other needed features.

On the full screen display, arrows, pointing in all four directions, are provided. Clicking on the appropriate arrow moves to the next map up, down, left, or right. In a finished system, the user could move around the state or even across state lines by clicking on successive arrows.

Buttons are also provided to move directly back to county, state and regional levels. It is important to notice the advantages of being able to move around the system without having to follow a strict tree structure.
The maps were drawn using three methods. First, an attempt was made to use a digital scanner on commercial maps. This did not produce satisfactory maps. The scanner copied the critical features of the map and also copied many features not wanted. The cleanup time to produce an acceptable map was far in excess of the following procedures.

The second method used a digital tablet. The map displayed below was drawn by placing a copy of the map on the tablet and tracing the desired features. This method was much more efficient, but limited the size of the drawing to an 8 \times 11 inch section.

The third method uses both the use of a digital scanner and another software program named MacDraw II. This proved to be the most efficient method and had the added advantage of being able to produce large, detailed maps on the laser printer.

MacDraw allows continuous images of up to 8' by 8'. Sections of the map were scanned and imported into MacDraw and pasted on a base layer. Details were then overdrawn onto a second layer, much like drawing on a piece of clear acetate over the map. Finally the base layer was deleted, leaving the finished map. Pieces of this large map are then transferred to the Hypercard stack.
Township Maps
Under the present proposal, township maps, as shown to the right, would be produced only for areas of special interest. In this case, the shaded area contains many equines. The other areas are either urban or do not have a substantial population of horses. Over time, the database should contain several maps showing general areas for cattle, swine, poultry, etc.

As geographic features change over time, new roads and housing areas can be added to the map. As urban areas encroach, the animal population shading can be reduced.

With very limited training, all the changes can be made by field or clerical personnel.

Section Maps
Section maps will be produced as needed on site. Enough details will be provided on each map to enable personnel to drive to a specific location and to identify some standard legal coordinates. Range, township and section numbers are often available. Longitude and latitude could be an alternative. Grid coordinates, such as used in the "Thomas Guides" found in California, could be used.

At this level, the need for a system that is easily taught to non-computer professionals is critical. The database will become very specialized to meet local needs. Changes and additions will be frequent.

The usefulness of this geographic information system lies in its ability to provide timely information to a working field force and middle and upper level managers. The additions and changes must be able to be made quickly and on site.
Plat Maps

If the need arises, plat maps could also be produced on site. The sample to the right shows simple solid outlines for homes and outbuildings. Other shading patterns and a corresponding legend could be substituted. Features such as waterways or major geographic barriers could be superimposed on the same image.

In the event of an outbreak of a disease of domestic animals, it would be important to identify the locations of other susceptible species on near or adjoining premises. Several maps covering a larger area might be needed. In this event arrows, connecting to adjoining maps, would be provided.

Property Maps

Property maps would be produced on site for premises of special interest. The sample to the right shows some of the graphic possibilities. Most of the patterns and outlines can be quickly drawn using tools available within Hypercard. If an object needs to be moved, it does not need to be redrawn. Instead, the object is selected with a special tool and moved intact to the new location.

Please notice the wide range of points the user can proceed directly to with a simple click of a button. This feature is largely user-controlled. In an active working environment, more buttons could be added to cross-relate other items of importance.
Parting Thoughts

All of the above hopefully demonstrates the need for a fast, flexible, adaptable, portable, geographic information system. The pilot system demonstrated here does not meet all the needs of a field task force operation, but it covers more critical aspects than any other commercially available system I've seen to date.

As I pointed out earlier, during the development of the project several managers pointed out that the same maps, hypertext capabilities and other information could be used in many day-to-day management activities. Recent demonstrations have also indicated that there are many other areas, both in and out of the government, that could benefit from a similar system.

The following material covers the application of the pilot system to a task force operation in southern California.
The primary dairy area in southern California is centered around Chino, California. Los Angeles is approximately 50 miles to the west. San Bernardino is approximately 20 miles to the east. The area is densely populated. One interstate and several other major highways run east and west. One interstate highway and several other major highways run north and south. Most of the dairies are located south of Highway 60 and east of Highway 83 in a state-designated agriculture preserve.

This area became reinfected with brucellosis in 1972. Since that date, over 175 herds have become infected with brucellosis. Eradication efforts have been successful in the great majority of the herds, but reinfection occurs frequently. In the last two years, the herd infection rate has risen from 10 to over 30 herds as of January 1, 1989. The State of California established a special task force to eradicate the disease from this area in April 1989.

Most of the dairies are located within a five-mile radius of the Chino Airport. On January 1, 1989 all of the infected premises were located in the area shown below. The cattle population in this small area exceeds 200,000 dairy cows and heifers. Many of the dairies have adjoining fences and those that don't utilize open fields and pastures in between for dry cows and bred heifers.

Transparent buttons have been placed over each of the shaded areas showing the location of reactor herds. Clicking on any button displays a detail map of the premises as partially shown below. The full screen display includes an information field with data about the infected premises. This field can be hidden to display a larger map area. Both the maps were prepared by Dr. John Belfrage as part of his epidemiology study of the area. Farm names have been changed.
Several options can be made available on detail maps. The sample below shows a general layout of the infected premises, and the adjoining premises are shown as shaded blocks.

Another alternative would be to provide details on all the adjoining premises and add shading only to the infected premises.

If necessary, additional buttons can be added to display information and/or graphics about any pen or other premises detail. These will be added as more epidemiological information is gained about the area.

**Special Visual Effects**

Much of the job of the epidemiologist is concerned with education of the livestock owners. In order to convince the dairyman that there is a problem, you must be able to demonstrate the problem. The following five small sections out of full screen maps show how visual effects can be useful. They show the changes in herds under hold order from January 1986 to April 1986. The entire series shows the changes over a much larger area from January 1986 to November 1987. The number of herds in the area increased from 7 to 17 herds.

The full screen display has buttons for displaying specific months and a special field shows the number of herds under hold order for any given month. Shaded areas are herds under hold order.
The first picture above dissolves to the second, showing that a change will be taking place to the Frey herd.

In the fourth image, three herds are highlighted. One herd will be released from hold order and two others will be placed on hold order.

After holding for about one second, the third image appears showing the final result.
The final image in this series shows the status of the herds as of April 1986.

MacDraw II
Mapping
Applications

The following pages contain examples of maps drawn with MacDraw II on a MacIntosh II microcomputer. MacDraw II allows continuous images of up to 8' by 8'. Sections of the map were scanned and imported into MacDraw and pasted on a base layer. Details were then overdrawn onto a second layer, much like drawing on a piece of clear acetate over the map. Finally, the base layer was deleted, leaving the finished map.

The map shown on the page to the right is a 50% reduction. The full-sized map is 16" x 22". The scale was chosen to match the "Thomas Guide" maps that are popular in the California area. MacDraw prints out the map in 8.5" by 11" sections on a laser or dot matrix printer. A plotter could also be used to obtain larger unbroken paper sizes.

The base map took about 6 hours of work. In a production environment, this time could be short-
MacDraw prints out the map in 8.5" by 11" sections on a laser or dot matrix printer. A plotter could also be used to obtain larger unbroken paper sizes.

The base map took about 6 hours of work. In a production environment, this time could be shortened considerably. From this scale, the size of the image can be enlarged or reduced as needed, either on the laser printer or within the computer. For example, it is possible to print the present map out at a 200% enlargement that corresponds to a 40" by 45" aerial photograph being used in the task force headquarters.

The map displayed on the next page has four additional layers beside the base map:

1) Outlines of the dairies. Not all the dairies in the area are shown at this time. When we started comparing the aerial photograph with the previous maps, several images did not match and several dairies showed up in unexpected places. Verification of the location and boundaries of all dairies is presently in progress.

2) Herd numbers.

3) Dairy herd names are not shown. Several dairymen made a request that only dairy herd numbers be shown to certain audiences.

4) Shaded areas over current reactor herds.

The individual layers can be shown or hidden in any combination. Up to 500 layers can be generated. As the eradication effort progresses, we will add to, or delete from, the map at will. With a minimum of additional labor we will be able to produce numerous map's progress, status, adult vaccinated herds, quarantine areas, etc. In turn, these can be transferred to the Hypercard data base and text information added and cross-indexed for research and other uses.

One of the most interesting aspects of the epidemiology of this area is the extreme density of the cattle. The ebb and flow of brucellosis in this area over the years tends to indicate that there is much exposure due to fence line contacts. Aerial photographs of the area have been obtained and will be used to establish the boundaries of each dairy and where the animals move within the property lines.

MacDraw II will be used in conjunction with digital scans of the aerial maps. In the following pages there are two examples of techniques under consideration. In both techniques the starting point is a 300 dots per inch scan of the aerial photograph. MacDraw II is then used to outline or label the features of interest. In the first technique, the map is placed side by side with the scanned image. In the second technique, the map is printed directly on to a clear acetate sheet and laid over the scanned image.

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GEOGRAPHIC INFORMATION SYSTEM

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For the past twenty years the natural resource agencies of state and federal governments have been developing and standardizing systems to collect, manage, analyze, store and display, spatially referenced data. Such a system is called a Geographic Information System (GIS). Some of the earliest work in this field was in the area of digital cartography, that is the use of computers to compile i.e. create maps. There is still some confusion as to whether an automated mapping system is in fact a complete GIS. Automated mapping systems generally do not support analytical procedures which are the most important functions of a GIS. Another more recent addition to the field is the technique of digital image processing. Although there is a new application of digital image processing which treats text material such as letters and reports as an image, the original development of the technology involved aerial and/or space images.

The art of human photo-interpretation has been a part of mapping for many years. There are really two important and different skills in photo-interpretation. The first involves the mechanical skill of drafting. The interpreter draws on transparent overlay material, transferring features that he sees on the photo to the overlay. If he is working with color photography, two adjacent features are separable because there is a color difference between them. It may only be a difference in tone or intensity, but there is a discernible boundary. On panchromatic photography there is a gray scale difference. The draftsman is merely tracing the observed boundary with his pen. The other skill is more truly the interpretation skill. The image analyst, using information from many sources is able to identify the features. In a panchromatic scene he must rely on the tone and texture of the feature. In a color rendition, in addition to texture, he has the advantage of the dimensionality of color; chroma, hue and value. He also knows where the image was taken, the time of year as well as the time of day. All of these clues plus his own experience, especially familiarity with the terrain, allow him to identify the feature. The complete job, therefore is to both draw the overlays and label them. These products are then used in the compilation of maps. Whether this is done by a human or by a machine the technique is referred to as feature extraction.

Normally before feature extraction is performed the scene must be rectified. This operation takes the raw input image and transforms it to a cartographic product, a so-called photo-map. Both satellites and aircraft are not completely stable platforms which look straight down. If they were there would be no need to correct the scene. They are both subject to a variety of motions about the axis of flight, but the two most important are pitch and roll. These motions distort the scene so that the recorded image is no longer square but oblong. Both motions often occur together. The
simplest correction technique involves the use of an enlarger with an articulating base. The original film negative is put in the enlarger and instead of putting the print paper on the base board you put a map on the area. This base is then moved such that the tip and tilt of the base matches the pitch and roll of the aircraft. The map is then removed and the paper put in its place and exposed. The resulting print is now rectified and ready for interpretation and feature extraction. Computer rectification is a more complex operation which uses the aircraft or satellite navigational computer information instead of the scene, but accomplishes the same end.

There is no question that maps convey information to a map user in a manner that is perfectly matched to the inherent spatial pattern recognition skills of the human eye-brain combination. Compare a descriptive narrative about how many livestock or poultry farms there are in a specific geographic region, to a map displaying the same information. Comprehension and retention are many times easier and faster with the map. There is also a wealth of additional information that can be available on the same map. The topography, surface water, soils and vegetation types, roads, etc., can easily be displayed.

Where GIS technology differs from conventional mapping is that the computer has the ability to display, as a derived map, the results of complex arithmetic operations on individual map overlays. You can add two maps or you can subtract one from the other. You can extract parts of one map and add it to parts of another. For example, you can combine in the computer a cultural features map with a topographic map, a soils map, and a hydrologic map to display and/or to plot the response to the following query:

"Display all of the clay soils below the 20 foot contour line along both sides of the river for this entire map sheet."

If you had an animal agriculture overlay, you could modify the above query, "Display all the poultry farms on clay soils, etc." The term cultural features refers to all the works of man; ordinarily roads, dams, airports, cities and towns, etc. We customarily think of them as road maps. However if they are large enough in scale they can easily show farm buildings. Since they are virtually always line drawings derived from the interpretation of satellite and/or aerial photography, they frequently form the base map on which other features, such as vegetation or soils, are displayed.

As a point of clarification the concept of scale needs to be understood. Most people know that the scale of a map is published on the map. Shown in one corner or in the center at the bottom of the map one may see, for example, Scale = 1:63,360. This means that one unit on the map equals 63,360 units on the ground. Thus one inch of the map equals 63,360 inches (1 mile) on the ground. This is referred to as "inch to a mile" map, and is said to be a large scale map, whereas if the Scale = 1:1,000,000 or greater, the map is said to be a small scale map. The terms large and small refer to how large or small the features are on a map, and not how much territory the map portrays. Indeed a large scale map covers a small geographic region,
GEONOGIC INFORMATION SYSTEM

whereas a small scale map covers a very large geographic region. A large scale map can display very small features, such as individual farm buildings and even stock ponds. However, a small scale map can only display very large features such as major roads, airports, town and cities. Most important, scale virtually always refers to the output product (the map). Scale is a determinant on the input side only in the amount of detail drawn on the overlay by the image analyst, or extracted by the digital image processor.

The resolution of the image that forms the base material for the map is the controlling factor. If a feature cannot be seen on the image, it cannot become a feature on the map, whether a man or a machine is doing the interpretation. In our work in agriculture we get material from a variety of photographic/image sources. On the average, USDA acquires new aerial photography of the states each 5 years. The photography is flown at an altitude of 10,000 feet with a 6 inch focal length camera using 9 inch film. The original film has a contact scale of 1:20,000 and a resolution of about 2 feet. The term “contact scale” refers to the practice of making a contact print that is the same size as the film. If you enlarge the print, you change the scale, making it larger. If the contact scale is 1:20,000, and the film is 9 inches wide, the distance on the ground along one edge of the film equals 9x20,000 inches, or 15,000 feet. If you square that number you get about 8 square miles of land coverage, or 5120 acres on each photograph. By comparison the high-flight photography from the U2 aircraft is acquired on 9x18 inch color or color-infrared film with a 12 inch focal length camera from an altitude of 68,000 feet. This product has comparable resolution but has a contact scale of 1:68,000 and covers 9x68,000 inches (51,000 feet) on one side and 18x68,000 inches (102,000 feet) on the other. The resulting land coverage is over 119,000 acres. When you consider that the camera was designed as a metric mapping camera it is clear that the high-flight photography is a more cost effective approach for mapping.

Satellite data is used for many applications and useful coverage can be acquired as often as twice a day. The Advanced Very High Resolution Radiometer (AVHRR) on the weather satellites “sees” the entire earth once during daylight hours and once at night. It has a resolution of 0.8 kilometers. These weather satellites have only recently been used for the monitoring of earth features but their application is very important in looking at regional data. The most important application of interest to animal agriculture is the monitoring of vegetation condition. Since the satellite looks every day, the cloud cover problem is minimized. Indeed it is clearly possible to monitor vegetation condition objectively on a weekly basis with a very high expectation of complete coverage of the area.

Landsat, with a resolution of 30 meters, “sees” the whole earth once each 16 days. Since the Landsat imagery has many agricultural applications, it is interesting to compare its coverage to the above aircraft data. Each Landsat scene covers approximately 845,780 acres and the satellite sensor
acquires data in a number of spectral bands (colors). The data can be prepared as color film, color-infrared film or false color film using any combination of bands the analyst wishes. Furthermore since the data is acquired in digital form it can be processed directly by image analysis computers without having to go to the film format. If it is possible to perform the required analysis using imagery from any of the platforms then it is useful to look at efficiency factors. If we set the low altitude aircraft efficiency factor to 1 then:

- Conventional aircraft = 1
- High altitude aircraft = 23
- Landsat = 165

In spite of the obvious advantages to using the satellite imagery, it has been in foreign countries, particularly in the developing world, where Landsat has seen its greatest use. For the most part the reason is the lack of current and accurate natural resource information of any kind in those countries. However, there has been a great reluctance on the part of US government agencies to commit to the new technology. Once again, something we have invented is being developed by others. In this case it is the French with their 10 meter resolution SPOT satellite. In a recent study in Florida, SPOT was used to update county land use/land cover maps with an accuracy of 0.2 acres. Imagine having vegetation maps with that accuracy in a Heartwater campaign. Fortunately, current US policy permits the civilian agencies to build 5 meter resolution systems, and it now appears that we will do so.

Having discussed briefly what a GIS is and how resource information is derived and stored in the system, the question arises, why should APHIS and the individual states be interested? The simple answer is that if we are going to invest money in the electronic storage of animal health information, then we should most certainly store the data in a system that allows us to ask the machine to tell us not just how many animals there are but where they are. Disease for us is not a static phenomenon. Veterinarians in private practice deal principally with the individual animal, less often with herd health, although that is changing. Those of us in government practice deal principally with herd health, and frequently with community health, at least at the political community level, (counties and states).

In trying to encourage veterinary students to become interested in government practice it is particularly discouraging to see their eyes glaze over at the mention of the word epidemiology. On the other hand, the mere mention of the word ecology seems to capture their interest. It is not important whether they are all closet ecologists or whether the term is just more fashionable these days, what is important is their level of interest in hearing lectures about “The Ecology of Disease.” One could argue that there is no difference between ecology and epidemiology as we apply the latter. That may be true, but epidemiology is after all, a branch of biology, whereas
ecology is not. Thus the ecology of disease is a better paradigm for what we are about. It is, after all, difficult to think about the study of an animal transportation system as a biological thesis, but it is clearly part of the interaction between the animal and its environment.

It is convenient to apply the notion of scale to the ecology of disease. Further it is important because GIS technology facilitates the handling of features with different scale components. In the ecology of disease there are two important scale components, space and time. GIS technology can handle them both. The spatial component is the one that we deal with all the time. We are more or less familiar with these components, depending on our orientation, training and experience.

1. The Animal.

This is the area where we receive our greatest training at the undergraduate level and where the private practitioner spends the bulk of his career.

2. The Herd.

We receive adequate undergraduate training on herd health matters but it certainly isn’t emphasized to the degree that the individual animal is. In government practice our focus is on the herd and much of the epidemiological training that we receive deals principally with the herd.

3. The Community.

About the only undergraduate training we receive at the level of the community, deals with poisonous plants and soil deficiencies and toxicities. There are virtually no lectures dealing with the “setting” of the herd in the context of the environment. Little attention is given to the importance of roads and rivers and very little more to the subject of climate as it impacts the animal or the plants it lives on. It is left to the specialty interest of the student or to the government agency that employs the graduate for this material to assume any importance.

GIS technology, by its very nature permits the veterinarian to visualize the spatial component with ease. It is possible to handle the herd as a spatial entity and to study the movement of disease in the herd. This is rarely done. A much more common application deals with the movement of disease in the community as it spreads from herd to herd. It can be done on the video display of the computer where each presentation can be saved as a file, or sent to some hard copy output device, such as a printer or plotter.

The temporal component is one that can give us great insight in the disease process as it works in each spatial component. It is convenient to allocate time into blocks.

1. Minutes to Hours.

This block is the one we are concerned with when we are dealing with the individual animal and much of its physiological response to stress factors.
2. Hours to Days

This block concerns both the individual and the herd. The immune response is most generally in this time frame and the response of both the animal and the herd to microbiological stress.

3. Days to Weeks

This block concerns principally the herd and the community. Most of the time we are dealing with microbiological stresses and the problem of disease spread mechanisms. It is possible to extend the temporal component to years in the case of slow viruses but for the purpose of this paper the three blocks are sufficient.

If you consider the dimensionality of GIS technology, it is clear that the output product, a map, is a two dimensional sheet of paper. But we also know that the contours on a topographic map can display relief, a third dimension. The question is, how do we display a fourth dimension, that of time? There are two methods in common use, one of which we see every day on television. The moving cloud photography that we see on the weather reports is a simple technique. Individual images taken each 30 minutes from geosynchronous satellites are made into a continuous movie loop and played through a standard movie camera. This is nothing more than cosmic time lapse photography. Imagine how instructive it would be to display the spatial spread of disease over time. Just as we can see the clouds move, we could see the disease move. While this is clearly the most effective, the other method of displaying time is just as easy. In this case one uses color to code events as a function of time. Conventionally one would assign a color to all of the events that occurred on a particular day, or week, or even month, depending on the speed with which events occurred.

The Geographic Information System is a fully mature technology and one in which the states and APHIS should invest. It is not the purpose of this paper to review the hardware and software that is available, but it should be pointed out that the most important limiting factor in the selection of software would be the requirement to have the identical software on both mainframe and microcomputers. If transportability of files is all that is required, there are many packages to choose from.
REPORT OF THE COMMITTEE ON
ANIMAL DISEASE SURVEILLANCE AND
ANIMAL HEALTH INFORMATION SYSTEMS

Chairman: Dr. R. E. Bohlender, North Platte, NE
Vice Chairman: Dr. D. Hueston, Ft. Collins, CO

J. A. Acree, MD; J. L. Alley, AL; L. A. Anderson, IA; C. W. Beard, GA;-
D. L. Berndt, MD; S. L. Diesch, MN; C. R. Dorn, OH; T. R. Drake, PA; D.
Galbreath, MD; H. S. Gosser, MO; F. D. Gregerson, CO; C. M. Hibbs, NM;
D. W. Hird, CA; J. F. Hudelson, CO; M. E. Hugh-Jones, LA; L. Hutchin-
son, PA; N. E. Hutton, OR; L. J. King, MD; P. B. Ladd, CO; H. Lloyd, FL;
L. D. Mark, VA; E. H. McCauley, MT; D. Meeker, IA; W. R. Miller, AL;
L. G. Morehouse, MO; J. C. New, TN; S. R. Nusbaum, NJ; W. J. Owen, IA;
J. C. Paige, MD; A. B. Park, MD; M. Parkhie, MD; P. A. Pickerill, TX;
E. I. Pilchard, MD; G. C. Poppensiek, NY; J. C. Prucha, MD; P. F. Ross,
IA; L. H. Russell, TX; M. D. Salman, CO; V. A. Seaton, IA; R. C. Swanson,
CO; R. L. Tharp, MO; W. Utterback, CA; J. M. Williams, CO.

The Animal Disease Surveillance and Animal Health Information Sys-
tems Committee focused its attention on the development of the National
Animal Health Monitoring System (NAHMS).

Dr. Lonnie King, Deputy Administrator for Veterinary Services, set the
tone of the meeting by reemphasizing the U.S. commitment that the
NAHMS program is being developed to meet the needs of its potential
users. He reiterated the question raised by Phil Bradshaw in his address
Monday night: “How much is it worth to protect America’s 80 billion dollar
animal agriculture industry?” The NAHMS program, ahead of its time at
its inception 6 years ago, is no longer ahead of its time now. Dr. King
welcomed the committees input regarding user needs and concerns. Phil
Bradshaw echoed these concerns, encouraged continued commitment of
resources to the program — the need for reliable data is critical.

A wide representation of potential users of NAHMS addressed the com-
mittee. Dr. David Meeker from the National Pork Producers Council
stressed the critical need of the swine industry for sound scientific informa-
tion on which to base research priorities and policy formulation. Phil Dukas
of National Dairy Herd Improvement Program challenged the NAHMS
program to answer 4 basic questions: 1) Why is the information being
collected important? 2) who will interpret the information for the practi-
tioners, producers, and industry?; 3) Where are other existing databases
which can be shared to enhance the NAHMS objectives; and 4) When will
producer organizations be willing to actively support the NAHMS develop-
ment. I, Bob Bohlender, spoke on behalf of the beef industry. Beef produc-
ers, i.e. cowboys, are obsessed with individual cow diseases, but have been
unwilling to collectively take action in support of the NAHMS program.
Cattlemen have been slow to accept new technologies, in part due to an

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incomplete understanding of the import of disease. The NAHMS program must quantify disease costs to the cattle industry. The NAHMS program should forge links with the IRM program to facilitate delivery of information to the producers.

Industry information needs were also addressed. Mary Gale of IDEXX emphasized the two measures of success for an information intensive program like NAHMS; 1) strong production economics and 2) safe food. Successful utilization of information assists farm managers in profiling herd/flock health and making wise economic decisions. Collaboration between the agriculture industries, government and technology companies provide a tremendous opportunity for progress. Fred Guillo of CODON focused on biotechnology company needs. With an investment averaging $4 million for the development of each new biotechnology product, industry needs objective data on the relative importance of specific diseases. These companies cannot find new answers to old problems if the problems themselves are not carefully defined. The NAHMS program is needed to help industry guide their animal health research investment. Dr. Doug Armstrong of Norden reminded the committee that the focus of NAHMS is improving the well being of animal health. Describing animal health conditions require accurate and specific diagnoses, consequently the diagnostic laboratory capabilities must be strengthened and standardized.

University/diagnostic laboratory needs were addressed by two speakers. Dr. Bill Hooper of the Association of American Veterinary Medical Colleges stressed the important collaboration potential for not only Veterinary Colleges, but also Departments of Veterinary Science and Animal Science Departments. The areas of information needed are 1) to help guide development of educational programs; 2) to facilitate cooperation in the development of agricultural policy; 3) to direct research priorities; 4) to provide a communications interface for industry/veterinary colleges and government; 5) to facilitate epidemiologic modelling and research; and 6) to forge collaboration with diagnostic laboratories. The agricultural community is suffering a public perception problem — NAHMS can provide crucial information to counter these perceptions. Dr. Dan Lein of the New York Diagnostic Laboratories, commented on the need for population based incidence and prevalence information to complement diagnostic laboratory results. Recent experiences of the diagnostic laboratories reinforce the importance of field data collection in conjunction with diagnostic testing.

Dr. Dale Groteluschen, University of Nebraska, related results of a survey of producer and veterinary reactions to NAHMS participation. The participating producers from Colorado’s second and third years of beef cattle monitoring were surveyed: A total of 73% felt that NAHMS was economically beneficial to them. About 1/4 of the producers changed their management as a result of NAHMS involvement. Most of these owners’ veterinarians expressed an interest in increasing their participation in NAHMS.
Dr. Scott Hurd, Michigan State University, discussed the flow of data and information from NAHMS back to its users. Using Michigan dairy data, several examples were presented of information useful for pharmaceutical companies and producers.

An overview of the upcoming NAHMS National Swine Survey was presented by Dr. Jeff Farrar of the NAHMS staff. A total of 1400 herds in 18 states will be included. The focus of the survey is farrowing sows and piglets from birth to weaning. Dr. Jim Alexander of the NAHMS staff discussed the subsampling activities planned in conjunction with the swine survey. Dr. Will Hueston, NAHMS program leader, then provided insight into the future planning for NAHMS. The Strategic planning process and development of operational plans were reviewed. The responsiveness of NAHMS to user needs was stressed.

A report from the Ad Hoc Committee on Information Technology was presented by Dr. Archie Park of the Maryland Department of Agriculture. The potential for Geographic information systems was discussed as well as concerns regarding the need for diagnostic laboratory software which supports information technology. Reports of two surveys of information technology resources will be included in the proceedings. In addition, a summary of a survey of diagnostic laboratories will be included in the proceedings.

The committee meeting concluded with the passage of two resolutions concerning NAHMS. These will be presented to the resolutions committee.
Oysters and salmon were vital staples for Native Americans in the Northwest long before white man entered the scene. They were critical to the new arrivals as subsistence until agriculture could be established but soon became a valuable cash crop for export out of the Oregon Territory. Their natural abundance attracted early exploitation. First the native oyster was shipped, along with lumber, to support the California gold rush. Soon after, when fish canning became practical, salmon became an inexpensive protein source to be sold throughout the nation and beyond. But by the turn of the century, both salmon and oysters had suffered serious depletion by unrestricted overfishing.

Solutions to the dramatic decline in the natural resource appeared in the form of public and private fish hatcheries for enhancement, and through private stocking of the Pacific Oyster, imported from Japan. This was the early beginning of aquaculture and much of that early work set the stage for subsequent expansion of culture and harvest as opposed to simple extraction.

The Pacific Oyster became the species of choice due to its rapid growth and large size. Japanese seed provided the needed stock to replenish the beds since oysters reproduced erratically in the cold waters of Puget Sound, Grays Harbor and Willapa Bay. The seed supply was interrupted by World War II, so a few shallow, warm bays became critical to the West Coast industry since spawning did occur with some regularity. Oyster spat was caught and transported to suitable grow out beds in Western Washington, and the industry prospered until the 50s and 60s. Conflicts with industrial pollution, agricultural runoff and expanding urbanization severely limited the production and natural spawning, reducing the industry by 30 – 50 percent during that period.

The development of oyster seed production and expansion of new culture techniques has reversed the negative trend to a certain degree, but suitable water quality still remains a limiting factor in the expansion of oyster production in the state.

Presently the US imports about 55 percent of the oysters we consume. With disease and pollution problems in the Gulf and East Coast, Washington has assumed a more prominent role in domestic production. Last year, 35 percent of the domestic production was from the West Coast, with more than 85 percent of that coming from Washington State. Our shellfish industry is poised to expand production even further, if alternative off bottom, longline and rack and bag culture methods can be sited in intertidal areas in the state. User conflicts and fear of competition with the wild resource are the primary stumbling blocks to further expansion.
AQUATIC FARMING IN WASHINGTON STATE

Salmon species met with similar problems. Dams on the Columbia River and loss of spawning habitat in rivers throughout the state compounded the over-exploitation. State and federal hatcheries produced fingerlings for enhancement, but in many cases strains were totally lost in many river systems.

Salmon farming in net pens started in the early 1970s with the rearing of small, pan-sized Coho Salmon and the industry has grown slowly since that time. Presently there are 13 farms in Puget Sound producing primarily Atlantic Salmon. Expansion of the industry faces stiff resistance as it is perceived as an impairment to view and a use conflict for recreation and commercial fishing.

The typical commercial operation covers about two surface acres and produces 500,000 to a million pounds of salmon per year.

Trout is also grown in relatively small quantities in private freshwater hatcheries, but the trout industry is dwarfed by the Idaho industry. Washington State boasts a major international trout egg producer who provides about 10 percent of the world trout egg supply.

By State Law, commercial salmon ranching is not allowed in Washington State. Native Americans, due to their sovereign status, can & do ranch salmon on a limited basis using the delayed release marine net pen system. One tribe accounted for 25% of the South Puget Sound coho salmon catch in 1987, using ranching techniques. Sports groups in cooperation with Washington Dept. of Fisheries use delayed release techniques to enhance Ice Sport Fishery.

John L. Pitts
Nov. 1, 1989
Wash. Dept. of Agriculture

AQUACULTURE DISEASE CONTROL 220-77-020
Chapter 220-77 WAC
AQUACULTURE DISEASE CONTROL

WAC
220-77-010 Intent.
220-77-020 Definitions — Aquaculture disease control.
220-77-030 Finfish aquaculture disease control.
220-77-040 Shellfish aquaculture disease control.
220-77-050 Amphibian aquaculture disease control.
220-77-060 Marine plant aquaculture disease control.
220-77-070 Aquaculture disease control — Emergency provisions.

WAC 220-77-010 INTENT. The intent of this chapter is to establish rules to protect the aquaculture industry and wild stock fisheries from a loss of productivity due to aquatic diseases or maladies. These rules will identify the conditions that will be required for transfer and importation of live
acquaculture products and the circumstances when action will be taken to control disease. These rules have been developed jointly by the department and the department of agriculture.

WAC 220-77-020 DEFINITIONS — AQUACULTURE DISEASE CONTROL For purposes of this chapter, the following definitions apply:

(1) “Aquaculture products” are defined as private sector cultured aquatic products propagated, farmed, or cultivated on aquatic farms under the supervision and management of an aquatic farmer, or such products naturally set on lands under the active supervision and management of aquatic farmer.

(2) “Disease” is defined as infection, contagious disease, parasite, or pest, occurring on or within the aquaculture product or on or within the water and substrate associated with the aquaculture product, or an occurrence of significant mortality suspected of being of an infectious or contagious nature.

(3) “Finfish” is defined as live fish, fish eggs, or fish gametes, but not to include aquaria species commonly sold in the pet store trade when raised in indoor containers, indigenous marine baitfish, or mosquito fish.

(4) “Shellfish” is defined as all members of the phyla mollusca, arthropoda, and echinodermata.

(5) “Epizootic” is defined as the occurrence of a specific disease which can be detected in fifty percent of the mortality or moribund individual fish in an affected container, and which results in an average daily mortality of at least one-half of one percent of the affected individual fish for five or more days in any thirty-day period.

(6) “Marine plant” is defined as nonvascular plants belonging to the phyla Chlorophyta, Phaeophyta, or Rhodophyta and vascular plants belonging to the family Zosteraceae when growing in marine or estuarine waters, and includes the seeds, spores, or any life-history phase of the plants. “Marine plants” do not include aquaria plants or phytoplankton.

(7) “Working day” is defined as any day other than Saturday, Sunday, or a Washington state holiday.

(8) “Department” is defined as the department of fisheries.

(9) “Quarantine” is defined as isolation of the organism in a department approved facility.

(10) “Pest” is defined as parasite, parasitoid, predator, or fouling agent.

WAC 220-77-030 FINFISH AQUACULTURE DISEASE CONTROL. (1) It is unlawful for any person to import into or transport within the state of Washington finfish aquaculture products without first having obtained a permit to do so issued by the department. A copy of the permit shall accompany the finfish aquaculture products at all times within the state of Washington, and must be presented upon request to department employees.
AQUATIC FARMING IN WASHINGTON STATE

(2) The director may impose permit conditions as necessary to ensure the protection of aquaculture products and native finfish from disease when the director concludes that there is a reasonable risk of disease transmission associated with the finfish aquaculture products.

(3) Upon confirmed diagnosis of viral hemorrhagic septicemia, or confirmed diagnosis of whirling disease, infectious hematopoietic necrosis, or infectious pancreatic necrosis in a previously uninfected lot, the department must be notified by the end of the following working day after diagnosis by an accredited pathologist.

(4) The director will issue, upon request, a pamphlet containing policy guidelines for importers and transferors of finfish aquaculture products.

(5) The director will issue or deny a permit within thirty days after a completed application containing all requested information is received by the department.

(6) Violation of these rules or the conditions of the permit may result in the suspension or revocation of the permit:

(7) In the event of denial, suspension, or revocation of an importation or transfer permit, the affected person may appeal the decision to the director. Additional appeals may be made through the Administrative Procedure Act (chapter 34.04 RCW). A suspended or revoked permit will remain suspended or revoked during the appellate process.

WAC 220-77-040 SHELLFISH AQUACULTURE DISEASE CONTROL.

(1) It is unlawful for any person to import into or transport within the state of Washington shellfish aquaculture products for planting in Washington waters, without first having obtained a permit to do so issued by the department. A copy of the permit shall accompany the shellfish aquaculture products at all times within the state of Washington, and must be presented upon request to department employees. Possession of an oyster transfer permit issued under RCW 74.24-.110 will meet the requirements of this subsection.

(2) The director may impose permit conditions as necessary to ensure the protection of aquaculture products and native shellfish from the disease when the director concludes that there is a reasonable risk of disease transmission associated with the shellfish aquaculture products.

(3) For established species and established routes of commerce, the department will issue import and transfer permits if the following criteria are met:

(a) A regular pattern of importation with no more than a one-year time lapse between importations.

(b) Documentation of recent mortality and disease history of the shellfish aquaculture product in the area of origin showing a lack of significant mortality.
(c) Verification that there has been no introduction of diseased stocks into the area of origin.

(d) Documentation that the shellfish aquaculture product proposed for import is from the approved area.

(4) For established species not from established routes of commerce, the department will additionally require the following before deciding whether to issue an import or transfer permit:

(a) Documentation of mortality and disease of the shellfish aquaculture product for the past ten years from the area of origin, together with similar information for closely related species, if deemed necessary.

(b) A history of those diseases in the area of origin that may affect aquaculture products or native fauna and flora.

(c) When applicable, documentation of an agreement with the appropriate governmental agency with management responsibility in the area of origin.

(5) For nonestablished species, the department will additionally consider the following criteria, which will require the importer to provide a detailed life history and comply with the requirements of SEPA:

(a) The capability of the receiving facility to hold the shellfish aquaculture product in quarantine.

(b) The ability of the shellfish aquaculture product to naturally reproduce or interbreed with endemic species in the state waters.

(c) The ability of the shellfish aquaculture product to compete with or prey upon endemic species.

(6) For purposes of verification of the disease-free status of shellfish aquaculture products in subsection (3), (4), and (5) of this section, the department may require sufficient samples for histological evaluation either prior to or after subjecting the shellfish aquaculture products to stress tests to detect latent disease conditions. In the event of failure to obtain permit approval, consideration will be given to introduction after hatchery production of a second generation stock.

(7) Violation of these rules or the conditions of the permit may result in the suspension or revocation of the permit.

(8) In the event of the denial, suspension, or revocation of an importation or transfer permit, the affected person may appeal the decision to the director. Additional appeals may be made through the Administrative Procedure Act (chapter 34.04 RCW). A suspended or revoked permit will remain suspended or revoked during the appellate process.

WAC 220-77-050 AMPHIBIAN AQUACULTURE DISEASE CONTROL.
(1) It is unlawful to import into the state of Washington amphibian aquaculture products without having first obtained a permit to do so issued by the director.

(2) It is unlawful to possess African clawed frogs for aquaculture.
WAC 220-77-060 MARINE PLANT AQUACULTURE DISEASE CONTROL

(1) It is unlawful for any person to import into the state of Washington marine plant aquaculture products without having first obtained a permit to do so issued by the department. A copy of the permit shall accompany the imported marine plant aquaculture products at all times until the initial point of entry into the marine environment, and must be presented upon request to department employees.

(2) The director may impose permit conditions as necessary to ensure the protection of aquaculture products and native marine plants from disease or pests when the director concludes there is a reasonable risk of disease or pest transmission associated with marine plant aquaculture products.

(3) For *Porphyra yezoensis* and *P. tenera*, the director will issue import and transfer permits if the plants are in the form of:

(a) Unialgal conchocelis culture of free living material; or

(b) Conchocelis-phase culture in shells after the shells and conchocelis have been washed and soaked in fresh water for at least twenty-four hours; or

(c) Blade phase on netting after two weeks at a temperature of minus twenty degrees centigrade or lower.

(4) For import of other species, the department will consider at least the following criteria, which may require the importer to provide a detailed life history and comply with the requirements of SEPA:

(a) The ability of the marine plant aquaculture product to naturally reproduce or interbreed with existing species in state waters.

(b) The ability of the marine plant aquaculture product to compete with existing species.

(5) Importation of marine plant aquaculture products for scientific study in a laboratory or under other controlled conditions is allowed without having obtained a permit when measures are taken to prevent release of the products or release of their gametes, spores, or tissue fragments into state waters. The director may inspect facilities to ensure appropriate control measures.

(6) For purposes of verification of the disease-free status of the marine plant, aquaculture product in subsections (3), (4), and (5) of this section, the department may require sufficient samples for evaluation. In event of failure to obtain permit approval, consideration will be given to introduction after laboratory production of a second generation.

(7) It is unlawful to transfer marine plant aquaculture products between any of the following geographic areas without having first obtained a transfer permit: Columbia River; Pacific Ocean waters; Willapa Harbor; Grays Harbor; Puget Sound. No transfer permit is necessary for transfer within any of the geographic regions described above. When required, a copy of the transfer permit shall accompany above. When required, a copy
of the transfer permit shall accompany the marine plant aquaculture products at all times until the products are reintroduced into state waters, and the transfer permit must be presented upon request to department employees.

(8) Violation of these rules, or the condition of any permit may result in suspension or revocation of the permit.

(9) In the event of denial, suspension, or revocation of an importation or transfer permit, the affected person may appeal the decision to the director. Additional appeals may be made through the Administrative Procedure Act (chapter 34.04 RCW). A suspended or revoked permit will remain suspended or revoked during the appellate process.

WAC 220-77-070 AQUACULTURE DISEASE CONTROL — EMERGENCY PROVISIONS. (1) The director may take the following emergency enforcement actions when evidence indicates these actions are necessary to protect aquaculture products and native stocks from disease or severe mortality from an unexplained source:

(a) Deny issuance of an import or transfer permit.

(b) Quarantine the aquaculture products.

(c) Confiscate or order the destruction of the aquaculture products.

(d) Require removal of the aquaculture product from state waters.

(2) Confiscation or destruction will be ordered without a hearing if confirmed diagnosis by an accredited pathologist is made that finfish aquaculture products are infected with the causative agent of viral hemorrhagic septicemia (Egtved virus).

(3) For finfish, shellfish, amphibian, and marine plant aquaculture products:

(a) Isolation may be ordered without a hearing when aquaculture products are transferred without appropriate inspections or permits or transferred in violation of the conditions of a permit.

(b) Isolation may be ordered without a hearing when evidence demonstrates that aquaculture products, previously imported, may introduce a disease not known to occur in Washington.

(4) For finfish aquaculture products, an epizootic of whirling disease, infectious hematopoietic necrosis or infectious pancreatic necrosis may result in quarantine, confiscation, or destruction, subject to the aquatic farmer’s right to an emergency departmental hearing, if confiscation or destruction are ordered.

(5) For shellfish aquaculture products, an outbreak of serious mortality in which contagious disease is suspected may result in quarantine or require removal of the suspected disease shellfish aquaculture products from state waters, subject to the aquatic farmer’s right to an emergency
departmental hearing, if removal from state waters is ordered.

(6) When there is evidence that continued presence of aquaculture products in state waters may cause disease that would harm other aquaculture products or native fauna or flora, the director may order quarantine, confiscation, destruction, or removal from state waters. Except as provided for in subsections (2) and (3) of this section, the aquatic farmer has a right to a departmental hearing. In the event the director has ordered emergency action of confiscation, destruction, or removal from state waters, the director shall give notice to the affected aquatic farmer. At the time of notice of emergency action, the affected aquatic farmer may request an emergency departmental hearing. If requested, the hearing will take place no later than the third working day after notice is received by the aquatic farmer. The hearing will be presided over by a hearing officer appointed by the director, who will consider the severity of the disease outbreak, remedies, and alternate courses of action. The hearing officer shall present a recommendation to the director. The director will then review the emergency action and, if appropriate, order confiscation, destruction, or removal from state waters. If so ordered, the emergency action will take place no sooner than forty-eight hours after the order. If no request for an emergency departmental hearing is received, the emergency action of confiscation, destruction, or removal from state waters, may take place immediately after the third working day after the notice is received by the aquatic farmer.

(7) If the department refuses to issue an import or transfer permit, or orders quarantine or isolation of aquaculture products, the aquatic farmer has a right to a hearing under the Administrative Procedure Act (chapter 34.04 RCW).

[Ch. 220-77 WAC — p. 6] (3/27/87)

WASHINGTON AQUATIC FARMING

Today and for the Future

U.S. consumption of fishery products was a record 15.4 pounds of edible fish per person in 1987, up 0.7 pounds from 1986. If you consider there are 240 million people in the United States, that increase amounted to 168 million pounds of fish. Since 1982, per capita consumption of seafood has risen 25 percent. U.S. Department of Commerce

How does the U.S. meet increasing seafood demands?

For the most part, the United States has satisfied increased demand through imports. Since 1980, the United States has imported an average of 65% of its seafood consumption each year. Of natural resource imports, the U.S. fishery trade deficit is second only to the U.S. oil trade deficit. Our
Fishery trade deficit increased from $6.2 billion in 1986 to $7.1 billion in 1987. A record $8.9 billion of fishery products was imported in 1988. U.S. Department of Commerce

Can we reduce imports by increasing U.S. fishery production?

We can, but not by simply catching more fish. Catch trends for the world's principal fishery resources indicate that they are at or close to their biological limits, or that certain stocks have been overfished. Aquaculture has provided an increasing percentage of the world fishery harvest. Development of aquatic farming in the U.S. can lead to: reduction of trade deficit; increase in a stable supply of domestically produced, top quality seafoods; creation of new jobs; improvement of recreational and commercial fishing potential. Sports Fishing Institute

How does Washington rank in aquaculture production?

- The Pacific Coast supplies 25% of total U.S. oyster production. Washington oyster farmers provide more than 90% of that Pacific Coast production.
- Salmon production based in Washington, Oregon, California, and Maine, and trout production centered in Idaho are the second and third largest aquacultural food fish industries.
- Washington boasts the nation's largest single farmed-clam producer, located in Mason County, and the largest mussel farm, located in Island County.
- Washington is #1 in trout egg production in the United States. One Washington farm produces 10% of the world's live trout egg supply.
- Washington has the world's largest oyster hatchery located at Quilcene. Washington oyster hatcheries supply 85% of the oyster seed in our state and over 60% of all the seed on the Pacific Coast from Mexico to Alaska. Washington oyster farmers and the University of Washington are world leaders in shellfish research and technology.
- The University of Washington School of Fisheries is the center for California, Oregon, Idaho, Alaska and Washington aquaculture research. This Regional Aquaculture Consortium is one of five nationwide and is funded by the U.S. Department of Agriculture.

How important is aquaculture to the Washington State economy?

- Washington aquaculture employs an estimated 2,000 people in direct farm jobs and an equal number in upland support jobs. Employment ranges from on-the-job-trained skilled laborers, to community college-trained technicians, to university-educated scientists.
- Washington aquaculture equaled nearly 30% of the total value of Washington's marine fisheries catch from 1980-85. That proportion is expected to rise in the future.
How is the world aquaculture production changing?

In 1975, aquaculture accounted for 5% of the world fishery harvest. By 1987, that figure had risen to 12%. The United Nations Food and Agriculture Organization predicts that by the year 2000, 1/4 of the world's seafood will be produced by aquatic farms and, by 2010, 1/3 of our seafood will be farm-raised.

The 1987 World Economic Summit, held in Venice, Italy, selected aquaculture as a top economic priority along with human health, mass transit, solar energy and space exploration.

Who will farm this food, U.S. farmers or someone else?

The United States has lagged behind other countries in aquaculture. In 1986, U.S. aquaculture production accounted for only 5% of U.S. fishery production. The U.S. Department of Agriculture says there is a strong possibility that aquaculture will eventually produce more protein-rich food than traditional agriculture in years to come.

Can Washington grow seafood that the United States now imports?

The United States imports significant amounts of many seafood products that grow or can grow in Washington.

Oysters: In 1986, The U.S. consumed 90.5 million pounds of oysters, 40.5 pounds of domestic production (78 million) and 50 million pounds of imports ($96 million). U.S. Department of Commerce

Clams: In 1987, the domestic harvest of clams was 134.4 million pounds valued at $132.9 million. U.S. Department of Commerce.

Fresh Salmon: U.S. imports of fresh salmon have grown from near zero in 1980 to 39 million pounds valued at $125.5 million in 1987. Imports of all salmon products equaled $191.5 million in 1987. U.S. Department of Commerce

Sea Vegetable (Nori): In 1987, we imported $18 million worth of this nutritious sea vegetable used for sushi. The first commercial production of nori in the Western Hemisphere occurred on Vashon Island in 1988. This included the cultivation, harvest and primary processing of this high-value product. American Sea Vegetable.

All these seafood products are farmed in Washington waters. We also rear trout, steelhead and mussels, and have great potential for abalone, scallops and sturgeon.

Why isn't Washington's aquaculture industry expanding to meet the growing market demand?

Water pollution has reduced the number of suitable farming areas. One-third of our traditional shellfish growing beds have been closed due to bacterial pollution (coliforms) from failing septic systems, agriculture
runoff and other sources. Industrial pollutants, such as toxic waste and heavy metals, prevent salmon and shellfish farming in some bays in Puget Sound. Aquatic farming may compete with other users in some areas.

**Are there environmental or health concerns associated with aquaculture?**

Aquaculture must have clean water in order to survive. The key to successful farming is locating the farm where water quality conditions are best. Correctly sited and managed aquaculture does not pollute. Aquaculture projects must receive local, state and federal permits before they can operate. The state of Washington has developed the most comprehensive salmon farming guidelines in the world. They are the world model for environmental protection.

Our wild salmon and steelhead resources are protected by the Washington Departments of Fisheries and Wildlife. Claims that our wild salmon resource will be lost due to farmed salmon disease or genetic conflict is not consistent with scientific data and extensive experience in Washington and around the world. The recent discovery of a European virus (VHS) in wild salmon returning from the open sea, resulted in extensive testing of wild, hatchery and farmed fish. The virus has never been found in farmed salmon or trout in Washington. The disease affects farmed rainbow trout in central Europe and has never been found in farmed or wild Atlantic salmon. The virus does not reside in eggs.

Shellfish and salmon aquaculture are "canaries in the mine" with regard to water pollution. Adverse impacts on cultured species indicate problems that can have negative impacts on marine creatures that could otherwise go undetected.

Properly regulated aquaculture activities do not adversely affect public health. Fish disease does not cause or lead to disease in man. Salmonids do not carry coliform bacteria which are found in man and other mammals.

"It becomes increasingly difficult to extract those additional supplies required to meet rising (fishery) demand. We must promote further growth in supplies from aquaculture and give close attention to the effects of environmental degradation." Director General, Edouard Saouma, United Nations Food and Agriculture Organization, 1989.
AQUATIC FARMING IN WASHINGTON STATE

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<th>Year</th>
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<td>1986</td>
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<td>1987</td>
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Source: Washington State Department of Fisheries
# Washington Aquatic Farming Production 1985 - 1988

## Finfish

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## Total Aquaculture Production

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<td>22,983,622</td>
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Source: Washington State Department of Fisheries

8/8/89
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CULTURE METHODS AND DISEASES OF CHANNEL CATFISH
ICTALURUS PUNCTATUS

Michael R. Johnson
College of Veterinary Medicine
Mississippi State University
Mississippi Agricultural and Forestry Experiment Station, Delta Branch
Stoneville, Mississippi 38776

The farm-raised catfish industry is still a very young industry, which developed in the early 1960s. Today channel catfish culture is the backbone of the aquaculture industry in the United States. The catfish industry accounts for almost half of the United States aquaculture production. In 1988, 295 million pounds of catfish were processed with a farm gate value of 225 million dollars. The next largest United States aquaculture industry is trout culture with 59 million pounds processed in 1988. Channel catfish represents the fourth largest volume of edible fish products behind tuna (883 million), pollack (697 million) and salmon (603 million).

There are 1,922 commercial catfish operations in 17 states accounting for over 140,000 acres. Mississippi is by far the largest producer of farm-raised channel catfish with over 91,160 acres of catfish ponds, followed by Arkansas with 18,600 acres, and Alabama with 14,932 acres of catfish ponds. Mississippi has less than 18 percent of the catfish producers (314) controlling 63 percent of the U.S. acreage, and accounts for 75 percent of the total output. Many factors make Mississippi an ideal state for channel catfish culture: vast expanses of flat land, heavy clay soils, abundant high quality water supplies, a climate that provides a catfish growing season in excess of 200 days, and large farms which can afford the initial large capital investment needed to start raising channel catfish (approximately $5,000 per acre).

METHODS OF CHANNEL CATFISH CULTURE

There are at least 39 species of the family Ictaluridae in North America. The most commercially important species in the United States is the channel catfish, Ictalurus punctatus. The channel catfish is a desirable fish for commercial culture for many reasons. The channel catfish does not reproduce readily in a controlled culture pond environment yet is very easy to spawn. Sufficient numbers of fry are produced for restocking and the fry readily accept artificially prepared feeds at 3 days of age. The channel catfish is very hardy, tolerates crowding, and adapts well to various culture environments. Finally, the most important attribute of the farm-raised channel catfish is its mild sweet flavor, light texture with no “fish” odor.

The vast majority of the cultured channel catfish are raised in 15-20 acre ponds with an average water depth of 4 feet. Soils in pond sites should have a high clay content to prevent excessive seepage. Water is supplied from
CHANNEL CATFISH *ICHTALURUS PUNCTATUS*

wells of 100-150 feet depth. Soil pesticide levels are checked before pond construction. If cotton was grown at any time after 1940 the solid would be analyzed for chlorinated hydrocarbon residues, such as toxaphene and endrin. The maximum suggested concentration in soil is 0.5 ppm. Fortunately, most of the toxaphene is found in the surface soils and can be removed and placed into the outside levees.

Channel catfish production is divided into four production segments: broodstock, fry, fingerling, and food fish. Channel catfish reach 1 - 1 1/4 pounds (market size) in 15 – 18 months, and sexually mature as early as 2 years. However, for reliable spawning broodstock of 3 years or older weighing approximately 3 pounds or more are used. Brood fish are sexed, and stocked into ponds at about 1,000 lb/acre in the spring. The female channel catfish spawns once a year, but the male channel catfish is capable of 2-3 spawnings a year. Spawning begins when water temperatures rise above 70° F. Spawning only occurs in sheltered areas or special “spawning containers”, i.e. 10 gallon milk cans, ammunition cans, or small kegs or drums. Two to three containers are used per four pairs of broodstock since not all fish will spawn at once. Containers are checked every other day for the presence of spawns. Approximately 3,000 eggs per pound of female are produced. The male remains in the container to protect and oxygenate the fertilized eggs. Spawns are removed from the container as soon as they are found and transported to a hatchery.

Commercial hatcheries in Mississippi generally operate for about 10 weeks beginning in late April or early May. Optimal temperature for hatching eggs and rearing fry is between 78° and 82°F. Lower temperatures delay the hatching time and lead to fungal invasion of the egg masses. Temperatures greater than 28°C may lead to malformation of the embryo or invasion of the eggs by bacteria. Water supply is an important consideration in designing a hatchery. Although either surface or ground water sources are used, ground water is preferred and is most commonly used. Ground water quality and temperature are constant, free of turbidity, and is less likely to be contaminated with pesticides.

Egg masses are usually dipped in a 10% betadine solution before being placed in the egg-hatching trough. Egg masses are placed in baskets typically constructed of galvanized or rubber-coated hardware cloth. Paddles are slowly rotated between each egg basket creating a current that gently rocks each egg mass, circulating fresh oxygenated water. Channel catfish eggs hatch after 6 to 8 days depending on the temperature. Egg mass color indicates the stage of development. During normal development they change from yellow to pink to red-brown. Newly hatched fry (called sac fry) fall through the egg basket to the bottom of the trough. Three to four days later the fry will have absorbed their yolk and begin swimming (swim-up fry). Swim-up fry must be fed at 2-4 hour intervals; they are usually fed a commercial fry starter ration containing 50% protein. The fry are fed for about seven days after swim-up and then stocked into nursery ponds.
Fry are stocked into nursery ponds at approximately 75,000-100,000 fry per acre. The receiving pond must be rid of all resident fish and insects that can prey on the fry. The nursery ponds should be fertilized to increase phytoplankton growth which prevents growth of aquatic weeds and promotes the growth of zooplankton which provide a natural food source for the fry.

Catfish fry and fingerlings are fed 2 to 3 times daily. Feed is distributed around the shoreline and fry begin to accept supplemental feeds once the natural food supply becomes limiting. Several weeks may go by before the fry are seen feeding on the supplemental feed. At this stage they are classified as fingerlings. Survival from fry to fingerling of 80% is considered good.

After fingerlings reach 5-8 inches, they are harvested and stocked into food fish growout ponds. Stocking rates are usually 5,000-8,000/acre but can vary between 2,000 and 17,000/acre. Higher stocking rates require a higher level of management skills, sufficient aeration equipment, adequate water supplies, and quick disease recognition and treatment.

Two production strategies are used in raising channel catfish to food size fish; single harvest and continuous production. The single harvest production scheme consists of growing a single cohort of fingerlings and then seining all the fish in the pond. The pond is either drained or not, depending on the production schedule. A new cohort of fingerlings is then restocked for growout. In the continuous production strategy, large fish are selectively harvested with a grading seine and then the pond is restocked with small fingerling to replace those removed. However, this method has its inherent problems. After a few years in production the farmer may be unable to make an accurate estimate of the number of fish of a certain size, the stocking density, or standing crop of fish in that pond. Therefore good records are essential and computer programs are available. However, accurate estimates of harvest size and numbers are difficult and time consuming. Diseases are also easily transferred from different groups of fish in ponds managed under the continuous production strategy.

Fingerlings are fed a 32% protein floating feed during growout to food-sized fish. The feed consists of soybean, corn, wheat, fish meal, vitamins, and minerals. Typically fish are fed at 3% body weight. About 1 pound of fish is produced for every 1.7 to 1.9 pounds of feed consumed. Annual production averages about 5,000 lb of fish/acre.

DISEASES

Annual losses of channel catfish to infectious diseases are estimated to cost the channel catfish industry 10 to 20 million dollars annually. These values do not take into account losses due to morbidity, poor feed conversion, and decreased growth. Fingerling-sized fish account for over 80% of the total number of disease losses.
Many fish pathogens are found normally in channel catfish production ponds without incidence of disease outbreaks. It is not until the fish are stressed that disease outbreaks occur. Stress is caused by poor water quality in the ponds, excessive handling, and other factors.

Serious disease losses can occur due to bacterial, fungal, viral, or parasite infections. However, bacterial infections account for over 50% of all the disease losses in the channel catfish industry.

**BACTERIAL DISEASES**

**Enteric Septicemia of Catfish**

The most devastating disease problem in the catfish industry, Enteric Septicemia of Catfish (ESC), is caused by an obligate bacterial fish pathogen, *Edwardsiella ictaluri*. ESC was first identified as a bacterial pathogen in 1976. First cases were seen in Alabama and Georgia. Subsequently, ESC has been reported in every state where channel catfish are cultured.

ESC accounts for over 25% of fish kills in the catfish industry, and 50% of all deaths due to bacterial infection. Fingerlings and yearling fish are most commonly affected, and mortality rates in a population can run as high as 50%. The disease is seasonal occurring in the spring and fall when the water temperatures are between 72°F (22°C) and 82°F (28°C). When temperatures are outside this range, the incidence of ESC significantly declines.

*Edwardsiella ictaluri* is a gram-negative, cytochrome oxidase-negative bacterium that is weakly motile. Motility is lost above 86°F (30°C). *Edwardsiella ictaluri* is fairly host specific for channel catfish but has been rarely isolated from other species of catfish. It appears to be non-pathogenic in bighead carp, golden shiner, and largemouth bass. However, *E. ictaluri* has been isolated from the green knife fish and Bengal danio.

ESC may occur clinically as an acute or chronic disease. Acute ESC is characterized by a typical bacterial septicemia, with necrosis and inflammation of multiple organs. Chronic ESC is characterized by the classical "hole in the head" or a bump on top of the head at the cranial foramen. Necrosis and inflammation of the internal organs may not be as evident in chronic ESC.

Gross lesions of channel catfish with ESC include petechial and ecchymotic hemorrhages around the mouth, fins, opercular flaps, and ventral surface. Slightly raised to depressed punctate tan to white nodules may also be evident covering the dorsal and lateral trunk. Infected catfish may often have exophthalmia and ascites. An ulcer or white soft swelling may be present on the dorsum of the head. Slight depigmentation with underlying hemorrhage may be evident over the cranial foramen.

Gross lesions of the internal organs are evident in the liver, anterior, and posterior kidney and spleen. These tissues may appear congested, mottled, swollen, and necrotic with multiple hemorrhages. Histological lesions are
characterized by necrosis and inflammation of the various organs. Principal lesions are usually evident in the intestine, olfactory sacs, liver, skin, and brain.\textsuperscript{9} Meningioencephalitis may be evident involving the olfactory bulb, olfactory tracts, and brain. Nephritis, ocular lesions, and myositis may also be observed. The pathogenesis of \textit{E. ictaluri} is not known. \textit{Edwardsiella ictaluri} can survive in water for 8 days and mud for 4 months but it is not known if \textit{E. ictaluri} can reproduce in water or mud. Histological findings suggest the intestinal mucosa and olfactory mucosa may be the sites of entry.\textsuperscript{8,9,10,11,12} Recent evidence suggest a possible carrier state in which \textit{E. ictaluri} may be carried in the gastrointestinal tract of apparently normal channel catfish.

\textit{Edwardsiella ictaluri} can be readily isolated from the kidney, liver and brain by using brain heart infusion or blood agar. Following incubation of 48 hours at 82°F, small white (2 mm diameter) bacterial colonies develop. Diagnosis is made by physical and biochemical characteristics, indirect fluorescent antibody techniques, or ELISA.

\textbf{Columnaris}

\textit{Flexibacter columnaris} accounted for 32\% of the bacterial disease outbreaks seen in cultured channel catfish in Mississippi in 1988.\textsuperscript{17} Columnaris infections can be internal or external and most often occur following stress caused by poor water quality or handling.\textsuperscript{3}

Epizootics of columnaris occur most often in the spring and fall in Mississippi and other southeastern states when water temperatures are between 50°F and 75°F. However, disease outbreaks can occur throughout the year.

\textit{Flexibacter columnaris} is a long, slender, pigmented gram-negative rod that is strictly aerobic. Columnaris most often affects fingerlings, however, larger fish can also be affected. The disease usually is less severe in older fish.

Lesions are most commonly seen on the gills, however, they may also be evident on the head, body, fins, or in the mouth. Gross lesions on the gills are characterized by yellow-brown necrotic tissue at the distal end of the gill filament. Lesions extend to the gill arch as the disease progresses. Initial gross lesions on the skin begin with pale, discolored gray-white plaques which can progress to large necrotic ulcers with extensive loss of the surrounding tissue and musculature. Frequently, the pale discoloration begins at the base of the dorsal fin, covering as much as 20-25\% of the fish, giving the characteristic shape of a saddle hence the frequently used name "Saddle Back." Histologically, lesions are characterized by sloughing of the gill epithelium or the gill filaments, congestion, and multifocal hemorrhages and vast necrosis of the gills and dermis.

A rapid presumptive diagnosis can be made when sufficient numbers of the bacteria are present from a skin or gill scraping. \textit{Flexibacter columnaris}
bacteria move by flexing or gliding and form 'haystacks' or column-like masses (hence the specific epithet, columnaris). Isolation of F. columnaris can be made on a low nutrient agar such as Ordal's or Cytophaga agar incubated between 77°F to 85°F. Colonies are flat, spreading, with rhizoidal edges, and a clear, characteristic yellow pigment.

**Motile Aeromonad Septicemia**

Motile aeromonad septicemia accounted for approximately 16% of the bacterial diseases seen in commercially cultured channel catfish in Mississippi during 1988. The highest incidence of *Aeromonas* infections occurs in the spring and the fall, but disease outbreaks can occur throughout the year. As with columnaris, predisposing stress is usually associated with *Aeromonas*. Environmental factors associated with *Aeromonas* epizootics include high nitrite, high temperature, low dissolved oxygen, high ammonia, and excessive handling and crowding.

In Mississippi during 1988, 82% of the aeromonad infections were caused by *Aeromonas sobria* and 18% as *Aeromonas hydrophila*. There seems to be no apparent difference grossly or clinically in channel catfish infected with either *Aeromonas sobria* or *Aeromonas hydrophila*. Furthermore, both organisms have been isolated from the same fish.

Species of *Aeromonas* are short, gram-negative, fermentative rods, which are motile by a single flagella. They are ubiquitous in most natural waters and are more prevalent in waters with high organic loads. They can routinely be isolated from water, mud, and the digestive tract of channel catfish. Controversy exists in the classification of motile aeromonads due to the existence of phenotypically, genotypically, and antigenically diverse members. *Aeromonas liquefaciens* and *Aeromonas punctata* are now grouped into the species *Aeromonas hydrophila*. *Aeromonas hydrophila* differs from *A. sobria* by hydrolizing esculin and fermenting both salicin and arabinose.

There may be several clinical forms of this disease: skin lesions with internal infections, skin lesions only, and acute mortalities with no lesions. Gross clinical signs of aeromonad infections are characterized by multifocal petechial and ecchymotic hemorrhages over the body. Dermal ulceration with hemorrhage and inflammation may be evident. Erosion of the underlying musculature may be extensive. Exophthalmia, ascites, and frayed fins are also common characteristics seen with aeromonas infections. Petechial hemorrhages and congestion may be observed. The liver and kidney may be pale, swollen, and friable. Histologically, there is extreme tissue destruction and necrosis.

There is evidence of a carrier state in channel catfish and that the virulent aeromonads are carried in the intestinal track and shed when fish are stressed. Experimentally, the route of infection has been demonstrated to be via the digestive track or uninjured skin under crowded conditions at high temperatures.
Isolation of *Aeromonas* from liver or posterior kidney can be made on TSA, blood agar, or any general-purpose bacterial culture medium. Incubation temperatures are best at 77°F to 85°F for 24 to 48 hours. Biochemical characteristics are used to confirm the diagnosis.

**ANTIBIOTIC TREATMENTS**

Presently there are only two antibiotics registered by the Food and Drug Administration for use in channel catfish reared for food; Romet-30® and Terramycin®. Both are registered for use against systemic bacterial infections, i.e., *Edwardsiella ictaluri* and *Aeromonas* spp. and both antibiotics are bacteriostatic.

Romet-30® (RO-5) is a 1 to 5 mixture of ormetoprim and sulfadimethoxine. The premix is incorporated into feed which is fed to deliver a dosage rate of 50 mg of active ingredient per kilogram of fish per day for 5 days. There is a 3-day withdrawal period following treatment.

Terramycin® (oxytetracycline) is added to feed and fed to deliver 55 to 82 mg of active ingredient per kilogram of fish per day for 10 days. There is a 21-day withdrawal period following treatment. Terramycin® is also effective against internal columnaris. Terramycin® can only be fed in a sinking feed because the heat needed during extrusion of floating pellets destroys Terramycin®.

**VIRAL DISEASES**

There are only two known viral diseases of channel catfish: channel catfish virus (first discovered in 1968) and catfish reovirus (first described in 1984). The pathogenicity of catfish reovirus is low and it does not cause high mortalities. Very little is known about the pathogenesis or epidemiology of this disease, and catfish reovirus has not been reported outside of California.

*Channel catfish virus disease*

Channel Catfish Virus Disease (CCVD) is a highly virulent herpesvirus that can cause up to 100% mortality in channel catfish fry and young fingerlings. CCVD occurs during the spring and summer when water temperatures are above 75°F. Acute epizootics are often associated with stress, such as handling or poor water quality.

Gross clinical signs are characterized by a distended abdomen, exophthalmia, and petechial hemorrhages on the body and base of the fins. The abdominal cavity is filled with a clear to yellowish fluid, the liver is usually pale and the spleen enlarged and congested. However, all or none of these clinical signs may be present. Secondary bacterial infections are common. Histologically the kidney, spleen, and liver are edematous and necrotic. Hepatocytes develop intracytoplasmic inclusion bodies.

Channel catfish virus is very host specific and other species of catfish
CHANNEL CATFISH Ictalurus punctatus

seem to be refractory to natural infections. Different strains of channel catfish show differential susceptibility.

Isolation of channel catfish virus from the liver, spleen or kidney is performed using a channel catfish ovary (CCO) cell line. However, brown bullhead (BB) cells can also be used. The characteristic cytopathic effect (CPE) of syncytial formation occurs within 12–24 hours if cells are incubated at 77°F.

In theory, CCVD can be managed by lowering the water temperature, but catfish farmers have little control over hatchery and pond water temperatures. At present, management of the disease relies upon hatchery sanitation and quarantine and disposal of infected fry.

PARASITES

Protozoan parasites account for almost all of the parasitic disease losses seen in channel catfish culture. Although some protozoans that affect channel catfish are obligate parasites, most are facultative and only under stressful conditions do they pose health problems to the fish.

Ordinarily most protozoan parasites cause no significant damage when present in small numbers. However, in large numbers they may decrease oxygen transfer at the gill surface. Some may feed on the epithelial cells and mucus and a few parasites actually attach or burrow under the epithelial cells (e.g., Ichthyophthirius multifilis) severely damaging the gills.

Bacterial disease outbreaks often are diagnosed with concurrent infestations of large numbers of protozoan parasites. The bacterial disease probably weakens the fish making it more susceptible to infestation by the protozoans. However, at times the parasites may be the primary agent in causing fish mortality.

Treatment of parasitic infestations usually is warranted only if there are large numbers of parasites. Treating fish for parasitic infestations that are concurrently infected with a bacterial or viral pathogen can stress fish and increase mortality. Therefore, a thorough diagnosis is imperative before treating for external parasites. The exception to this rule is Ichthyophthirius multifilis. This parasite must be treated immediately if found on fish.

Proliferative Gill Disease (PGD)

“Proliferative gill disease” (PGD) was first described in commercially cultured catfish in 1984. PGD can occur throughout the year but is most prevalent in spring and fall when the water temperatures are between 60°F to 70°F. The disease occurs most often in new ponds or ponds that have been drained, dried, and refilled.

Almost 9% of the 2,529 fish disease cases reported in Mississippi in 1988 were diagnosed as PGD. The total incidence is probably higher since subclinical infections are difficult to diagnose unless tissues are examined histologically. Additionally, some farmers may not submit fish to diagnostic
JOHNSON

labs from PGD outbreaks since there is no cure.

The proposed etiologic agent of PGD is a protozoan parasite. An exact classification of the parasite has not been made, although the parasite is presumed to be of the genus *Sphaerospora*.\(^\text{18}\)

The life cycle has not been determined; but it appears the gills are the initial route of infection. After reproducing in the gills, the organisms spread to other organs including kidney, liver, heart, brain, spleen, and intestines. The most common gross clinical sign is the swollen, friable gills. Gill lesions reduce respiratory efficiency and fish may be at the surface gasping for air, even when dissolved oxygen concentrations are high. The fish may be weak and observed resting in large numbers at the water bank. Mortalities may be very acute and occur within 2 days of exposure. Histologically there is a moderate to severe branchial hyperplasia and branchitis. Chondroplasia and extensive hemorrhage may also be prevalent in the gills. PGD parasites may or may not be abundant.

A presumptive diagnosis can be made by microscopic examination of gill filaments demonstrating filament cartilage hyperplasia, chondroplasia, and necrosis. However, PGD can only be confirmed by identification of the causative parasite after histologic examination. There is no treatment; however, good water quality and high dissolved oxygen levels must be maintained to keep mortalities to a minimum. Treatment with chemicals commonly used for external protozoan parasites may cause a significant increase in mortality.

*Ichthyophthirius multifilis* (*Ich*)

Ich is the most pathogenic external parasite of cultured channel catfish. Ich is a ciliated protozoan characterized by a large C-shaped macronucleus. Infestations of this organism are often grossly visible as characteristic small, raised white spots on the skin and gills. Depending on the temperature and health of the fish, mortalities may be as high as 100%.\(^\text{3}\)

Ich has a biphasic life cycle and is susceptible to treatment only during two life stages within this cycle. The free-swimming infective tomite burrows under the epidermis of the skin or gills. Within days the parasite develops a mouth and is called a trophozoite. The trophozoite then feeds on the body fluids and epidermal cells. The mature trophozoite then leaves the fish and swims until contacting substrate suitable for cyst formation. Cell division begins as early as one hour after encystment. Up to 1,000-2,000 theronts are formed within the cyst. When cell divisions complete, the cyst ruptures and the theronts are then called tomites which actively search for a fish host. Tomites have 48 hours to find a host or the tomites will die. The life cycle can be completed in less than four days if the ambient water temperatures are between 70°F to 80°F. Treatments are only effective in the two free-swimming stages and not when the parasite is on the fish or encysted.
Epizootics occur most often in the spring (April) when water temperatures are between 70°F to 80°F. However, epizootics have occurred in the winter when water temperatures were as low as 40°F. The infective stages of Ich do not survive above 83°F, therefore, epizootics of this disease are rarely seen in the summer.

Three chemical treatments are available to treat Ich: copper sulfate, potassium permanganate, and formalin. Ponds are usually treated every other day until mortalities stop. During colder temperatures, treatment intervals are extended due to the protracted life cycle which may take 35-40 days to complete at 45°F.

**Trichodina**

*Trichodina* is the most common parasite of channel catfish and is often considered an ectocommensal causing minimal damage. However, in large numbers this parasite can cause extensive mortalities.

Many species of Trichodinids can infect a fish at the same time. The genus are easily recognized by their denticular ring. There is a seasonal incidence of heavy infestations of *Trichodina* in the spring and fall. The pathogenesis of *Trichodina* infestations is unknown, but heavy infestations of the gills can cause increased mucus production. Increased mucus production and large numbers of parasites residing on the gill surface probably reduces oxygen transfer at the gill surface, thus debilitating the fish. Treatment of *Trichodina* with copper sulfate, potassium permanganate, or formalin is usually effective.

**Ichthyobodo (Costia)**

*Ichthyobodo* is a small flagellated protozoan, about the size of a red blood cell, and looks like a tear drop when attached to the gill or skin of the fish. *Ichthyobodo* can occur throughout the year, but is most common during colder weather. *Ichthyobodo* is an obligate parasite that is often found on the gills or skin of apparently healthy fish. Only when this parasite occurs in large numbers does it seem to cause problems. Stress is a major predisposing factor for the occurrence of epizootics. Increased mucus production on the skin of fish infected with *Ichthyobodo* give the fish a characteristic white-gray to bluish coloration.

Evidence suggests that this parasite penetrates and feeds upon the live cells of the host. Histologically, there is hyperplasia and necrosis of the gill epithelial cells. Edema or spongiosis may be evident in the epidermis of skin infestations.

Diagnosis is best made from gill or skin scrapings while the protozoan is still alive. Treatment with copper sulfate, formalin, or potassium permanganate is usually effective.

**Trichophrya**

*Trichophrya* is a suctorian protozoan with a broad, round body covered
with tentacles. A few of these parasites are commonly found on the gills of channel catfish with no apparent ill effects. However, in moderate numbers, *Trichophrya* may cause severe swelling and ulceration of the gill tissue. The resulting blood loss may cause anemia. Diagnosis is made by microscopic examination of gill scrapings. Copper sulfate is the only effective treatment of *Trichophrya*.

**DISEASES OF UNKNOWN ETIOLOGY**

**Winter Kill**

Winter kill, winter fungus, or winter mortality describes a generalized syndrome affecting pond-cultured channel catfish. Winter kill was responsible for approximately 11% of the total disease cases diagnosed in Mississippi in 1988. Prevalence of winter kill is probably much higher because many farmers do not report their losses because there is no cure. Mortalities are chronic and highly variable.

Winter kill occurs in the late fall to early spring when water temperatures are below 60°F. The causes of winter kill are unknown; however, the underlying factor appears to be stress. Previous disease outbreaks during the summer and fall seem to predispose fish to winter kill in the late fall or winter. Fish may appear to have recovered from previous disease outbreaks, but subsequently develop winter kill. Poor water quality may also stress the fish and predispose them to winter kill.

Gross characteristics of fish with winter kill can include dry depigmented areas of skin, endophthalmia, and areas of external mycosis on the skin and gills. All or none of these clinical signs may be evident. Occasionally, bacteria can be isolated from the posterior kidney and liver, and a variety of external parasites may be present.

There is no effective treatment. Treatment with potassium permanganate to remove external fungal infections is usually ineffective at cold temperatures. Treatment with potassium permanganate may temporarily remove the fungus, but at cold water temperatures the immune system of channel catfish is suppressed, healing is slow, and the fungal infections often reoccur. Effective fish disease treatments during warmer months and proper water quality management may reduce subsequent losses to winter kill. Fish should be fed throughout the winter to insure optimum nutritional status.

**NON-INFECTIOUS DISEASES**

**Hypoxia**

Water quality management is the key to successful culture of channel catfish. The most important component of good water quality is dissolved oxygen concentrations (DO). When dissolved oxygen concentrations in the water fail to meet the oxygen requirements of the fish, a state of hypoxia
CHANNEL CATFISH *ICHTALURUS PUNCTATUS*

occurs. There is no absolute value for the minimum requirement of dissolved oxygen for successful catfish culture because the dissolved oxygen requirements of catfish are affected by many variables. Some of the factors influencing the dissolved oxygen requirements by the fish include: feeding status, weight and age of fish, water temperature, length of exposure, water chemistry, and health of the fish. Hypoxia should be considered whenever dissolved oxygen concentrations fall below 4 mg/l. Dissolved oxygen concentrations below 4 mg/l adversely affect weight gain, feed consumption, feed conversion, reproductive performance, and disease resistance. Dissolved oxygen concentrations below 1 mg/l are lethal if exposure lasts more than a few hours. Wide fluctuations of dissolved oxygen occur daily in channel catfish ponds. Values may exceed 15 mg/l in the afternoon and fall below 3 mg/l by dawn. These daily changes in dissolved oxygen are common and are a result of the dense algae (phyto-plankton) populations found in commercial catfish ponds.

Concentrations of dissolved oxygen at dawn are usually highest in the spring. They decline as the growing season progresses. The lower feeding rates and fish standing crop and lower water temperatures (oxygen solubility is greater at cooler temperature) account for the higher oxygen concentrations in the spring. During the summer the dissolved oxygen levels are at their lowest at dawn due to the higher water temperature, higher feeding rates, and higher fish standing crop. All these factors combine to make the period of mid-July through September the most critical time for oxygen depletion.

Management of low dissolved oxygen is very critical during the summer and early fall. Monitoring dissolved oxygen levels several times daily and throughout the night is a routine practice in all ponds.

The use of emergency aeration to raise dissolved oxygen concentrations is essential in preventing fish kills. The use of emergency aeration is common during the summer and early fall in heavily stocked culture ponds. Commonly used aeration equipment to combat low dissolved oxygen levels are pump-sprayers, large paddlewheels, and diffused air systems.

**Ammonia**

Channel catfish excrete ammonia and not urea or uric acid, as the principal nitrogenous waste product following protein catabolism. The un-ionized ammonia (NH₃) passively diffuses across the gills. This occurs rapidly and is energy efficient. Another advantage of ammonia excretion is the osmoregulatory benefit. Ionized ammonia NH⁺, is actively transported across the gill in exchange with sodium. The disadvantage of excreting ammonia is that un-ionized ammonia is very toxic to channel catfish. High levels of un-ionized ammonia in the environment make passive diffusion of ammonia across the gills difficult leading to ammonia toxicity.

Increasing pH and temperature greatly affect the toxicity of ammonia by
increasing the fraction of un-ionized ammonia in the ponds. However, pH and temperatures change diurnally in catfish ponds. Thus, the concentrations of un-ionized ammonia fluctuate making a diagnosis of ammonia toxicity difficult.

The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills. The actual mechanism of ammonia toxicity is unknown, however, many mechanisms have been studied. High ammonia levels do decrease growth rates, reduce feeding activity, increase water uptake, and serum corticosteroid levels, increase dissolved oxygen requirements and possibly cause degenerative changes in the gills.

Treatment of high ammonia levels is not feasible in large channel catfish culture ponds. Exchanging the water has little effect due to the dynamic biological processes in the pond. The best approach is the use of reasonable feeding rates and not to feed over 100 pounds of feed per acre per day.

Methemoglobinemia

Methemoglobinemia also called brown blood disease occurs in channel catfish when concentrations of nitrite (NO₂⁻) are high in the culture ponds. Nitrite occurs in ponds through the processes of nitrification and denitrification which occur following ammonia excretion by fish. Nitrite concentrations in the pond may vary from 0 to 4 mg N/l or more. The highest concentrations of nitrites occur in the cooler months due to a decrease of ammonia assimilation by phytoplankton populations and the differential effect low temperatures have upon bacteria involved in nitrification.

In channel catfish the nitrite ion (NO₂⁻) is actively transported into the circulatory system by lamellar chloride cells in the gills. Channel catfish concentrate nitrite in the blood so concentrations found in the plasma are greater than concentrations found in the surrounding water. Once in the blood, nitrite causes the oxidation of the heme iron in hemoglobin. This results in methemoglobin formation, and methemoglobin is not capable of combining with oxygen. This creates a functional anemia, in which there is no loss of red blood cells, but a loss in the ability of the hemoglobin in the red blood cell to bind with oxygen.

Clinically the blood turns a characteristic dark brown color, thus the gills appear chocolate-brown. As with proliferative gill disease the fish suffer from hypoxia even when dissolved oxygen concentrations are high. Fish may be listless, on the pond bank or gasping for oxygen.

The methemoglobin concentration may vary from 5 to 89% in channel catfish. Slight browning of the blood and gills is evident when methemoglobin levels range from 25 to 30%. Sublethal levels of nitrite have been shown to increase the susceptibility of channel catfish to Flexibacter columnaris and Aeromonas infections.

This is one of the few diseases that is preventable and easily treated once it occurs. Chloride and nitrite compete for the same uptake and methemoglobin formation is influenced by the ratio of nitrite to chloride in the
CHANNEL CATFISH  
**ICTALURUS PUNCTATUS**

external water environment. A molar ratio of 1:10 nitrite to chloride will keep channel catfish hemoglobin levels near normal. So channel catfish farmers are advised to keep the chloride concentration in their ponds above 20 to 25 ppm chloride. The use of sodium chloride is an economical simple management plan to prevent methemoglobinemia.

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REPORT OF THE COMMITTEE ON AQUACULTURE

Chairman: *Dr. Harvey L. Rubin, Kissimmee, FL  
Vice Chairman: **Dr. Mark P. Dulin, Hyattsville, MD

J. A. Brock, HI; R. C. Goetz, AR; T. D. Goodrich, WA; E. E. Grass, MD; W. E. Ketter, MD; D. D. King, MD; A. Langston, MD; A. D. Liggett, GA; J. B. Malone, AR; H. F. McCrory, MS; R. B. Miller, MD; V. F. Nettles, GA; C. Palmer, CA; A. R. Smith, IL; J. S. Walker, DC; S. J. Wechsler, WY.

The USAHA Committee on Aquaculture met on November 2, 1989 with 52 members and guests in attendance.

The Chairman provided the committee with some statistics compiled by the USDA, Economic Research Service on US beef, pork, lamb and poultry production and average per capita consumption of red meat and poultry over the past 20 years. This data showed relatively slow growth or declines in beef, pork and lamb production but steady increases in both production and consumption of poultry and aquatic species.

Data was also presented comparing US landings of wild caught species vs production by aquaculture industries. This information showed that catching wild caught species has remained relatively constant while US production has increased steadily over the past 10 years. The committee discussed the seafood trade imbalance between US exports vs imports. This imbalance has resulted in trade deficits of over 4 billion dollars per year for the last several years which is the second largest entity responsible for US dollar drain in the natural resource category (oil is number 1).

The agenda for this year’s committee focused on the opportunities and constraints in US aquaculture production.

Dr. Althaea Langston, USDA, APHIS presented an overview of APHIS responsibilities, current involvement in aquacultural activities, and areas where more involvement is needed to fill voids in the federal sector. The committee felt very strongly that APHIS and USDI, Fish and Wildlife Service (FWS) should cooperatively establish control mechanisms to prevent the introduction of foreign aquatic animal pathogens through quarantines, testing and/or certification and that these controls should not only apply to live animals and germplasm but also to all products and substances derived from aquatic species. The committee expressed disappointment that APHIS has not obtained the necessary authorities/funding to get more involved in aquatic animal health issues and emphasized the urgent need for restrictions on the importation and use of fish pathogens by the research community. While the committee did not want to stifle research efforts by US aquatic animal scientists, committee members agreed that

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*Dr. Mark P. Dulin was Acting Chairman on Dr. Rubin’s behalf.  
**Dr. Tom Goodrich, Bellevue, WA was Acting Vice-Chairman.
requirements must be established to minimize the risks associated with the importation and distribution and use of pathogens which could cause severe harm if they escaped from laboratories.

Dr. Robert Miller, USDA, APHIS provided an update on fish biologics currently licensed by USDA. Although the trout and salmon industries are better equipped to prevent diseases with the use of effective immunizing agents, Dr. Miller anticipated the emergence of several new vaccines to combat diseases affecting catfish.

Dr. Donald Gable, FDA. Center for Veterinary Medicine listed the 5 therapeutics approved for use on food fish and discussed the problems/expenses for manufacturers to obtain FDA approval for therapeutics used to treat diseases of aquatic species. Dr. Gable provided an update on research currently being conducted in an attempt to get more therapeutics approved for use in fish and shrimp production. The committee was informed of an upcoming FDA-sponsored workshop on aquaculture to be held in Rockville, Maryland on March 12—13, 1990.

Dr. John Nickum, USDI, FWS discussed the FWS role in protecting native fish populations from species which could harm or destroy native populations should they become established in US waters. The FWS also expressed a commitment to remain strong in the arena of aquatic animal health by providing expertise and diagnostic assistance to the federal, state, and private sectors. The FWS currently has 9 fish health centers strategically located throughout the United States. Dr. Nickum summarized the FWS “notice of intent to revise” Section 16.13 of Title 50 Code of Federal Regulations. The committee discussed each of the 12 specific topics outlined in the FWS “notice of intent to revise” and decided to submit a letter to FWS from the USAHA aquaculture committee expressing support for 9 of the 12 items outlined in the August 17, 1989 proposed rule. The public comment from the aquaculture committee will include specific recommendations for modifications on the 3 items which were not supported by the committee.

Dr. Joe Blair, USDA, FSIS summarized the 6 bills currently being considered by Congress proposing mandatory federal inspection of food fish, invertebrates and shellfish. No decision has been made whether Congress will approve funding for inspection of aquatic animal species but it is clear that consumers are pressing for a federal inspection program to ensure the same sanitation and wholesomeness standards which they receive for red meat and poultry. If approved, it is not clear whether responsibilities for carrying out the program would go to USDA, to FDA, or to a combination of USDA, FDA and the Commerce Department. It is also not clear whether the inspection program would be on a fee-basis or whether the program would be supported by tax dollars. The Mississippi Board of Animal Health submitted a written comment to the Committee stating that the catfish industry is under voluntary inspection by the Meat Inspection Division of the Mississippi Department of Agriculture. They
stated that a need exists for public information on the good quality of sanitation carried out at catfish processing plants and that there are minimal public health concerns from fish. The Mississippi Board of Animal Health does not believe continuous inspection is needed for every fish processed, but if consumers demand inspection of operations and facilities then Mississippi has no objections. If fee-basis inspection were required, the consumer would have to pay increased prices at the retail level.

Dr. Jerry Walker, USDA, Office of International Cooperation and Development (OICD) gave an overview of some of the more important aquatic animal health research currently going on in foreign countries. Dr. Walker pointed out that the United States is lagging behind many countries in both production technology and disease control. The OICD recognizes the need for research in aquaculture and will continue to support foreign research that is likely to obtain results which could benefit US producers.

Dr. Mike Johnson, Mississippi State University, Delta Branch Experiment Station provided an update on the problems threatening US catfish production. The primary problem to catfish producers is "off-flavors" in the flesh. Blue-green algae are responsible for most of the problems in Mississippi ponds but other substances (such as decaying fish) may result in the absorption of undesirable compounds into the musculature system thereby producing an unmarketable product. Mississippi produces more catfish than any other State and currently 88% of the ponds are "off-flavor." Producers face tremendous losses by holding fish until they come "on-flavor", particularly if the fish grow beyond 3 lbs. at which time the processing plants pay a reduced rate for the less desirable, larger fish.

Predatory birds such as cormorants, heron and egrets are the second biggest cause of economic loss to Mississippi catfish producers. It is estimated that Mississippi farmers lose between 5 to 10 million dollars a year from these predatory birds and the problem appears to get worse every year. Producers appreciate the assistance from APHIS, Animal Damage Control but are disappointed that the FWS permits do not allow killing adequate numbers of cormorants to get the upper hand on their battle against predators.

Fish diseases also cause devastating losses. Enteric septicemia is currently the most significant infectious disease affecting catfish production in Mississippi.

Dr. Jim Brock, Hawaii Department of Agriculture was unable to make it to the meeting but provided the Committee with a written report on the impact of IHHN virus on marine shrimp production. Aside from severe death losses, IHHN causes a runting deformity syndrome resulting in production of less than optimum sized shrimp. Hawaii has been able to market the smaller shrimp (under 15 grams) but top prices are paid for the 20 gram size. Currently, only 3 facilities in the United States are testing negative for IHHN. Unfortunately, the virus has also been widely distrib-
uted to the major marine shrimp producing countries throughout the world. The US Shrimp Consortium is working to reestablish IHNN-free populations of marine shrimp in Hawaii, and research is underway to improve the diagnostic test for detecting the virus.

The four resolutions passed by the committee are summarized below:

1. Request for USDA, APHIS and USDI, FWS to cooperatively provide control of the importation and dissemination of live aquatic animals, germplasm, and all products and substances derived from aquatic species which could represent a disease-risk to US species.

2. Request for USDA, USDI and other appropriate federal agencies to develop, define and outline (in cooperation with State Departments of Agriculture, State Natural Resources Agencies etc.) a program to promote and enhance the provision of services to and the development of aquaculture in the United States.

3. Request for USDA and USDI to officially recognize aquaculture as a US commercial agricultural industry. This recommendation included the controlled, commercial aquacultural production of fish, amphibians, reptiles, invertebrates and products of these animals. This official recognition is needed to obtain services such as disease control, export certification and provisions for inspection, grading and marketing.

4. Recommendation for expansion of veterinary college training programs in aquaculture for both students and in continuing education programs for graduate veterinarians and aquatic animal health professionals.

This concludes the report of the USAHA aquaculture committee.
IMPLEMENTATION OF THE AMENDMENTS TO THE
VIRUS-SERUM-TOXIN ACT

David A. Espeseth, D.V.M., M.S., Deputy Director
Peter L. Joseph, D.V.M., M.S., Senior Staff Veterinarian
Biotechnology, Biologics, and Environmental Protection

INTRODUCTION

Veterinary biological products play a vital role in the protection of the Nation's multimillion dollar livestock and poultry industry. The United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) has the responsibility for assuring livestock owners, veterinarians, and the American public that there is an ample supply of pure, safe, potent, and effective biological products for this purpose.

For over 70 years, USDA has successfully regulated these products under the statutory authority provided by the Virus-Serum-Toxin (VST) Act of 1913. In the early 1980's, however, it became evident that this legislation was no longer adequate to provide the controls needed to ensure that all biological products offered for use in animals in the United States met the necessary basic standards. The VST Act limited the jurisdiction of USDA to imported products and those distributed interstate. Products produced for intrastate distribution or for export were not included. This limitation in the VST Act was confirmed by court cases in 1915 and more recently in March 1980.

BACKGROUND

A USDA survey conducted in 1984 revealed that only 15 States required that animal biological products used within their boundaries be licensed by USDA. Only two States actively regulated the intrastate production and sale of animal biologics. It must be assumed, therefore, that “intrastate” animal biological products were almost entirely free of any official control in the remaining States. Products purportedly prepared for shipment only in intrastate commerce were often found in interstate commerce. Unlicensed producers of animal biological products were subject to regulation under the Food, Drug and Cosmetic Act (21s U.S.C. 301 ET SEQ.) administered by the Food and Drug Administration (FDA), Department of Health and Human Services. However, FDA had not developed a regulatory program for this purpose.

The Nation needed uniform standards to ensure the purity, safety, potency, and efficacy of all animal biological products shipped in or from the United States. The major national livestock producer groups, the American Veterinary Medical Association, and other interested parties, therefore, supported the passage of new legislation. The VST Act was amended on December 23, 1985, by passage of the Food Security Act (P. L. 99-198) in order to accomplish the following:
1. Ensure uniform standards for veterinary biological products,
2. Eliminate the artificial limitation on USDA jurisdiction, and
3. Provide effective enforcement authority for USDA.

PROVISIONS OF THE AMENDMENT

The Food Security Act of 1985 expanded the authority of the VST Act to
give USDA the responsibility for the regulation of products shipped
intrastate and products to be exported. Thus, uniform regulatory require-
ments were established for all animal biological products shipped in or from
the United States.

The amendment provided for the issuance of special licenses under an
expedited procedure. A special license may be issued to meet an emergency
condition, a limited market or local situation, or other special circumstance
and may be issued based upon assurance of purity, safety, and only a
reasonable expectation of efficacy.

Additional authority for detention, seizure, and condemnation was
added along with the authority to obtain injunctions.

Except as specifically exempted by statute, the amendment requires that
all veterinary biological products shipped in or from the United States be
produced in an establishment licensed by the Secretary of Agriculture and
be distributed in accordance with regulations promulgated by the Secretary.
The applicable regulations appear in Title 9, CFR 101-118. Products
specifically exempted from the requirement of being produced under
license are:

1. Products prepared by a person, firm, or corporation solely for admini-
   stration to animals of such person, firm, or corporation;
2. Products prepared by a person, firm, or corporation solely for admini-
   stration to animals under a veterinarian-client-patient relationship in the
   course of the State licensed professional practice of veterinary medicine by
   such person, firm, or corporation; and
3. Products prepared solely for distribution in the State of production,
   pursuant to a license granted by such State under a program approved by
   USDA.

The amended Act provided for a transition period of 4 years to allow
producers of unlicensed veterinary biological products an opportunity to
obtain USDA licenses for their products. Manufacturers of unlicensed
products that were being produced for intrastate distribution or export
during the 12-month period ending December 22, 1985, could be exempted
from licensing requirements until January 1, 1990. The deadline for
manufacturers to apply for exemptions was January 1, 1987.

Following passage of the Amendment, APHIS realized the need to inform
interested persons and the general public of the changes to the VST Act.
APHIS instituted the actions summarized below.
AMENDMENTS TO THE VIRUS -SERUM-TOXIN ACT

A Press Release was issued in January, 1986, announcing that the VST Act was amended by the passage of the Food Security Act of December 23, 1985. This was followed by publication of several Notices and rulemakings in the Federal Register including:

a. Notice of March 20, 1986, giving the new licensing requirements and informing interested persons that requests for exemptions must be made prior to January 1, 1987;

b. Interim Rule published October 22, 1986, listing the procedures for claiming the exemptions;

c. Final Rule published April 7, 1987, explaining the provisions of the Act related to autogenous products and the provisions for issuing conditional licenses;

d. Final Rule of August 13, 1987, related to experimental products and exempted products;

e. Final Rule published September 14, 1987, adding new authorities to the regulations regarding detention, seizure, and condemnation;

f. Notice of May 15, 1989, outlining procedures for requesting extensions; and,

g. Notice published May 15, 1989, announcing a public meeting to discuss regulations and policy related to the amended Act.

EXEMPTIONS GRANTED

In accordance with the amended Act and accompanying regulations,APHIS has granted exemptions to 57 unlicensed manufacturers. The exemptions cover 2,199 unlicensed products being produced solely for distribution within the State where manufactured. Since the exemptions were granted, 15 of these manufacturers have received USDA Veterinary Biologics Establishment Licenses and have received Veterinary Biological Product Licenses for 76 previously exempted products. Of the 57 manufacturers who received exemptions, 8 own and operate a central production facility in one State and also own and operate satellite facilities in other States. Exemptions were granted on an individual basis to the central facility and to the satellite facilities to manufacture products solely for intrastate distribution. All of the exemptions granted by APHIS will expire on December 31, 1989.

EXTENSION OF EXEMPTIONS

The amended Act also provides that the exemption may be extended by the Secretary of Agriculture for a period up to 12 months (that is, until January 1, 1991) in an individual case on a showing by a person, firm, or corporation of good cause and a good faith effort to comply with the Act with due diligence.

Neither the statute nor the legislative history provides guidelines as to how the Agency should determine whether a manufacturer has shown
"good cause and good faith effort to comply with due diligence." APHIS has developed criteria for making this determination to reflect the intent of Congress.

In order to inform the public of the criteria used, the Agency published two Notices in the Federal Register on May 15, 1989. One Notice announced that the Agency was holding a public meeting in Ames, Iowa, on July 6–7, 1989, to discuss current regulatory and policy issues related to veterinary biological products with special emphasis on the policy for granting extensions. The second Notice described procedures to assist persons, firms, or corporations who wish to claim extension of the exemption.

POLICY FOR GRANTING EXTENSION OF EXEMPTIONS

In accordance with the criteria developed by the Agency, a manufacturer may be considered eligible for an extension of an exemption if:

1. The manufacturer obtains an Establishment License and at least one product license prior to December 31, 1989.

2. The manufacturer files a product license application, Outline of Production, research protocol, and supporting data for each product for which an extension is requested.

3. The protocol and data demonstrate that all requirements for licensure can be completed prior to December 31, 1990.

In developing the policy for implementing the amendment, the Agency has had to determine the disposition of products manufactured during the 4-year exemption period, but which remain in inventory as of January 1, 1990. Specifically, APHIS has had to address the disposition of exempted products that would not qualify for an extension. It has been determined that a biological product manufactured under the provisions of the exemption shall be treated the same as a product manufactured under license insofar as distribution is concerned. That is, products manufactured under license may continue in distribution channels throughout dating even though the license has been voluntarily terminated. Products prepared under license and products produced under the exemption were legally produced and therefore are eligible for distribution throughout dating. In the case of veterinary biologicals produced under the exemption, the Agency has determined that the end of dating for such products shall not be later than December 31, 1990.

EXTENSIONS GRANTED

The Notice published in the Federal Register on May 15, 1989, states that persons wishing to request an extension must do so by October 1, 1989. This will allow the Agency time to review each request and determine whether an extension could be granted before the exemption expires at midnight, December 31, 1989. The Agency has received requests for extensions from 13 manufacturers for 179 products. Seven of these manufacturers already have obtained Establishment Licenses; and, for another six manufactur-
AMENDMENTS TO THE VIRUS -SERUM-TOXIN ACT

ers, licensure is pending. Many of the combination products for which extensions were requested contain individual components which have already been licensed. In accordance with the amended Act, requests for extensions will be reviewed on an individual basis.

IMPACT OF THE AMENDMENT

As was stated earlier, APHIS granted exemptions for the manufacture and intrastate distribution of 2,199 products. For some of these products, it would not appear to be financially feasible for the producer to meet all the requirements for a regular license. Some of the products are used to treat local disease conditions in a specific geographic location. For some such products, an autogenous biologic license may be a means of making some products available provided the producer can satisfy the requirements for an Establishment License. The amendment provides that an Establishment License may be issued based on a Product License for an autogenous biologic. In order to meet an emergency condition or provide for a limited market or local situation, the amendment provides for the issuance of a conditional license upon demonstration of purity, safety, and reasonable expectation of efficacy. It appears, therefore, that Congress did not intend for the removal of all such products from the market provided certain basic requirements can be met.

CONCLUSIONS

Since the turn of the century and probably earlier, establishing proper standards for veterinary biological products used in the treatment of diseases of animals has been of concern. The dialogue recorded in the legislative history of the USDA Appropriations Bill for fiscal year 1907 indicates that Congress was concerned about the value of veterinary biologicals being used at that time for treating food-producing animals. Questions were raised concerning the value of such products in general and of hog cholera products in particular. As a result Congress later passed the Virus-Serum-Toxin Act of 1913.

In amending the Act in 1985, Congress recognized that all veterinary biological products are in intrastate as well as interstate commerce, and therefore should be regulated under uniform standards. During the 4-year period of exemption, several options are available to unlicensed manufacturers to obtain licenses, such as an autogenous product license, a conditional license for further manufacture, or a regular license.

It was APHIS's responsibility to develop the policy for implementing the exemption and extension provisions of the amendment. The Agency has tried to be as fair as possible to both licensed and unlicensed manufacturers, and to offer ready opportunities to assist unlicensed manufacturers in making the transition to Federal licensing. The intent of APHIS is to implement this transition in a manner consistent with the wishes of Congress. During this transition, however, we intend to use all resources available to ensure compliance with the Act.
REPORT OF THE COMMITTEE ON BIOLOGICS

Chairman: Dr. R. W. Loan, College Station, TX
Vice Chairman: Mr. J. N. Huff, Denver, CO

J. B. Addison, MO; D.C. Alexander, CAN; G. A. Anderson, KS; W. H. Beckenhauer, NE; R. W. Behan, IA; G. M. Buening, MO; M. L. Chapek, NE; G. S. Colgrove, NE; M. L. Crandell, VA; S. R. Ellsworth, NE; D. A. Espeseth, MD; W. H. Fales, MO; J. E. Finnell, IL; R. H. Fulker, IA; E. P. J. Gibbs, FL; J. S. Gloyd, IL; T. D. Goodrich, WA; J. A. Gourlay, CA; F. Gvillo, CA; B. B. Hancock, IA; D. D. Hancock, WA; S. K. Harris, IA; M. Huff, CO; W. L. Kadel, KY; E. Keahey, TX; L. H. Lauerman, Jr., AL; L. Leach, VA, H. M. Lefler, CA; R. L. Levings, IA; D. F. Long, MD; S. McConnell, TX; H. A. McDaniel, MD; R. Mead, WA; T. R. Mickle, GA; L. F. Moore, KS; J. B. Payne, TX; C. W. Purdy, TX; D. C. Randall, Jr., IA; J. A. Schmitz, NE; R. Schultz, IA; R. R. Simonson, MN; R. C. Stewart, KS; J. R. Taylor, GA; R. F. Taylor, NJ; O. H. Timm, CA; J. D. Todd, KS; P. R. Turner, TX; H. E. Vanderslice, DE; M. A. Vanier, DC; E. E. Wedman, OR; D. L. Weiss, IA; G. B. E. West, CA; G. Wilder, MO; J. M. Williams, MO; R. D. Williams, IN; W. H. Wohler, TX.

Thirty-two members and 25 other interested persons attended the meeting of the Committee on Biologics November 1, 1989. The Chairman announced that current members had been polled to determine their continuing interest in serving on the committee. Other persons interested in committee membership were asked to indicate this. Executive Committee adoption of a 1988 committee resolution on product labeling requirements of individual states was noted.

Dr. David A. Espeseth, Senior Staff Veterinarian, USDA, updated the committee on the biologics program of USDA for 1989. He reviewed the 1985 amendment of the Virus-Serum-Toxin Act, the allowed exemptions, the published rules and the implementation program. Originally, 57 manufacturers requested exemptions for 2199 products. To date 15 manufacturers have been licensed and licenses for another 6 are pending. Dr. Espeseth outlined the criteria for extension of the above exemption up to one year. Extensions have been requested by 13 manufacturers, 7 of which are licensed. The licenses of 6 additional manufacturers are pending and must be completed by December 31, 1989.

During 1989 USDA issued 9 new establishment licenses and 1 import permit. Four establishment licenses were terminated. Currently, there are 103 licensees including 5 permitees.

During 1989 USDA issued 132 product licenses and 45 product licenses were voluntarily terminated. There are 1797 licensed products. Dr. Espeseth showed graphs depicting steady growth in the number of licensees and licensed products. Thirty-four biotechnology products have been licensed.

In 1989 27 billion doses of poultry biologics and 2 billion doses of biologics...
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for other species were produced. One billion doses of poultry biologics and 100 million doses of biologics for other species were destroyed because they were outdated or were unsatisfactory. This product destroyed represents a very acceptable percentage of total product. During the past year the Veterinary Biological Field Office in Ames, Iowa conducted 81 inspections, and handled 84 consumer complaints, 46 regulatory actions and 16 investigations. The National Veterinary Services Laboratory testing activities were discussed.

Dr. Espeseth outlined current issues which stimulated much discussion within the committee. These issues included the nomenclature of blood and milk origin products, companion tests for pseudorabies virus vaccines, the January 1, 1990 deadline on intrastate products, free trade agreements, co-culture for the production of veterinary biologics, mixing biologics in feeds and drugs, the wistar vaccinia vectored rabies vaccine, state biologics programs and initiatives, recombinant-DNA product field trials in foreign countries and a foot and mouth disease vaccine bank. Dr. Espeseth indicated USDA has no legislative authority to regulate animal blood for transfusion.

Dr. J. B. Katz, Veterinary Medical Officer, Biologics Virology Laboratory, NVSL presented the topic, “Applications of Biotechnology to in vitro Assessment of Potency and Purity of Biologics.” He indicated that two-thirds of new biologics are evaluated for release in other than the natural host. Methods Dr. Katz discussed in some detail included the Enzyme Linked Immunosorbent Assay (ELISA), electrophoresis, radio-immunoassay, single radial immunodiffusion, immunoblotting and hemagglutination and hemagglutination inhibition. Most of the newer methods for in vitro testing of biologics make use of monoclonal antibodies.

Dr. Laurie Leach, Animal Health Institute, Washington, D.C., discussed distribution restrictions placed on some federally licensed veterinary biologics in three states. The state restrictions evolved from concerns over mail order distribution of vaccines, lack of proper diagnoses, conflict with eradication programs, concerns about efficacy and about the use of vaccines in areas where the disease was not present, and improper use of the products.

The Animal Health Institute’s position is that these restrictions impose unnecessary burdens on interstate commerce, may result in loss of small volume products, and will increase costs to the consumer. The Animal Health Institute is also concerned about the redundancy of regulatory efforts when states impose restrictions on federally licensed products. Initiatives to classify veterinary biologics as “dangerous drugs” in the context of model veterinary codes were felt to be improper.

Dr. H. Michael Chaddock, Director and State Veterinarian for Michigan presented his perspective on regulation of the distribution of veterinary biologics. At the present time in Michigan, permission of the State...
REPORT OF THE COMMITTEE

Veterinarian is required for the importation of veterinary biologics. Regulations are being promulgated and the need for restricting the sale of specific veterinary biologics to licensed veterinarians is being studied. Among the biologics being considered for restricted use are brucellosis, paratuberculosis and rabies vaccines. Dr. Chaddock indicated that there is no active coordination of distribution initiatives among the various states. Discussion followed with several perspectives being presented.

A brief discussion of the need for a ringworm vaccine was held. Russia has used such a vaccine with apparent success. No definitive action was taken.

A resolution on state regulation of the distribution and sale of veterinary biologics was prepared and forwarded to the Resolutions Committee.
HARMONIZATION OF BIOTECHNOLOGY REGULATIONS
EQUAL OR EQUIVALENT

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INTRODUCTION

I am honored to be here today to present my views on the harmonization of biotechnology regulations. The impact of biotechnology on veterinary medicine and animal health is already significant and it continues to grow at an exponential rate. For example: recombinant vaccine construction has been carried out with viruses; gene cloning has been used in animal breeding; monoclonal antibody production and production of hormones and drugs has been used to increase growth and productivity. Monoclonal antibody production and recombinant DNA technology are areas of biotechnology uniquely applicable to the control of infectious disease and improved animal health. Infections are controlled by both passive and active protection. Monoclonal antibodies are used for passive protection and active protection is effected by the administration of safe and effective vaccines.

Accompanying the new veterinary animal health care products of biotechnology are the legal, ethical, social, and economic controversies that have surrounded the development and use of this technology. Evidence for these concerns have shown up in several areas. In the area of recombinant vaccines, the Animal and Plant Health Inspection Service (APHIS) was sued in 1986, over the issuance of a veterinary biologics license for the first recombinant derived pseudorabies vaccine. More recently, the Wistar Institute's request to perform a field trial a vaccinia-vectored rabies oral vaccine for animals in the wild has generated considerable debate. Although the proposed test was approved by the U.S. Department of Agriculture-APHIS and the State of Virginia as safe, the State of South Carolina denied its approval on the basis of legal liability, site security, and zero human health risk. Further, private owners of the island proposed for the approved Virginia test have yet to approve use of the island.

In the area of animal growth hormones, especially Bovine Somatotropin (BST), the news headlines are frequent and the polarization of views evident. Although the Food and Drug Administration (FDA), which is reviewing the BST drug application, has allowed consumption of milk derived from clinical test animals, finding a market for this milk is another matter. Some States have taken action to ban human consumption of milk containing BST. A well-known Vermont ice cream maker refuses to purchase milk from cooperatives which accept milk containing BST. In the Washington, DC area, several large supermarket chains announced that they would not purchase milk containing BST. Internationally, we are well
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aware of the European opposition to growth hormones.

Lastly, in the area of "transgenic animals," although the U.S. Patent and Trademark Office on April 12, 1988, issued the first patent of a living animal—Patent Number 4,736,866—"The Harvard Mouse," the issue is far from being settled. The U.S. Congress is actively considering H.R. 1556, the Transgenic Animal Patent Reform Act of 1989. On September 13 and 14, hearings on H.R. 1556 were held before the House Subcommittee on Courts, Intellectual Property, and the Administration of Justice. Representative Kastenmeier of Wisconsin, introducer of H.R. 1556, stated that in his opinion, Congress has moral obligation to set limits on what can be patented. Testimony at the 2 days of hearings, was received from various groups including farm groups, Federal agencies, the academic/research community, and animal rights, religious, and environmental groups. Testimony ranged from discussions about H.R. 1556's farmers' exemption, both pro and con, ethical questions about the impact of biotechnology on the reverence of life, to H.R. 3247, a bill that would impose a 2-year moratorium on the patenting of "any vertebrate or invertebrate animal that is modified, altered, or in any way changed through genetic engineering . . . ." Clearly, this matter is far from being resolved and I do not expect that we at this meeting can resolve it.

Similarly, I do not feel that harmonization of regulations can resolve conflict, be they economic, social or moral, characterized by polarization of beliefs. However, I do believe that harmonization of regulations and their underlying scientific basis can assist in resolving some of the questions associated with development, use, and commercialization of products of biotechnology, especially veterinary animal health care products produced through biotechnology. These questions center around human, environmental, and host animal safety; product purity, potency, and efficacy standards; patent protection; and elimination/prevention of trade barriers.

The remainder of my talk will focus on some U.S. efforts to harmonize the regulation of products of biotechnology.

DISCUSSION

In my view, the "harmonization" of regulations, nationally or internationally, means to bring such regulations into harmony or agreement with each other. The basis for achieving such harmony does not require identical regulations but similar and equivalent regulations. The overall . . . negotiating objective of the Trade Act of 1974, updated by the General Agreements on Tariffs and Trade (GATT), was to obtain more open and equitable market access and the harmonization, reduction, or elimination of devices which distort trade or commerce (9 U.S.C. 2113). In our discussions concerning implementation of the United States–Canada Free Trade Agreement for biological products, we have focused on the necessary equivalent regulatory procedures for free trade of veterinary biologicals products. Therefore,
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when I refer to the "harmonization" of regulations, I am referring to similar and equivalent regulations.

In an effort to produce harmony among the Federal agencies responsible for regulating the products of biotechnology, an interagency working group was formed in April 1984, within the Executive Office of the President. The working group's charge included identifying the existing laws and regulations applicable to biotechnology and determining their adequacy for regulating the products of new technologies. The results of these efforts were first published for public review and comment in December (49 FR 50856, (1984)), and in final form in June 1986, as the "Coordinated Framework for Regulation of Biotechnology" (51 FR 23302-23393, (1986)). The Coordinated Framework included an index of laws applicable to biotechnology products in the various stages of research, development, marketing, shipment, use, and disposal. This index of laws was published in final form November 14, 1985, (50 FR 47177-47195, (1985)).

The U.S. Federal policy has been, and continues to be based on several conclusions: (1) the products of biotechnology will not differ fundamentally from unmodified organisms or from conventional products; (2) the product, rather than the process should be regulated; (3) regulation should be based on the end use of the product and conducted on a case-by-case basis; (4) and the existing laws provide adequate authority for regulating the products of biotechnology. An important corollary to this policy is the Federal commitment to promoting the safe development of the products of biotechnology. Each Federal agency is committed to ensuring protection for public health and the environment from any potentially harmful effects of the technology.

The Coordinate Framework, published in June 1986, contained among other policies the final policy statements by the U.S. Federal agencies that share a major responsibility for regulating veterinary animal health care products produced through biotechnology. The agencies are FDA and USDA.

FDA Policy for Regulating Biotechnology

FDA, which is part of the Health and Human Services Department of the U.S. Federal Government, regulates foods, human and animal drugs, cosmetics, and medical devices under the authority of the Food, Drug, and Cosmetic Act of 1938, as amended (the Act). Key amendments to the Act have given FDA authority to require premarket approval of the food and color additives used in food, and premarket safety and efficacy testing for all drugs. While the Act generally limits FDA jurisdiction to products involved in interstate commerce, interstate commerce is broadly defined to include importation and exportation. FDA's statutory and regulatory authority place the burden of proof of product safety on the manufacturer.

FDA policy is that regulation of products developed through biotechnology should be based on scientific evaluation of products and case-by-case review. FDA does not consider that recombinant derived products require
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a special or unique review procedure based on process. FDA’s organizational units like the Center for Veterinary Medicine and the Center for Food Safety, review products developed through many different processes, with attention to scientific concerns, specific tests, and “points to consider.” “Points to Consider Documents” have been made available on such subjects as interferons, monoclonal antibodies, recombinant DNA-derived products, and use of mammalian cell lines. FDA has approved several products of new biotechnology that can be viewed as medical milestones. These include alpha-interferons for the treatment of a lethal leukemia, a monoclonal antibody preparation for preventing rejection of kidney transplants, a new generation hepatitis vaccine, and a clot-dissolver for treating heart attacks. FDA has approved or licensed over 200 monoclonal antibody-based diagnostic kits and half a dozen each of therapeutic drugs and recombinant DNA probes for infectious agents. In addition, FDA’s Center for Food Safety and Applied Nutrition has filed three Generally Recognized as Safe (GRAS) affirmation petitions for products developed using genetically engineered microorganisms.

USDA Policy, Authority, and Organization for Regulation Biotechnology

USDA policy on the regulation of biotechnology has been consistent with the overall Federal policy. In policy documents published in December 1984, and June 1986, and in practice: (1) USDA has not viewed genetically engineered organisms and products as fundamentally different from those produced by conventional methods; (2) USDA has used existing laws to regulate the products of biotechnology; (3) USDA has attempted to focus attention on the product and the risk, rather than on the process used in production.

In keeping with this policy, USDA, like other Federal agencies, has developed an administrative structure under its existing authorities to deal with all aspects of biotechnology research and regulation. Within U.S. agencies, the research and regulatory activities are generally separated administratively.

The USDA Agency directly concerned with regulating biotechnology veterinary animal health care products (e.g. veterinary biologics) is the Animal and Plant Health Inspection Service (APHIS). Within Biotechnology, Biologics, and Environmental Protection (BBEP), APHIS has combined a number of existing staffs to create the Department’s lead division for biotechnology regulation. BBEP is composed of the following:

a. The Biotechnology Coordination and Technology Assistance (BCTA) staff coordinates biotechnology regulatory activities within USDA. BCTA takes the lead in liaison between APHIS and other Federal agencies on biotechnology regulatory matters.

b. The Biotechnology Permits staff is responsible for issuing permits for the field testing of certain genetically engineered plants and microorganisms, maintaining liaison with State departments of agriculture, the
HARMONIZATION OF BIOTECHNOLOGY REGULATIONS

academic community and scientific societies, and providing technical information for environmental analyses used to issue permits allowing field tests of regulated articles.

c. The Veterinary Biologics staff issues licenses for veterinary biologics products produced through biotechnology and conventional methods.

d. The Veterinary Biologics Field Office inspects and monitors veterinary biologics production establishments.

e. The Environmental Documentation staff ensures that APHIS programs comply with the applicable environmental laws.

In the area of animal health, the Virus-Serum-Toxin Act (VSTA) of 1913, as amended, provides USDA's APHIS with the authority to regulate all veterinary biologics that are imported into the United States, shipped or delivered for shipment interstate, intrastate, and that are exported. USDA also has enforcement mechanisms such as the power to detain and seize products. The VSTA is administered by APHIS in the same manner for genetically engineered and naturally occurring organisms and products. APHIS issues U.S. Veterinary Biological Product Licenses after satisfactory completion of all requirements to assure purity, safety, potency, and efficacy. Veterinary biological products produced by recombinant methods are evaluated on a case-by-case basis using the same stringent standards for licensing employed for conventionally produced biologics.

The APHIS application procedure for licensing veterinary biological products requires that an “outline of production” describing the procedures used to produce each serial of a product accompany each application. For recombinant-derived products, the manufacturer must provide the specific cloned nucleotide sequence coding for the product or other DNA segments. Licensees are required to establish a Master Seed of bacteria, viruses, or other microorganisms at a specific passage level to be used as the source of seed materials. Immunogenicity of vaccines must be approved by statistically valid host animal immunization, challenge, and safety studies. Firms are required to show that they can produce each product in a consistent manner. Three consecutive satisfactory serials of a product must be produced in the licensed production facility. To confirm results, samples are sent to the National Veterinary Services Laboratories in Ames, Iowa, for testing.

A three-category classification scheme for hybridomas and recombinant-derived products based on biological characteristics and safety concerns was published in the Federal Register as a part of the final USDA policy statement on biotechnology in June 1986 (51 FR 23339, (1986)).

The first category includes inactivated recombinant DNA-derived vaccines, bacterins, bacterin-toxoids, virus subunits, or bacterial subunits. These nonviable or killed products pose no risk to the environment and present no new or unusual safety concerns. Monoclonal antibody (hybridoma) products used prophylactically, therapeutically, or as components of
diagnostic kits are included in this category.

The second category includes those products containing live microorganisms that have been modified by the addition or deletion of one or more genes. Precautions must be taken to insure that the addition or deletion or specific genetic information does not import increased virulence, pathogenicity, or survival advantages in these organisms which are greater than those found in natural or wild-type forms. Modifications must not impart undesirable new or increased adherence or invasion factors, colonization properties, or intrahost survival factors.

The genetic information to be added or deleted must consist of well-characterized DNA segments. Required licensing data may include base pair analysis, sequence information, restriction endonuclease sites, and phenotypic characterization of the altered organism. A comparison is also required between the genetically engineered organism and the wild-type form for factors affecting pathogenicity.

The third category includes products using live vectors to carry recombinant-derived foreign genes that code for immunizing antigens and/or other immune stimulants. Live vectors may carry multiple recombinant-derived foreign genes because they can carry large quantities of new genetic information.

APHIS has issued 34 licenses for veterinary biological products manufactured from biotechnological processes. Thirty are from Category one, which includes bacterins (5), monoclonal antibodies for therapeutic or prophylactic use (2), and diagnostic kits (23). These Category one products have been used successfully since the first, a bacterin, was licensed in October 1983. Four Category two product licenses have been issued, all for recombinant-derived pseudorabies virus vaccines for use in swine.

Coordination

Intra-agency and interagency coordination have been key elements in the successful implementation of the U.S. Federal policy for regulating biotechnology. Within USDA, coordination has been achieved at the administrative level through the activities of the Committee on Biotechnology in Agriculture. Interagency coordination of policy issues have been assigned to the Biotechnology Science Coordinating Committee, which includes high level representation from each Federal agency with research or regulatory authority for biotechnology.

APHIS uses the existing working relationship with State veterinarians to assure that State authorities are kept informed about pending biotechnology applications which involve the field trial of experimental veterinary biologics within State boundaries. The approval of appropriate State officials is required for APHIS approval. APHIS also utilizes an interagency committee for the review of certain applications prior to approval. In its review of the field test request for the genetically engineered live vaccinia virus for rabies, APHIS also held a public hearing to receive comments.
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The preparation of policy documents on biotechnology safety issues by U.S. Federal agency representatives for presentation to the Organization for Economic Cooperation and Development has also fostered cooperation and communication and hopefully aids in harmonization. A paper being finalized on Good Industrial Large Scale Practices (GILSP) for the industrial application of genetically engineered microorganisms could have applicability to the production of certain veterinary animal health care products. It is also hoped that our participation in the activities of the Office of International Epizooties will assist in harmonizing the various regulations for the development and use of biotechnology veterinary health care products.

The success, to date, of the U.S. Federal regulatory effort to promote the safe development of products through biotechnology has been verified in assessments prepared by the U.S. Office of Technology Assessment (May 1988), and the U.S. Government Accounting Office (June 1988).

CONCLUSION

APHIS is committed to providing the resources necessary to review field test and product applications for veterinary biologics developed through biotechnology. We feel that the procedures that have been established for conducting these reviews and analyses are reasonable, both from the perspective of the applicant and the concerned public. The information gained from the first field tests and product approvals is of vital importance to the future development of safe and beneficial products. It is in the interest of all concerned to maintain the relationship developed among industry, Federal/State governments, the research community, and public interest groups on an international basis.

It is unequivocal that the stakes are high. A 1987 report entitled, "Agricultural Biotechnology Strategies for National Competitiveness," begins by concluding that "a National strategy for biotechnology in agriculture must focus on solving important scientific and agricultural problems, effectively using the funds and institutional structures available to support research, training researchers in scientific areas, and efficiently transferring technology" (emphasis added). In my opinion, efficient technology transfer of a global nature is facilitated through international harmonization of regulations.

Harmonization of regulations was a critical concern for the 12 European countries which agreed in 1987 to push ahead with earlier plans and complete the Herculean task of integrating their national economics into one "single market" by 1992. The new "Community" will encompass a population of 320 million and a gross domestic product (GDP) of $4 trillion. The European Community (EC) predicts that a complete integration will increase GDP by 4.5 percent, add 1.8 million jobs to the economy, and reduce consumer prices by 6.1 percent.

From the EC perspective, 1992 should be a boom for both the Community
and its trading partners. The EC points to the elimination of 12 frontiers and corresponding sets of regulations.

APHIS is committed to promoting national and international harmonization and cooperation in the development and regulatory oversight of biotechnology veterinary health care products. We are fully aware of these stakes and the need for harmonization in regulations to achieve protection of human, environmental and animal health and to foster a framework for efficient technology transfer.

REFERENCES

 CONSTRAINTS ON DEVELOPMENT OF RECOMBINANT VACCINIA VIRUS VACCINES

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There has been a substantial amount of concern and anxiety surrounding the development of live recombinant vaccinia viruses for use as vaccines. The amount of anxiety is surprising when one considers that the principles applicable to the development of these vaccines are similar to those applicable to the development of any live virus vaccines, and upon comparing traditional smallpox vaccine to recombinant vaccinia viruses. Traditional smallpox vaccine was produced by techniques as crude as those ever used to produce any live viral vaccine. For hundreds of years there were virtually no criteria used to define vaccine acceptability, and even the source of the virus used to produce the vaccine remains unknown. In contrast, recombinant vaccinia viruses are cloned viruses that have undergone extensive molecular and biological characterization, are produced by state-of-the-art manufacturing procedures, and undergo rigorous testing and characterization. Extensive research has been accomplished to document the safety of these recombinant viruses, criteria for regulatory control are well developed, and clinical trials have been performed successfully. Reasons for anxiety regarding development of recombinant vaccinia virus vaccines have largely been mitigated.

Some potential reasons for concern over the development of recombinant vaccinia virus vaccines are listed in Table 1. One of the greatest reasons for concern has been anxiety, or fear of the unknown. Such anxiety is based on a feeling that risks may exist which cannot be defined or anticipated and against which adequate precautions cannot be taken. In the absence of an adequate knowledge base, on which to evaluate risk, such fear is appropriate. However, as will be indicated subsequently, sufficient research has been accomplished on recombinant vaccinia viruses that risk can be assessed and adequate precautions can be taken. As with any vaccine there exists the remote possibility of unanticipated rare adverse effects, but adequate precautions can be taken so that the probable benefits of vaccine development can outweigh the attendant actual and theoretical risks.

Specific areas of concern regarding development of recombinant vaccinia viruses as vaccines are their potential for environmental impact, risks to humans infected by vaccination or by contact, and the potential for limited effectiveness. Risks of adverse effects on the environment can be quantitated by assessment of virus transmissibility. Risk of adverse effects on
QUINNAN

humans can be assessed based on past experience with smallpox vaccine and studies of virulence of recombinant vaccinia viruses in animal models. The potential for benefit must always be taken into account, since the probable benefit of research should outweigh the risk. The greater the likelihood of public health and individual benefit indicated by previous research, the stronger the justification for assuming risk.

TRANSMISSIBILITY OF VACCINIA VIRUS

The potential for sustained transmission of vaccinia virus in nature is very low if existent at all. Some observations which support this assessment are listed in Table 2. First of all, pox viruses in general are often transmitted with difficulty. Were smallpox transmitted as readily as measles virus, for example, it would never have been eradicated by the vaccination programs employed. Monkey pox is rarely transmitted to man, and sustained transmission does not occur. Rabbit pox, the only orthopox virus known to be possibly endemic in the United States, has only been isolated twice. Vaccinia virus is transmitted by human vaccinees to contacts only rarely, and only as a result of intimate contact; secondary transmission of vaccinia virus from a contact case to a third person is virtually unknown. Even the fact that the origin of vaccinia virus is unknown is evidence of poor transmissibility. If the reason the source of vaccinia virus is unknown is that the virus is now extinct, then it was unable to sustain itself even in the natural host. If the reason is that the progenitor remains sequestered in some remote pocket in nature, then its lack of transmission in other natural circumstances is still evidence of very poor transmissibility. The simple observation that vaccinia did not establish itself as a widespread agent in nature during hundreds of years of unrestricted use in man and animals is dramatic evidence of its poor transmissibility.

The potential that introduction of recombinant vaccinia viruses into the environment might have adverse environmental impact should, therefore, be considered a very low risk a priori. Thus, studies of recombinant viruses can evaluate their behavior in a variety of animal species and their transmissibility under conditions of close contact. If the virus in question behaves like vaccinia virus, the risk of adverse environmental impact under circumstances of reasonably well controlled release should be considered negligible.

VIRULENCE OF VACCINIA VIRUS

The virulence of vaccinia virus for humans can be described on the basis of clinical experience with smallpox vaccine. The virulence of the virus in animals can be expressed quantitatively, for example as a 50% lethal dose, in a way which depends on the animal species and route of inoculation. In man and animals the virulence of vaccinia viruses is appropriately ex-
pressed by quantitative measurement of the effects it normally and usually induces, not rare effects.

The adverse effects attributed to smallpox vaccine are described in Table 3. The adverse experiences generally of greatest concern are post-vaccinal encephalitis and encephalopathy. These complications were widely considered to be effects of vaccine. However, there are many reasons to question their association. Most importantly, vaccinia virus has never been demonstrated in the central nervous system in association with these diseases. In addition, no animal model has ever been developed which mimics these conditions. The incidence of post vaccinal encephalitis and encephalopathy varied from place to place and from time to time. Some have attributed this variability to differences in vaccinia strains used in vaccines. However, much, if not all, of the decrease in incidence that occurred in the 1960s can easily be attributed to control of other infectious diseases known to be causes of encephalitis and encephalopathy, such as measles and pertussis. There is, at best, weak evidence to link smallpox vaccine causally to encephalitis or encephalopathy, and there are other more likely explanations for these events. If encephalitis and encephalopathy occurred as a result of vaccination they did very rarely.

There are four unusual complications that are potentially serious or life-threatening and that can be definitely attributed to vaccinia virus: progressive vaccinia, eczema vaccinatum, generalized vaccinia, and ophthalmic vaccinia. All are usually preventable and/or treatable. Progressive vaccinia begins as what appears to be a normal appearing “take” or infection at the inoculation site. Rather than the normal regression the infection progresses, at first locally and then generally. Progression is inexorable and the condition is fatal. Progressive vaccinia occurs in individuals with deficient cell mediated immunity. It can be prevented by not vaccinating people with such deficiency.

Eczema vaccinatum occurs in children with a past history of eczema. It can be fatal, but is usually treatable with vaccinia immune globulin. It can be prevented by not vaccinating children with a history of eczema.

Generalized vaccinia is an infrequent event with disseminated minor skin lesions. It occurs in healthy children, is not associated with severe symptoms, and resolves spontaneously.

Ophthalmic vaccinia results from contact of the eye with virus. It may result from autoinoculation, but usually results from close contact with another vaccinated person. Vaccinia immune globulin may be of value in treatment of this condition.

These four complications of smallpox vaccine are infrequent. With the exception of progressive vaccinia, they are all treatable or self limited. They can all be prevented by attention to the immune status of the vaccinee and prevention of contact spread. Because the infrequent, potentially serious complications of smallpox vaccination are preventable and/or treatable,
the most important adverse effects of vaccination are those which occur in most vaccinees, but are not regarded as serious.

The inoculation of vaccinia virus by scarification results in infection of the dermis and an associated inflammatory response which is referred to as a "take." The clinical manifestations of a take include local erythema, pain, swelling and induration. When the take is severe there may be extensive swelling, regional adenopathy, and systemic symptoms, such as fever, malaise, and myalgia. The severity of the clinical reaction to the take depends on the age and immune status of the donor, and the dose and strain of virus in the vaccine. The take which occurs on initial vaccination, the primary or major take, is more severe than that which occurs on subsequent vaccination, the secondary or minor take. When the time between vaccinations is short, for example two or three years, the secondary take is a very minor reaction typically one or two centimeters in diameter. When the interval between vaccinations is long, for example, 10 to 15 years, the secondary take is likely to be more severe and approach the severity of the primary take. Adults typically have more severe primary takes than children. Thus, non-immune or remotely immunized adults are likely to have relatively severe reactions, an expectation that has been considered in assessing risk of a recombinant vaccinia virus vaccine clinical study in humans and in designing the study.

The severity of reactions to smallpox vaccine are proportional to the dose inoculated. The potency of vaccines used during the smallpox eradication campaign were established to be sufficient to result in a high frequency of secondary takes when given one to two years after the last vaccination. While these doses induce relatively severe primary takes in adults, lesser doses can be selected that are less effective at establishing infection with a high frequency in previously vaccinated subjects, but that cause less severe reactions to occur.

Some strains of vaccinia are less virulent than others, in that they induce less severe takes than others. Examples of less virulent strains are the tissue culture adapted, attenuated strains CV-1 and LCM16. Animal studies of recombinant vaccinia viruses have indicated that genetic manipulation may reduce virulence of vaccinia. The virulence of vaccinia for humans is, thus, easily quantitated by dose response studies and reflects the ordinary replication capability of the virus in humans.

The virulence of vaccinia virus can also be quantitated in animal models. Animals may be inoculated by scarification or by various parenteral routes, including intracranially. It cannot be assumed that the virulence of vaccinia in any given animal model is directly related to its potential virulence in humans. However, comparison of a recombinant virus to its parent virus in a number of animal models should give a reasonable indication of its potential for increased virulence for humans, as well as its potential for increased transmissibility. Since its potential for virulence in man is
RECOMBINANT VACCINIA VIRUS VACCINES

reflective of its effects on the majority of people inoculated, its usual effects on a range of animal species can be considered a reasonable measure of the potential virulence of a recombinant vaccinia virus for man. The virulence of a recombinant vaccinia virus expressing the human immunodeficiency virus (HIV) envelope glycoprotein in a variety of animal species was considered adequate evidence of safety of the recombinant virus for use in clinical studies.

REGULATORY CRITERIA FOR RECOMBINANT VACCINIA VIRUSES

The principles applicable to control of manufacture, testing and quality control of recombinant live vaccinia virus vaccines are similar to those relevant to other live virus vaccines and products derived by recombinant DNA technology. In comparison to smallpox vaccine, it is very likely that all recombinant vaccinia virus vaccines for human use will be produced in cell culture using virus seed and cell bank systems. The same may be true for veterinary vaccines, although some scientists argue that local production in vivo in developing countries may still be essential for some vaccines. Principles applicable to regulatory control of human and veterinary recombinant vaccines are well developed.

FIELD TRIALS OF RECOMBINANT VACCINIA VACCINES

The first human clinical trial of a recombinant vaccinia virus vaccine was that performed by Collier et al, using virus which expressed the HIV envelope glycoprotein. Preclinical evaluation involved rigorous in vitro and molecular characterization and studies of safety and immunogenicity in a number of animal species including monkeys and chimpanzees. The first phase 1 clinical study was performed in a highly selected healthy population, as is generally the case in phase 1 vaccine studies. It was a dose response study which compared the recombinant virus to standard smallpox vaccine (Table 4). The purpose of this approach was to obtain a quantitative estimate of virulence so that judgements could be made about the potential safety of inoculating less extensively screened volunteers in phase 2. The study involved evaluation of the use of an occlusive dressing to prevent shedding of virus into the environment. The first few recipients of the recombinant virus were evaluated under containment conditions, until the effectiveness of the dressing was documented. The results of the study have not been published, so I will not discuss them in detail. In brief, the vaccine was well tolerated, but less immunogenic with regard to anti-HIV responses than had been hoped for. The potential to use the recombinant virus in conjunction with purified protein vaccine is of substantial ongoing interest, however. The validity of this stepwise approach which used progressively more open and large study groups and the safety of a recombinant vaccinia virus in man were well documented by this study.

The first substantial field trial of a recombinant vaccinia virus in animals
QUINNAN

was that of LeClerc and colleagues in Belgium (Table 5). These efforts were toward the development of vaccinia expressing the rabies virus G protein for use in bait to control sylvatic rabies. The combined efforts of the groups of scientists involved in development of vaccinia-rabies recombinants, including a large effort at the Wistar Institute, have involved studies of safety, efficacy, immunogenicity and transmissibility in hundreds of captive or laboratory animals of more than 35 species, including avian species and immunodeficient mice. The possibility that unanticipated adverse effects or sustained transmission could occur in some yet untested species is becoming vanishingly small. Further laboratory studies demonstrated that the expressed G protein was highly immunogenic (much more so than the HIV envelope protein), and highly effective upon experimental challenge. Moreover, oral infection was also efficient. Thanks to past studies of the use of live rabies vaccine in bait, methods for formulating bait that is taken up efficiently by the target species, foxes or raccoons, have been established.

The stepwise evaluation of this vaccine has involved a first phase that included testing infection, excretion and transmission in target and non-target species of animals in the geographic area where the field trial was planned. The field trial was performed in a rural area targeted at foxes using a bait known to be taken up with high efficiency. Its uptake by foxes, other wild animals and domestic animals was monitored through the inclusion of tetracycline in the bait. The study so far has been a great success, establishing the safety of the approach and its potential good species selectivity. Its efficacy in controlling rabies in the area will be monitored for a few more years.

Another example of the potential value of recombinant vaccinia viruses is the vaccinia-rinderpest recombinant vaccine developed by Yilma, et al. The studies done to date and planned are summarized in Table 6. Laboratory studies have shown that combined immunization with the F and HA proteins expressed in vaccinia confer complete protection against experimental challenge. Review activities are now ongoing to determine the acceptability of this vaccine for use in a large field trial in East Africa. The available living vaccine, propagated in cell culture is effective, but not heat stable and thus costly to apply in the tropics because of the expense of the "cold chain." Its broad use has not been successful in eradicating the disease from Africa because of economic, political and technical reasons. The availability of a safe, effective recombinant vaccinia vaccine against this disease could have substantial impact on food supply and human health.

SUMMARY

The principle concerns in the development of recombinant vaccinia virus vaccines regard human safety and environmental impact. The risks attendant to humans resulting from use of smallpox vaccine are well known.

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RECOMBINANT VACCINIA VIRUS VACCINES

Since the adverse effects are the direct result of the ordinary replication characteristics of the virus, the properties of a new recombinant virus can be estimated by testing in animal models in the laboratory. Transmissibility of vaccinia viruses is also low and can be estimated for new recombinants by testing in animal models, as well. The suggestion that human trials are necessary before field trials of recombinant vaccinia viruses for veterinary use can be performed is ethically and scientifically unacceptable. Criteria for manufacturing control and vaccine characterization and testing are sufficiently well developed at FDA and USDA to allow field trials to proceed. Both human and animal field trials have been performed demonstrating the safety, immunogenicity and potential for ready control of environmental effects of these viruses. Laboratory studies of some new recombinant vaccinia viruses indicate their strong potential to fill unmet human and animal health needs. Efforts to facilitate development of recombinant vaccinia virus vaccines which hold real promise can be facilitated based on the scientific data and approaches to evaluation that have been developed.

REFERENCES


### Table 1
**Potential Constraints of Recombinant Vaccinia Virus Vaccines**

1. Fear of the unknown
2. Safety concerns
   - virulence
   - environmental
3. Potentially limited efficacy

### Table 2
**Evidence for Low Transmissibility of Pox Viruses**

1. Eradication of smallpox
2. Poor transmissibility of monkey pox to man
3. Poor transmission of animal pox viruses (e.g. rabbit pox viruses)
4. Very low secondary spread of vaccinia
5. Lack of natural transmission of vaccinia

### Table 3
**Adverse Events Following Smallpox Vaccination**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Event</th>
<th>Caused by Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Rare</td>
<td>Encephalitis</td>
<td>Very doubtful</td>
</tr>
<tr>
<td></td>
<td>Encephalopathy</td>
<td>Very doubtful</td>
</tr>
<tr>
<td>Unusual</td>
<td>Progressive Vaccinia</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Eczema Vaccinatum</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Generalized Vaccinia</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Ophthalmic Vaccinia</td>
<td>Yes</td>
</tr>
<tr>
<td>Usual</td>
<td>Primary Take</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Secondary Take</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Table 4

<table>
<thead>
<tr>
<th>Phase</th>
<th>Evaluations</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Restricted population</td>
<td>In Progress</td>
</tr>
<tr>
<td></td>
<td>Dose-response study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical/Immunology effects</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excretion</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Safety in varied groups</td>
<td>Future</td>
</tr>
<tr>
<td></td>
<td>Efficacy in controlled studies</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Large scale safety and efficacy</td>
<td>Future</td>
</tr>
<tr>
<td></td>
<td>in open populations</td>
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</tr>
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Table 5

<table>
<thead>
<tr>
<th>Phase</th>
<th>Objectives</th>
<th>Status</th>
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<tbody>
<tr>
<td>I</td>
<td>Safety and efficacy</td>
<td>Completed</td>
</tr>
<tr>
<td></td>
<td>in target species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Safety in other species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>potentially at risk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virus excretion and transmission</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stability of formulation</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Restricted field trial</td>
<td>In Progress</td>
</tr>
<tr>
<td></td>
<td>Efficiency of bait uptake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Efficacy in target species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uptake by non-target species</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Open field trial</td>
<td>Future</td>
</tr>
</tbody>
</table>

Table 6

<table>
<thead>
<tr>
<th>Phase</th>
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<tbody>
<tr>
<td>1</td>
<td>Challenge</td>
<td>Plum Island</td>
<td>Completed</td>
</tr>
<tr>
<td>2</td>
<td>Spread to contacts</td>
<td>Plum Island</td>
<td>Completed</td>
</tr>
<tr>
<td>3</td>
<td>Field Efficacy</td>
<td>East Africa</td>
<td>Future</td>
</tr>
<tr>
<td></td>
<td>5 year</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE ON BIOTECHNOLOGY

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Vice Chairman: Dr. A. W. Strating, Washington, D C

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Advances continue to be made in biotechnology. It is now possible to express foreign genes in heterologous living organisms, including bacterial or yeast systems, several mammalian and insect viruses. Two of the most widely used vectors are baculo and vaccinia virus. Several experimental vaccinia vectored vaccines have been produced including rabies, rinderpest and vesicular stomatitis, however, obstacles exist in relation to application of such products. Some of these include fear of the unknown, safety concerns, virulence factors, environmental concerns and the potential limited efficacy of such vaccines. At the same time, vaccinia as a vector has many desirable attributes including 200 years of experience with its application as a vaccine against smallpox, low transmissibility, ease of production, ease of administration and its extreme stability. Many of the safety concerns of vaccinia as a vector already have, or are being addressed, including strain selection, strain or seed characterization, production of the virus in animals or tissue cultures, vaccine production techniques and criteria for vaccine development.

As concerns release of vaccinia into the environment, the recent incorporation of genetic material including a major protein of human immunodeficiency virus (H.I.V.) and its application in volunteers has produced no untoward effects even though the protection afforded to H.I.V. has thus far been disappointing. In Belgium, the vaccinia rabies glycoprotein product has been released in bait for the immunization of wildlife especially fox. There have been no untoward developments, such as persistence of the virus in other species or changes in its pathogenicity. In summary, the potential changes or bad effect from the product get smaller. Stepwise evaluation of vaccinia, as a vector, include safety for the target species, safety in other species, virus excretion and virus stability. The safety and efficacy of vaccinia as a vector can likely be demonstrated. While no release, other than the H.I.V. insert, has taken place in the U.S., release of vaccinia vectored rabies is still under study and planning towards its eventual release on an island location continues.

In recent times, the baculoviruses which are pathogenic to insects have received attention because of their potential use as a vector for production

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of proteins in insects and insect cell lines. One major advantage of these expression systems over bacterial, yeast or mammalian systems is the abundant expression of recombinant proteins which are antigenically, immunologically and functionally similar to their authentic counterparts. In addition, baculoviruses are not pathogenic to vertebrates or plants and do not employ transformed cells as do the mammalian systems. The committee heard a paper on the use of the baculovirus expression system for the development of diagnostic reagents and subunit vaccine for bluetongue virus. Highly immunogenic viral and subviral particles were synthesized in insect cells using these vectors. Protection was afforded sheep which had been given an experimental vaccine and a rapid latex agglutination test is under development by a commercial company.

While not produced by R-DNA technology, a report of a live mutagenized vaccine against Rift Valley fever was given and from all reports this vaccine offers to provide an effective, inexpensive vaccine which can be applied to livestock should the disease get into the U. S. and efforts to contain it are not successful. This development fulfills a long standing need for such a product. Because it is a living virus vaccine, longevity of immunity, not yet evaluated, is expected to be of long duration. Rift Valley Fever, because it is vector transmitted is potentially a very devastating livestock disease which results in almost 100% abortion of ewes and cattle.

Regulations for work in the area of R-DNA technology have been in place for more than a decade and have served us well. Now that products of the result of R-DNA studies are ready for release, those Federal agencies with regulatory responsibility, namely APHIS, FDA and EPA have formed a coalition to first decide which agency has jurisdiction over a given product. This system seems to be working well. As concerns product efficacy, the same standards for evaluating conventional products can be applied to products derived from R-DNA studies. At present, consideration of release is done on a case by case basis; however as experience is gained, there is a possibility that guidelines providing for exemption of certain categories of products will be developed.

Guidelines for review of research proposals within USDA and the Land Grant College system are also underway. These guidelines will supplement N. I. H. guidelines. There remains to be developed guidelines covering transgenic animals.

Many countries in the Hemisphere have not yet developed guidelines of any type. In recognition of this, the International Institute for Cooperation in Agriculture (IICA) organized and hosted a workshop last year where a generic guideline for work at the laboratory level was developed and it has now been released in English and Spanish. The same group will host a similar study next year where it is hoped agreement can be reached on guidelines for release of the products of R-DNA technology into the environment.
SAFETY AND IMMUNITY OF A MUTAGEN ATTENUATED RIFT VALLEY FEVER VIRUS IN PREGNANT CATTLE


Rift Valley fever (RVF), also called infectious enzootic hepatitis of sheep and cattle, is an acute febrile, insect-borne viral disease of animals and man characterized by a short incubation period, fever, hepatitis, and death in young animals and abortion. All domestic animals with the exception of pigs, guinea pigs, rabbits, and chickens are affected. Very young lambs, kids, calves, and puppies are highly susceptible. Significant morbidity and mortality occurs in sheep, cattle, and man. The most probable routes of introduction of RVF into the United States are via a viremic person who will be bitten by a mosquito or an infected mosquito aboard a plane. The infected mosquito will then infect a susceptible animal in which the virus will be amplified for the infection of more mosquitos.

Historically, RVF has been confined to sub-Saharan Africa and has occurred in epizootics about every 3 to 10 years in East Africa and every 20 years in South Africa. These epizootics generally have started in a dry area after a period of heavy rainfall. Researchers then found that in the interepizootic periods, the virus was present in the dormant eggs of the mosquito *Aedes lineatopennis* in the dry soil of grassland depressions (dombos). With adequate rainfall, the depressions filled with water and the infected mosquito eggs developed. The mosquitos infected ruminants which are amplifiers of the virus, and then the virus was spread by many species of mosquitos. In recent years, RVF has been diagnosed in West Africa.

The epizootic which started in 1970 spread into Sudan, Egypt, and the Sinai peninsula and caused severe loss of livestock and at least 600 human deaths in Egypt. Typically, in such an outbreak, abortion rates, stillbirths, and neonatal deaths can approach 100% in sheep and over 50% in cattle. Mortality rates of 40-60% in adult sheep and over 50% in cattle may occur. During the latest epizootic in Egypt, the following numbers of animals in millions (rounded to nearest 100,000) were lost:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cattle</th>
<th>Water Buffalo</th>
<th>Sheep</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>2.6</td>
<td>2.5</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td>1978</td>
<td>2.0</td>
<td>2.3</td>
<td>2.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

The effect of the RVF epizootic in Egypt was so devastating that it caused President Sadat to prohibit the slaughter of female sheep and cattle.

Control of RVF in Africa includes removal of stock from low-lying mosquito-infested regions to higher altitudes or stabling, spraying, and vaccinating. A vaccine that rapidly and effectively immunizes animals could be the first line of defense against RVF, because vaccination would protect not only the sheep and cattle, but also would prevent amplification...
of the virus and spread to the human population. Two types of vaccines have been developed: the Smithburn mouse brain which is an attenuated neurotropic vaccine for cattle and sheep, and an inactivated cell culture propagated Rift Valley fever virus vaccine that can be used in animals and man.

The advantages of the Smithburn vaccine are:

1. It can be easily produced in large volumes.
2. Protection in sheep and cattle is of long duration.
3. One inoculation produces protection 6 to 7 days after vaccination.
4. Vaccinated animals resist natural challenge.
5. Offspring of vaccinated animals which have received adequate colostrum are protected for about 5 months.
6. The lyophilized virus if refrigerated has good stability.

The disadvantages of the Smithburn vaccine are:

1. Vaccination of lambs less than 6 weeks old may cause an encephalitis.
2. Vaccination of pregnant ewes may cause abortion or encephalitic lambs.
3. The vaccine must be kept cold which is often a problem in developing countries.
4. Vaccinated animals develop a low level viremia, and thus, may be a potential source of virus for arthropods which could spread the virus to other animals and man.
5. The vaccine is infectious for man.

The advantages of an inactivated vaccine are:

1. The vaccination does not cause abortion.
2. The vaccination does not cause a viremia.
3. There is no problem of reversion to virulence or reassortment with related phleboviruses.

The disadvantages of an inactivated vaccine are:

1. Need large quantities of virus.
2. Has a short shelf life because it is liquid and absorbed to aluminum hydroxide.
3. Need two vaccinations
4. Onset of protection is slow
5. Need to vaccinate annually.
6. Does not always prevent viremia or fetal damage when animal is exposed to virulent virus.

The two injections of inactivated vaccine requires about 30 days for protection to develop. This slow onset of protection is a severe limitation,
for if RVF were introduced into the U.S. during the vector season, the disease could become widespread and cause severe loss before animals could be immunized.

Recently there has been developed and tested a mutagen attenuated RVF vaccine that produces rapid protection, has no adverse affect on newborn lambs, is safe for pregnant animals, and produces so low an order of viremia that the vaccinates are not a source of virus to infect mosquitoes. This paper presents the results of testing a mutagen-attenuated RVF vaccine in pregnant cattle at the United States Department of Agriculture, Plum Island Animal Disease Center.

Pregnant cattle were divided into groups and handled as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cattle</th>
<th>Gestation (months)</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6</td>
<td>3</td>
<td>Vaccinated subcutaneously</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>5</td>
<td>Vaccinated subcutaneously and challenged inoculated in 30 days</td>
</tr>
<tr>
<td>3.</td>
<td>10</td>
<td>5</td>
<td>Inoculated with vaccine diluent and challenge in 30 days (Controls)</td>
</tr>
<tr>
<td>4.</td>
<td>6</td>
<td>5</td>
<td>Vaccinated subcutaneously and allowed to calf (Controls for fetal inoculation; then to be used for colostrum protection study)</td>
</tr>
<tr>
<td>5.</td>
<td>6</td>
<td>8</td>
<td>Vaccinate subcutaneously</td>
</tr>
<tr>
<td>6.</td>
<td>6</td>
<td>3</td>
<td>Fetal inoculation of vaccine and allowed to calf</td>
</tr>
<tr>
<td>7.</td>
<td>6</td>
<td>5</td>
<td>Fetal inoculation of vaccine and allowed to calf</td>
</tr>
<tr>
<td>8.</td>
<td>6</td>
<td>4-5</td>
<td>Fetal inoculation of vaccine diluent and allowed to calf (Controls for fetal inoculation of vaccine and controls for calf challenge inoculation)</td>
</tr>
<tr>
<td>9.</td>
<td>10</td>
<td>lactating cows</td>
<td>Vaccinated subcutaneously</td>
</tr>
<tr>
<td>10.</td>
<td>12</td>
<td>steers</td>
<td>Vaccine titration</td>
</tr>
</tbody>
</table>

The animals were observed daily and body temperatures were recorded. On three to 10 days postvaccination, blood was collected for serum, virus assay, and serum enzyme assay, and for the lactating cows, milk was also collected for virus assay.

The challenge virus was the Zagazig-501 strain of RVFV.

Cows inoculated with the RVF vaccine remained normal and at 30 days postvaccination had plaque reduction neutralizing titers 80% (PRNT 80) of 1:160 to 1:10240. One of the six inoculated fetuses was aborted at about 8
months gestation. The fetus was malformed due to uterine adhesions; no RVFV or lesion of viral infection was observed. At birth, calves inoculated in utero had PRNT 80 antibody titers of 1:20 to 1:640. Ten cows, challenge inoculated 30 days postvaccination, remained normal while cows inoculated with diluent and challenge inoculated developed high fever and aborted. Calves from vaccinated cows had colostral derived RVF and antibody titers. These calves were vaccinated with no untoward effect and remained normal after challenge inoculation. Four neonatal naive calves inoculated with the mutagen attenuated RVF virus remained normal, seroconverted, and were protected when challenge inoculated.

The results of this experiment in cattle and previous experiments in sheep indicate that this mutagen attenuated RVF virus is a safe and effective vaccine for pregnant sheep and cattle, and neonatal lambs and calves. Test results for neurovirulence in monkeys suggest that the vaccine will also be safe for man. Thus, this mutagen-attenuated RVF vaccine has all the required characteristics of effective and safe vaccine.
REFERENCES


PROTOCOL TO CERTIFY GERMPLASM FREE OF BLUETONGUE VIRUS

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Some European countries (notably Italy, Germany and The Netherlands) are unable to import semen from the United States collected from bulls which are seropositive by the agar gel immunodiffusion (AGID) test for bluetongue virus. This restriction severely limits the germ pool available to these countries. The AGID test is the approved test and has been required by these countries for import. The disadvantage of this test is that it detects only past exposure to the virus. It does not differentiate between past and current infection.

Our laboratory, through negotiation with US and foreign regulatory and scientific experts, has developed a protocol which can distinguish between current and previous infection. It consists of a complex procedure illustrated schematically below.

Semen, blood and serum are collected from the donor bull. The semen and blood are inoculated into a sheep to allow virus amplification. Blood from the bull and the sheep is inoculated into embryonated chicken eggs (ECE)
for virus detection. Serum from the bull and sheep are tested by the AGID and serum neutralization tests for the presence of antibodies.

The use of the sheep amplification system for the detection of BTV is regarded as the most sensitive method known at the present time to detect BTV in the donor animal. Semen and blood from the bull are inoculated subcutaneously and intradermally into a seronegative sheep. Virus will multiply to detectable levels in this susceptible species, and the viremia will result in demonstrable seroconversion.

**Virus Detection System**

*(Embryonating Chicken Eggs and Cell Culture)*

```
BLOOD
   ↓
   ECE
   (1st passage)

No Embryo Death
(or nonspecific <2d)
   =

Embryo Death
   +/−

ECE
   (2nd passage)

<50% Death
   −

>50% Death
   +/−

Cell Culture
(Serotyping)
   +
```

The diagram of the virus detection system illustrates this procedure in which bull and sheep blood are inoculated intravenously into 11 day old embryonated chicken eggs for isolation. Any embryo dying between 3 and 7 days is submitted for the second (blind) passage in ECE, regardless of the absence of presence of "pathognomonic" lesions. An embryo death rate greater than 50% after the second passage is suggestive of the presence of BTV. The virus is adapted to cell culture. Serotyping by the disc neutralization method is confirmatory for BTV.

Serum from the bull and sheep are examined serologically by two methods. The AGID test is group specific, detecting the presence of antibodies in the bluetongue and epizootic hemorrhagic disease virus
(EHDV) group. The serum neutralization test is serotype specific, capable of quantifying antibody against the five serotypes of BTV (serotypes 2, 10, 11, 13, and 17) and the two serotypes of EHDV (serotypes 1 and 2) present in the United States. Seroconversion or a four-fold increase in titer indicate current or recent infection.

Semen Certification Time Line

<table>
<thead>
<tr>
<th>BULL</th>
<th></th>
<th>ECE</th>
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<th>Vector Season</th>
<th>AGID</th>
<th>SN</th>
<th>Serum, collected 1 x week</th>
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0 7 14 21 28 35 42 49 Post Inoculation Day

The semen certification time line shows the sequence of the scheduled events. Semen, blood and serum are collected from the donor bull for three weeks at twice weekly intervals. During vector season, the serum collection is extended an additional four weeks. Within 48 hours after the collection, the blood and semen are inoculated into a sheep. Serum and blood are collected from the sheep at weekly and twice weekly intervals, respectively, for 7 weeks. The serological testing is performed on all samples together at one time, at the completion of the collection period.

Thus, our protocol is able to verify whether bluetongue virus is present in the semen at the time of collection, regardless of serological status of the bull by AGID. Over a 4 year period, we have certified a total of 38 bulls (representing 228 collections) which were accepted for international export. None of the semen samples tested and exported have demonstrated any indication of BTV infection in the blood or semen of the bull by any of the criteria described.
BOVINE IMMUNODEFICIENCY-LIKE VIRUS INFECTION IN CATTLE

L. D. Miller¹, S. L. Carpenter², J. A. Roth², M. J. Van Der Maaten³ and C. A. Whetstone³

Department of Veterinary Pathology¹, Department of Veterinary Microbiology and Preventive Medicine², College of Veterinary Medicine, Iowa State University, Ames, IA 50011 and the USDA/ARS, National Animal Disease Center³, Ames, IA 50010.

Introduction

Bovine immunodeficiency-like virus (BIV) is a member of the family, retroviridae, and subfamily, lentivirinae. It is antigenically and morphologically distinct from bovine leukemia virus (subfamily oncovirinae) and bovine syncytial virus (subfamily ~pumavirinae).¹³ Synonyms for BIV include bovine visna-like virus³, bovine visna virus¹⁷, and bovine lentivirus.⁸ The virus was isolated from peripheral blood leukocytes and tissues of cattle suspected of having lymphosarcoma and was described in 1972.¹⁵ Its similarity to maedi, visna and ovine progressive pneumonia viruses of sheep was noted in the original papers on isolation and morphology.¹³ Recent interest in animal retroviruses, especially lentiviruses, has increased because of the need for animal models to study the human disease, acquired immunodeficiency syndrome, and the human immunodeficiency virus. At present, most of the work on BIV is still in experimental stages and preliminary reports of natural infections are just beginning to appear.¹,¹⁴,¹⁷,²⁰

Infection in Cattle

Van Der Maaten et al¹⁵ described intravenous inoculation of calves with either infected cultures of bovine embryonic spleen cells in suspension or with culture filtrates. After a transient leukopenia, the calves developed leukocytosis (up to 24,450/mm³) chiefly due to lymphocytosis (19,400/mm³). Concurrently there was enlargement of numerous subcutaneous lymphatic nodules, presumably hemal nodes. Many were visible grossly and they ranged from 2 to 20 mm in diameter. Microscopically the nodules were characterized as lymphoproliferative lesions primarily due to hyperplasia of germinal centers.

More recently tissue culture virus was inoculated into calves by intravenous and intrathecal routes.¹⁷,¹⁸ The calves were followed for 1–2 years but did not develop lymphocytosis or lymphoproliferative responses described in the initial report on cattle inoculation studies. In another experiment⁵, an aliquot of BIV from the original study was inoculated intravenously into a calf and serially passaged 6 times in 2–4 mo old calves by transfusions of 100 ml whole blood 2–3 wk postinoculation (PI). Two control calves and 2 calves that received 1 x 10⁷ frozen and thawed buffy coat cells from the passage 4 calf were also obtained. After BIV inoculation, the virus was isolated and viral specific antibody was demonstrated in calves at each
passage level. In most cases the calves were killed and necropsied 4–6 wk PI. Clinical signs following BIV infection were all transient and included pyrexia, neutropenia and lymphocytosis. In passages 2 and 3, marked one day temperature increases developed on day 10 PI (103.2 to 106.6 F) and day 9 PI (102.6 to 106.2 F) respectively, without signs of respiratory or enteric disease being noted. The 4th passage calf had a one day temperature increase from 102.4 to 104.4 F on day 27 PI. Other signs of illness were not noted. In contrast, the 5th passage calf had a temperature of 105.2 F and 105.0 F on days 4 and 5 PI in association with signs of respiratory disease. The calf received antibiotic therapy for 4 days beginning on day 4 PI and its temperature decreased to 102.6 F by day 7 PI. On days 16 and 17 PI its temperature was 104.0 F but other signs of illness were not seen. Temperatures in the 101–103 F range were recorded for the remaining days for these calves and throughout the observation period for passage 1 and 6 calves. Also temperatures in the 101–103 F range were recorded for the control calves and both calves that were inoculated with passage 4 buffy coat cells. Total leukocyte counts ranged from 4,100 to 19,000/mm³ for passage 1 through 6 calves. Usually 1 or more episodes of neutropenia occurred during the first 2 wk PI. Neutrophil numbers in the 500 to 1500/mm³ range were classified as neutropenia. Lymphocyte numbers in the 8,000 to 11,000/mm³ range were more common in passage 4, 5, and 6 calves than in control or passage 1, 2 and 3 calves. In the calves inoculated with buffy coat cells, lymphocyte numbers above 11,000/mm³ occurred frequently during weeks 3 to 6 of the observation period. The highest value for one calf was 26,520 lymphocytes (WBC 34,000/mm³) on day 24 PI and 18,748 lymphocytes (WBC 21,800/mm³) on day 28 PI for the other. Thus successive in vivo passage of BIV was associated with increased circulating lymphocytes; however, the short observation period precluded evaluation of persistence.

Macroscopic observations at necropsy for inoculated calves were normal or slightly swollen lymph nodes. Bulging of the parenchyma above the capsule was noted when the swollen nodes were incised. Hemal nodes were within the normal size range (2 to 14 mm) and varied in color from pink to reddish brown.

Microscopically, follicular hyperplasia was the prominent change seen in lymph nodes. Germinal centers contained many cells with large pale nuclei, mitotic figures were common and macrophages with stainable material in the cytoplasm ("tingible body macrophages") were increased in number. Often the follicular mantle of small dark lymphocytes was obscured by the more numerous, larger, pale staining lymphoblastic cells. The hyperplastic follicles were closely packed beneath the capsule and in the area adjacent to connective tissue trabeculae that penetrated into the nodes. Few primary follicles remained. Alterations in the paracortex, medullary cords and medullary sinuses were much less conspicuous or were absent. The hemal nodes contained hyperplastic follicles comparable to those seen in lymph.
nodes. The blood sinuses were reduced by lymphoid proliferation. This hyperplasia probably accounted for the macroscopic color change from normal reddish brown to pink that occurred in some nodes. Hyperplastic follicles were found in the spleen and mucosa-associated lymphoid tissue such as Peyer’s patches in the intestine. Morphologic changes in thymus and bone marrow were not noted.

Other reports indicate a similar transient course of clinical signs following experimental BIV inoculation. Thurmond et al. described subcutaneous inoculation of calves with a suspension of BIV-infected tissue culture cells. The calves were examined weekly for 3 months and monthly for 3 more months. One calf developed inspiratory dyspnea with wheezing and rales during the first 2 weeks PI and the other had posterior unilateral lameness with muscle damage about 5 months PI. Lymphopenia was observed 1 week PI and was followed by transient lymphocytosis lasting about 3 weeks. Natural infection was detected in a cow with intermittent lymphocytosis but without overt signs of illness.

Detection of Infection

BIV is detectable by the presence of syncytia following inoculation of peripheral blood leukocytes or other tissue specimens onto monolayer cultures of bovine embryonic spleen cells or other susceptible cells. Identity of the virus can be confirmed by reacting the cells with a known antiserum to BIV coupled with a suitable indicator system.

For detecting antibodies, the indirect immunofluorescent antibody test (IFA) has been the most widely used system. The procedure has been used to monitor the humoral immune response in experimentally infected cattle and has been the method used most frequently for assessing natural infections in cattle. In our literature search only one paper dealt primarily with testing of cattle herds of unknown status. Cattle in eight herds were tested for BIV antibodies by an IFA procedure. Of 235 serums tested, 16 (7%) were seropositive. A number of other procedures used in retrovirology are being used in BIV studies, chiefly as research tools. Western blot analysis has proven to be a useful and more sensitive method than IFA for monitoring humoral responses in experimentally infected calves. Other procedures and techniques being developed or utilized include enzyme-linked immunosorbant assay; reverse transcriptase assay; polymerase chain reaction; molecular cloning; monoclonal antibody production; in situ hybridization; lymphocyte, monocyte, and neutrophil function studies; lymphocyte subset analysis; immunohistochemistry; and use of other cell cultures and animals for virus replication. Information gained from other serologic tests will aid in evaluating the level of sensitivity and specificity for IFA assays currently in use. All of the procedures can contribute to our understanding of the pathogenesis of BIV infection.
BOVINE IMMUNODEFICIENCY

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REPORT OF THE COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUS

Chairman: Dr. B. I. Osburn, Davis, CA
Vice Chairman: Dr. L. D. Miller, Ames, IA

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The Bluetongue and Bovine Retrovirus Committee met from 1:30 to 5:20 p.m. on Tuesday, October 31, 1989, in Room C of the Riviera Conference Center in Las Vegas, Nevada. There were 44 in attendance.

The first part of the meeting on Bluetongue was chaired by Bennie Osburn of the University of California, Davis. Dr. Don Lein of the New York State Veterinary Diagnostic Laboratory, Cornell University, gave an overview of their Bluetongue Virus Program.

In July of 1985, New York State initiated a bluetongue virus control and herd certification program in order to assist the state’s producers in the exportation of their livestock. The program had two main components; 1) a herd certification program whereby herds could be tested on a yearly basis to document their bluetongue virus-free status and 2) a survey/research component which would strive to demonstrate the bluetongue virus-free status of the entire state. The lack of bluetongue virus activity in New York State had been suggested by yearly slaughter surveys conducted by the USDA. The following is a brief summary of the information available to support the bluetongue virus-free status of the State of New York. The survey/research component of the program was divided into three parts: 1) a survey of NYS cattle to determine the prevalence of BT antibodies as detected by the AGID test, 2) a Culicoides trapping program to determine whether resident gnats were infected with BT and 3) a laboratory exercise to determine whether resident Culicoides could be infected with BT. The results of these studies are as follows:

1. The survey plan was to obtain serum samples from the Brucellosis herd
REPORT OF THE COMMITTEE

certification program with an attempt being made to select sera from all counties of the state in proportion to the number of cattle in these counties. Of these, 6,683 were tested for BT and EHD. Of the sera tested, only 20 were positive for BT (0.3%) and 14 (0.2%) for EHD. All of the BT positive animals came from BT endemic states.

In addition to the cattle survey, attempts have been made to obtain serum samples from whitetail deer. These samples were obtained from animals taken during the fall hunting season. For the year 1986, 464 deer samples were tested for BT and EHD and none were positive. For 1987 and 1988, the number of samples tested were 55 and 52 respectively. Again, no BT or EHD reactors were found. The deer survey will continue for the foreseeable future.

2. With the demonstration by the serum survey that BT is not a frequent infection in cattle in NYS, it was important to determine whether the resident Culicoides in NYS were infected with BT. Light traps were located in representative areas as in the southern counties of NYS. The Culicoides trapped by this method were speciated. Pools of trapped gnats were processed for inoculation into sheep and tissue culture. In no instance was there seroconversion of the inoculated sheep and no cell cultures became infected with BT. As an added assurance, gnats were trapped on farms known to have had BT antibody-positive animals on the premises. These insects were also virus negative.

3. The final effort to demonstrate the BT virus-free status of NYS was to collect resident Culicoides and attempt to infect them with BT virus in the laboratory. Initial efforts to colonize the resident gnat were unsuccessful. Attempts to infect adult gnats trapped near Cornell did not result in the recovery of virus beyond the first day of infection. These data tend to support the idea that the resident gnat is an incompetent vector for BT virus. Without the control data, the infection data for the NYS gnats are in question.

Since the beginning of the BT herd certification program, 27,179 tests have been conducted for BT. Of this number, only 0.05% have been positive by the AGID test. All of the positive animals have come from BT endemic states.

In addition to the BT herd program, the Diagnostic Laboratory conducts BT tests predominantly for export requirements. From October 24, 1985, to October 18, 1989, a total of 20,484 BT tests for NYS animals were performed. Of these, 164 (0.8%) were positive as determined by the AGID test. The investigation of these positive animals by the NYS Department of Agriculture and Markets has failed to identify any New York State animals as having been infected with BTV.

A report from Veterinary Services, APHIS, Hyattsville, Maryland, was given by Dr. George Winegar. He reported that there has been a reduction in the BTV trade barrier of animals for export. Australia now accepts BTV
BLUETONGUE AND BOVINE RETROVIRUS

serologically negative animals from the U.S. who have two negative tests while at the quarantine facilities at Grey's Lake, Illinois. The cattle can then be shipped directly to Australia. Australia also accepts semen from the 18 Northeastern states that are AGID negative and SN negative to the five U.S. serotypes. The states of Montana, Nebraska and South Dakota have recently been added to the 18 BT free Northeastern states. AGID positive animals must also be tested for SN antibodies 40 to 60 days following collection.

Semen is now being accepted from AGID positive bulls which have been cultured for virus and certified free by a number of European countries. The U.S. policy for accepting embryos has been modified so that there are specific regulations on the health status of the donor cows or bulls. The collected embryos must be intact and the embryos need to be washed 10 times as per the Embryo Transfer Committee requirements.

Dr. James Pearson, NVSL, APHIS, Ames, Iowa, gave a report on activities at NVSL.

Bluetongue and epizootic hemorrhagic disease virus isolations over the last two years were reported. EHDV was isolated from deer in 13 midwest and mid-central states in 1988. Also, EHDV was isolated from cattle in Tennessee and Virginia. All isolations that have been typed were EHDV-2. BT was isolated from 6 states—Colorado, Illinois, Mississippi, South Dakota, South Carolina and Utah. All the isolations, except from the South Dakota deer and the Utah cows, were BT-17. There were more isolations of EHDV in 1988 than in any year since the NVSL started doing typing over 10 years ago.

There have been only a few isolations of EHDV in 1989 from Alabama, Louisiana and Nebraska. The Louisiana isolate of EHDV-2 was from a sentinel herd that had seroconverted. The Alabama BT isolates were from export animals. Brazil would have allowed importation of serologically positive cattle if they had been negative for virus isolation.

A BT survey of 19 northeastern states, western Washington, western Oregon, Alaska, and Hawaii was conducted in October and November 1988. A total of 16,171 samples were tested, of which 318 were AGID-test positive. Thirteen of the 16 geographic area's samples had 2.0 percent or less AGID-test positive samples. Indiana and Virginia failed to qualify over the last 3 years and North Dakota failed to meet requirements over 2 of the last 3 years. Eleven AGID-test positive samples were negative for neutralizing antibody against BTV types 1 through 20 and EHDV-1 and 2. Forty-four samples had neutralizing antibody against only BT. Of these, 7 had antibody against BT-11 and 1 had antibody against BT-13. Thirty-six samples had antibody titers against 2 or more types of BT with type 11 being the presumed infecting serotype in 24 of these samples. One hundred thirty-four samples had antibody against only EHDV. All of these were EHDV-2 positive, but there were 2 samples which were also EHDV-1
REPORT OF THE COMMITTEE

positive (1:10). Ninety-two samples had antibody against one or more types of BTV and EHDV. Of these, BTV-11 was the serotype with the highest titer for 33 samples and EHDV-2 was the serotype with the highest titer for 39 samples. Antibody was not detected against the exotic types of BT.

The states that have been sampled previously will be surveyed October 9–November 12, 1989, with a final report of AGID results by January 1, 1990. Other states were given opportunity to participate and will be sampled between January 8 and February 23, 1990. These states will be: Montana, Nevada, Utah, New Mexico, Nebraska, Kansas, Iowa, Louisiana, Alabama, Kentucky and South Carolina.

Dr. Ellis Greiner, University of Florida, reported on a bluetongue epizootiologic study in Central America and the Caribbean by the Arthropod-Borne Animal Disease Research Laboratory, Laramie, the Universities of Florida and Wisconsin, IICA, OICD, and ORISA. The objectives of the program were to 1) determine the prevalence of bluetongue in the region, 2) identify the BTV serotypes present, 3) isolate and characterize the virus(es), 4) identify the vectors and define their behavior. Previous serological studies suggested that BTV 1, 6, 12, 14 and 17 were present. To date the viruses isolated from sentinel animals have been serotypes 1, 3, 6 and 12. The pattern of infection in different locations varied and depended upon environmental and possibly other factors. For instance in the Central American Pacific Coast there were well defined periods when infection occurred and the age of animals at infection was 6–14 months of age. In contrast, the Atlantic Area has a high incidence of infection throughout the year and animals became infected at 4 to 8 months of age. The principle Culicoides vector is C. insignis which is the predominant vector in nearly all of the sentinel sites in Central America and the Caribbean. The second most common Culicoides is C. felasifer. A virus isolate has been made from C. insignis. C. insignis is closely associated with cattle and BT virus is capable of proliferating in this vector.

The genetic variation of BTV as determined by oligonucleotide fingerprinting was reported by Dr. N. J. MacLachlan of the University of California, Davis. One hundred and ten field isolates have been plaque purified and 35 of these are currently undergoing two dimensional oligonucleotide fingerprinting. A semi-automated system has been developed which permits evaluation of all 10 segments from two viruses per week. Of the 35 viruses examined so far, variations have been found in all segment and point mutations were present in some. There were between 2 and 4 constellations present in the 10 segments with variations ranging from 1+ to 4+. The indications are that BTV is a highly variable virus.

In another study carried out by Dr. MacLachlan, he described the pathogenesis of BTV infection in cattle. The cattle infected with plaque picked virus persisted without genotypic variation.

Virus was present in plasma through the first week post infection, in
BLUETONGUE AND BOVINE RETROVIRUS

mononuclear cells for 10–14 days and in erythrocytes for 6 weeks post infection. The virus replicated in macrophages but not in resting lymphocytes. Following inoculation, virus was reisolated from lymph nodes from 24 through 28 days post inoculation. A similar pattern was observed in the spleen. It was concluded that lymphoid tissue was the primary site of replication. Virus was found in endothelial cells in 2 of 5 calves. Bone marrow was not a site of replication. The pathogenesis appears to be that the initial replication occurs in macrophages followed by endothelial cells. The released virus attaches to RBC’s. The long term viremia is associated with the relatively long life span of bovine erythrocytes. Persistence does not occur.

Dr. William Wilson of the Arthropod-Borne Animal Disease Research Laboratory in Laramie, Wyoming, described the procedures used to refine the hybridization assay. The procedure has been shortened by eliminating the phenol extraction, treating with diethylpyrocarbonal, 50% glyoxol and binding to the solid matrix. The procedure takes 1.5 hours, followed by 2 hours of baking and hybridization overnight.

These dot blot hybridization procedures have been used to identify BTV and EHDV in pools of Culicoides. A cDNA probe of gene segment 3 from BTV 17 obtained from Dr. Polly Roy has been used in the hybridization assays. The sensitivity is such that 1 in 49 BTV infected pool of flies can be identified and 1 in 100 EHDV infected pool of flies.

Dr. Mary Sawyer of the University of California, Davis, described the semen certification protocol which was reported in the general session.

Dr. Osburn announced that plans were underway for a Bluetongue and Related Orbivirus Symposium at OIE in Paris, France, in 1991.

After a brief recess, Dr. Lyle Miller called the group to order and conducted the session on Bovine Retrovirus. Dr. James Evermann, Washington State University, Pullman, WA, presented a paper entitled “Impact of Bovine Leukosis Virus by Rectal Palpation: Herd Studies.” He listed management practices and other procedures that can result in transfer of blood and thus transmission of bovine leukosis virus (BLV). Included were gouge dehorning, ear tagging, tattooing, removal of supernumerary teats, other surgical procedures, and vaccinations. Regarding BLV transmission via the rectum, Dr. Evermann, et al., demonstrated that manipulation or trauma of the rectal mucosa was not required for transmission. Deposition of 2 ml of BLV-infected whole blood via catheter into the rectal lumen about 20 cm anterior to the orifice resulted in infection of 2 of 6 calves. Studies in commercial and university herds showed that intervention procedures as simple as rinsing the obstetrical sleeve with tap water between rectal examinations on different animals are effective in reducing BLV transmission. More elaborate procedures such as vigorous washing with detergent or single use of disposable sleeves may be desirable in some situations. The risk of transmission is greater as the number of rectal examinations per
animal increases. Other factors including herd infection rate, density of animals, antigen status of the donor, immune competence of the recipient, integrity of the intestinal mucosa, concurrent infections and experience of the palpator can affect the risk of transmission. Also, the cost of altering management and handling procedures is important.

Dr. C. A. Whetstone, USDA, ARS, NADC, Ames, IA, discussed the humoral immune response in cattle to the bovine immunodeficiency-like virus (BIV). This virus has been shown to have structural, genetic, antigenic and biological similarities to the human immunodeficiency virus. A BIV isolate propagated in bovine embryonic spleen cells was used to inoculate BIV sero-negative calves, and the humoral immune response of those calves was monitored up to 2-1/2 years post inoculation (PI) using both indirect fluorescent antibody (IFA) and Western blot assays. Antibodies to BIV could be detected as early as 2 weeks PI by Western blot, by 4 weeks PI using IFA, and up to 2-1/2 years PI using both techniques. Serum anti-BIV titers were variable in different animals over time, dropping to non-detectable levels in some animals. Fluorescence in the IFA test was typically diffuse and cytoplasmic. On Western blot, cattle sera reacted with BIV antigenic determinants on p110, p55, p26, p24, p18, p15, and p13. The strongest and earliest anti-body reactions were to p26, the putative major core (capsid) protein. Antibody reaction to p110, a glycoprotein, were weak and usually detected in samples after several months PI. Antibody responses to all BIV proteins were also found in sera from naturally infected cattle, with anti-p26 response being the strongest. Serum antibodies detected by both systems did not always correlate. Some IFA positive serum samples from infected cattle, especially from those that were naturally exposed to the virus, were negative on Western blot. Also, some weak responders on Western blots, i.e. showing only a minimal response to p26, were IFA negative. Pre-colostrum serum samples from twin calves born to an experimentally-inoculated, BIV antibody positive cow were negative for detectable BIV-specific antibodies, while post-colostrum serum samples were positive by both IFA and Western blot. Inoculation of cattle with material from low-passage BIV-infected tissue culture cells resulted in infection as determined by virus reisolation and viral-specific humoral immune responses. Inoculation of cattle with high passage BIV-infected cell culture material did not always cause infection. However, inoculation of calves with high passage BIV culture material followed by calf-to-calf passage, by blood transfusions, resulted in consistent infection with BIV as determined by virus reisolation and strong BIV-specific humoral immune responses both on IFA and Western blot.

These studies show that calves inoculated with BIV mount a BIV-specific antibody response that can be detected using either an IFA or Western blot technique. Anti-BIV sera from both experimentally inoculated and naturally infected cattle recognize BIV polypeptides of 110K, 55K, 26K, 24K, 18K, 15K and 13K on Western blot. The strongest and earliest antibody
response detected by Western blot in experimentally infected calves was to p26, the putative major core antigen. Anti-BIV titers may vary or diminish over time.

Testing of a limited number of serums from herds of unknown status by both procedures showed that fewer samples were positive by Western blot. Discrepancies were greatest among serums classified as weakly positive by IFA. Further study is required to establish the specificity and sensitivity of the tests.

Procedures used to establish BIV infected cell lines as steps in the development and application of an IFA test were discussed by Dr. John Black, American Bioreserch, Milton, Tennessee. Dr. Black obtained BIV infected canine thymus cells from Dr. M. Van Der Maaten, USDA, NADC, Ames, IA, and co-cultivated them with bovine kidney cells prepared in his laboratory (BBK) and with Madin-Darby bovine kidney cells (MDBK) through 10 passages. In BBK cell preparations, residual canine thymus cells were found to persist through 10 more passages. A pulse of canine parvovirus was used to destroy the canine cells. An additional 5 passages of BBK cells, which do not support canine parvovirus replication, permitted establishment of the BIV infected BBK cells for use in the IFA test. The cells had a high BIV infection rate but were poor as an IFA substrate. For the faster growing MDBK cells, an additional 10 passages permitted elimination of the slower growing canine thymus cells. The MDBK cells were found to have a low BIV infection rate. Thus BBK cells, which grow more slowly, were mixed 1:10 with MDBK cells and carried through 10 more passages. The resulting MDBK cell preparation had a high BIV infection rate, was devoid of BBK cells, was negative for bovine virus diarrhea virus infection and was a good BIV, IFA substrate.

Tests were conducted on serums from a bank of frozen samples collected over several years and from randomly selected healthy cattle, mostly dairy cattle. The results are as follows:

<table>
<thead>
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<tbody>
<tr>
<td>Tennessee</td>
<td>841</td>
<td>33</td>
<td>3.90%</td>
</tr>
<tr>
<td>Utah</td>
<td>400</td>
<td>0</td>
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</tr>
<tr>
<td>Texas</td>
<td>240</td>
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Dr. Lyle Miller, Iowa State University, Ames, IA, described BIV infection in calves used for rapid successive in vivo passage of BIV via transfusion of
whole blood. The information is included in the paper presented in the general session.

Dr. Osburn reviewed work on BIV by Drs. Mark Thurmond, University of California, Tulare; Jeff Stott, University of California, Davis, and other colleagues. Two calves were inoculated subcutaneously in the right prescapular region with 1 ml of cell suspension of BIV infected bovine embryonic spleen (BESP) cells. The calves were 55 and 69 days of age when inoculated. One calf was infected with bovine leukemia virus at the time of BIV inoculation. Both calves seroconverted to BIV by the IFA test and antibodies were undetectable after 124 days post-inoculation (PI) in one calf and 327 days PI in the other, which was the BLV carrier.

Reverse transcriptase activity and syncytium formation in co-cultivated lymphocytes and BESP cells were observed between 33 and 47 days PI. Presence of provirus was confirmed at 9–10 months PI with positive polymerase chain reaction (POLgene) results from peripheral blood lymphocytes. Within the lymphocyte population, there was transient loss of T helper cells beginning about 2 months PI and continuing for 2–3 months. Enlargement of peripheral lymph nodes or hemal nodes was not noted. The bulls remain in good physical health more than 1 year after BIV infection.

No resolutions were offered.
SUMMARY OF RESULTS OF BRUCELLOSIS TESTING FOLLOWING ADULT VACCINATION IN FLORIDA BEEF HERDS

E. Arza, D.V.M.¹
M. D. Salman, BVMS, MPVM, Ph.D.²
D. P. Warner, D.V.M., M.S.³

During the mid to late 70s, brucellosis vaccination with Strain 19 was being experimentally applied to adult animals in infected dairy herds in Florida in an effort to reduce losses and control the effects of the disease. Immediate positive results were achieved in reducing levels of infection and almost eliminating the greatest economical loss (i.e. abortions). Yet a serious problem surfaced which ultimately prevented wide acceptance of this procedure. Relatively high titer for brucellosis interfered with interpretation of serological tests.

During the first half of this decade many investigators diligently researched ways to improve the procedure in order to minimize the diagnostic dilemma. All this effort culminated in the development by the USDA of a new Adult Vaccination (AV) Program. This new program includes reduction in the dose, usage of the liquid form of the vaccine, and strict control of the handling, distribution and usage of the vaccine. The new program is applied to both dairy and beef herds with certain modification. Full details of this program were presented elsewhere.¹

In July of 1985, the present program was initiated in Florida; by the end of 1986, approximately 100 total herds had been whole herd vaccinated. In early 1987, Florida Brucellosis Program officials determined that it was necessary to implement a mandatory whole herd vaccination of all known infected herds, and the Adult Vaccination (AV) Task Force was initiated. The rationale for utilizing adult vaccination in beef herds was to:

1. Provide a practical solution to control the spread of brucellosis among and within herds.
2. Promote an efficient program that would reduce the number of reactors and the number of herd tests that were required to “clean up” infected herds.

Approximately 800 herds have been whole herd vaccinated in the present program. 600 of these were completed during the 1987 task force.

It is the objective of this paper to summarize the serological responses of 441 Florida beef herds vaccinate as adults with reduced dose (0.5 billion colony forming units) Strain 19 vaccine.

NATURE OF DATA

A data set was created for this study from individual herd records during the period June through August, 1988. Individual herd records were
reviewed from a list compiled from the AV task force files. Only herd records, with reliable and complete information, that were validated by the field epidemiologists were entered in the data set.

RESULTS

There were 441 herd tests and 56,707 animals entered in this data set. Three hundred herds were first post AV tested, 105 were second post AV tested, 28 were third post AV tested, and 8 were fourth post AV tested. The average herd size was 134 animals.

The following figures represented a summary of the analysis on this data set. The first series of figures correspond to results as they pertain to individual animals and the latter as they pertain to herds.

Figure 1: PERCENTAGE OF CARD POSITIVE ANIMALS CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

As expected, percentage of Card positive animals decreased as the time interval increased from vaccination to testing. However, even in those herds tested after 365 days there was still a substantial percentage of Card positive animals.

Figure 2: PERCENTAGE OF ANIMALS POSITIVE TO RIVANOL (≥ COMPLETE @ 1:25) CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

A substantial drop in Rivanol positive animals was noted in those herds tested at 3–6 month post AV and again in those tested 6–12 month post AV. It is the authors' experience that this slope gradually decreases and eventually plateaus, fluctuating at the 1%–3% level.

Figure 3: PERCENTAGE OF ANIMALS POSITIVE TO RIVANOL 5 (INCOMPLETE @ 1:100) CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

Even at the 3–6 month post AV interval, a relatively low percentage of "high" Rivanol titers were found. This is exemplified in those herds tested at >365 days post AV.

Figure 4: PERCENTAGE OF ANIMALS POSITIVE TO CF (≥ 1+ @ 40) CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

These results reiterate the fact that the Complement Fixation (CF) test is considered the "serological test of choice" by most experts. Many researchers have shown it to have the highest specificity of any existing serological test for brucellosis. The substantially low percentage of "high" CF titered animals found post AV makes the job easy for interpretation of results and illustrates the low number of infected animals found in these herds.
ADULT VACCINATION IN FLORIDA BEEF HERDS

Figure 5: PERCENTAGE OF ANIMAL REACTORS CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

As expected after reviewing the previous two figures, the percentage of reactors found parallels those results of the Rivanol and CF tests. It should be pointed out that final interpretation of test results depended on three major criteria: level of individual and herd titer (in both Rivanol and CF tests), time interval from vaccination to testing, and history of herd.

Figure 6: PERCENTAGE OF REACTORS (USING RIVANOL OR CF) BY YEAR FROM BIS

In order to review the short term effects of the program, the Brucellosis Information System (BIS—Ft. Collins, CO) was accessed. This graph represents all on-farm testing conducted over a 12-month period of time (June 1—May 31) in the 15 county area of highest incidence of brucellosis in south central Florida. A standard cut-off level was utilized for the Rivanol (≥ incomplete @ 1:100) and CF (≥ 1+ @ 40) tests. It is important to note there has been approximately a ten fold decrease in “high” CF titered animals between 1985–86 and 1988–89 while almost twice as many tests were conducted.

Figure 7: PERCENTAGE OF HERDS TESTED WITH AT LEAST ONE POSITIVE RIVANOL ANIMAL CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

During the first year post adult vaccination, the data indicates a relatively high percentage of herds with at least one Rivanol positive animal. By 12 months post AV approximately 75% of the herds had Rivanol positive animals and by 18 months, that level was reduced to approximately 60%.

Figure 8: PERCENTAGE OF HERDS TESTED WITH AT LEAST ONE POSITIVE RIVANOL 5 ANIMAL CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

The significance of this data is that by 6 months post AV only 50% of the herds tested had “high” Rivanol titered animals, and by 12 months post AV this level was reduced to 35%.

Figure 9: PERCENTAGE OF HERDS TESTED WITH AT LEAST ONE REACTOR ANIMAL CLASSIFIED BY TIME INTERVALS POST ADULT VACCINATION

As with the previous figure, a significantly low percentage of herds tested had any reactor animals. By 6 months post AV, the level was at 45%, and by 12 months post AV the level was reduced to 17%. It is worth noting that of the 8 herds reviewed that had a fourth post AV test, none (zero) of them had any reactors found.

CONCLUSION

The tremendous progress being achieved in the Florida Brucellosis
Program is the ultimate measure of the value of whole herd vaccination. Today there are less than 200 infected herds in the entire State of Florida which compares favorably with the fact that only four years ago there were in excess of 1,000 infected herds.

As stated in earlier presentations, "our present tools for control and eradication of brucellosis have to be used in the best possible ways to accommodate the cattle industry as long as the efficiency is kept at a maximum".¹

This summary of the serological response observed in Florida beef herds vaccinated as adults with reduced dose of Strain 19 vaccine clearly illustrates the effectiveness and efficiency of this tool and has proven to be a practical solution in controlling the spread of brucellosis among and within herds.

In conclusion, we realized that we were limited by the relatively short period of time post vaccination evaluated and that the long term significance of the program can only be measured by the continual analysis of the data being generated.

REFERENCES

5. Scanlan, C. M., et al., Laboratory Diagnosis of Bovine Brucellosis, Publication No. 1611, School of Veterinary Medicine, Auburn University, Auburn Veterinarian; 6–11.

¹Presently AAVIC, USDA, APHIS, V.S., Conyers, GA; from 4/85 to 8/88, Station Brucellosis Epidemiologist, Jacksonville, Florida.
²Associate Professor, Dept. of Environmental Health, Colorado State University, Ft. Collins, CO.
³Station Brucellosis Epidemiologist, USDA, APHIS, V.S., Sebring, Florida.
⁴All these herds had been diagnosed as being infected and Adult Vaccinated.
STATUS REPORT — 1989
COOPERATIVE STATE/FEDERAL BRUCELLOSIS ERADICATION PROGRAM

Jan D. Huber, D.V.M., M.S.
John D. Kopec, D.V.M.
W. C. Stewart, D.V.M., M.S.

Fiscal year (FY) 1989 saw the Brucellosis Eradication Program continue the accelerated progress that began in the early years of this decade. The number of brucellosis affected herds detected during the year and the number of animals classified as reactors both reached all time annual lows. During the third quarter, for the first time in the history of the program, the number of herds under quarantine in the United States dropped below 2,000. This decline continued, and when the FY ended on September 30, 1989, the number of quarantined herds stood at 1,411, a remarkable 39 percent reduction from the 2,345 a year earlier. Ninety percent of the bovine brucellosis detected during FY 1989 was located in just 8 states; and, conversely, 74 percent of the nation's mature cattle were located in the 44 states that had attained Class Free or Class A status.

On February 17, 1989, the additional restrictions on the interstate movement of certain cattle from Class B and Class C states or areas, went into effect in accordance with an earlier recommendation by the USAHA. The Class C restrictions became moot, however, when in June, southern Florida, the last Class C area in the United States, achieved Class B status.

Increased data processing costs at the Fort Collins Computer Center and the unavailability of additional operating funds resulted in the precipitous termination of the Brucellosis Information System (BIS) in July. This action caused temporary inconvenience in certain states, but had the beneficial effect of further accelerating conversion to the more economical decentralized Brucellosis Recording and Reporting System (BRRS) that was already in progress. Instead of installing 19 microcomputer systems for the brucellosis ring test (BRT) and vaccination as it had planned for FY 1989, the National Center for Animal Health Information, with the cooperation of local state and federal offices, responded to the needs created by the BIS situation by installing 26. By the end of the year hardware and software and training in their use had been provided to 39 States on the herd system and 36 states on MCI. During this time, all states still using the BIS BRT and vaccination programs were converted to microcomputer systems. Additionally, a networked data base was developed to resolve problems caused by the high volume of program activity in the high-incidence states. Implementation of this system will begin in the first quarter of FY 1990 with Texas and Florida having top priority for completion.

In June, meetings, jointly sponsored by the Livestock Conservation
Institute (LCI) and the American Farm Bureau Federation, were held in each of the six high-incidence states. These meetings of state, federal and industry representatives were to address local program concerns and to rally support for final eradication effort. Each of these meetings showed strong industry support for an accelerated effort that would quickly eliminate the remaining brucellosis and bring the brucellosis eradication program to an early and successful conclusion.

Slide 1

On September 30, 1989, 27 states and the Virgin Islands were Class Free, 17 states and Puerto Rico were Class A, and 6 states were Class B. In June 1989, Kentucky advanced from Class B to Class A, and Florida changed from a split Class B/Class C status to all Class B.

Slide 2

Twenty percent of all beef cows that have calved are now located in Class Free states, 48 percent in Class A states, and 32 percent in Class B states.

Slide 3

Of the nation's 10.2 million milk cows, 60 percent are in Class Free states, 31 percent in Class A states, and only 9 percent in Class B states.

Slide 4

Of the United States total cow herd, 30 percent of all beef and dairy cows are in Class Free states, 44 percent in Class A states, and 26 percent in Class B states.

Slide 5

There were 2,502 reactor herds found in FY 1989. This was a decrease of 29 percent from the 3,514 reactor herds detected in FY 1988. By considering Florida as all Class B status for the entire fiscal year, 82 percent (2,051 herds) were located in the Class B states. Of the remainder, 450 reactor herds were in Class A states and there was one case of brucellosis in a Class Free state. The latter was on a beef ranch in Wyoming that was discovered by an MCI traceback in February 1989. Following a thorough epidemiologic investigation, it was concluded that the most likely source was the infected elk or bison in the area. The herd was depopulated through a quarantined feedlot. Tests conducted on adjacent herds did not disclose additional infection.

Slide 6

Of the 2,502 reactor herds found during FY 1989, 95.6 percent were in 11 states. The remaining 4.4 percent were in 39 states and Puerto Rico. Texas had 1,037 reactor herds which represented 41.5 percent of the total. Florida, Oklahoma, and Louisiana had 249, 237, and 203 reactor herds.
respectively. These three states combined had 27.5 percent of the total. Seven states, with a range of 39 to 187 reactor herds each, had 26.6 percent of the total for the year.

**Slide 7**

Forty-one dairy herds were found affected with brucellosis as the result of BRT surveillance. This resulted from the blood testing of 1,136 BRT suspicious herds. There were a total of 2,096 suspicious BRT laboratory reports in FY 1989.

**Slide 8**

The 12 million MCI tests in FY 1989 were up 200,000 from the 11.8 million in FY 1988. Of this total, 6.2 million (51.5 percent) were samples collected at packing plants and 5.8 million (48.5 percent) were collected at stockyards, farms or ranches.

**Slide 9**

A total of 16.3 million cattle were tested for brucellosis in FY 1989, an increase of 200,000 (1.2 percent) over the previous fiscal year. There were 4.3 million animals sampled by farm or ranch herd tests versus 12 million MCI tests. Although there was a slight increase in the number of tests, the total number of reactors (38,000) for FY 1989 was 21 percent less than the 48,000 detected in FY 1988.

**Slide 10**

There were 9.2 million calves vaccinated for brucellosis in FY 1989. This was down 800,000 from the record 10 million vaccinated the previous fiscal year. This 8 percent decrease occurred while the number of calves eligible for vaccination increased by about 2 percent.

**Slide 11**

The number of swine tested for swine brucellosis in FY 1989 was 1.9 million, a decrease of 26.9 percent of the number tested in FY 1988. This total included 1.1 million tested under the Market Swine Testing (MST) Program (down 42 percent) and 780,035 tested on farms (up 3 percent). The increase in farm testing resulted from pseudorabies (PR) program development in several states where swine tested for pseudorabies were tested for swine brucellosis as well.

**Slide 12**

The number of Validated Brucellosis Free herds increased from 3,104 in FY 1988 to 3,445 in FY 1989. This is an 11 percent increase in the nation's validated herds.

Important indicators continue to show that the prevalence of swine brucellosis in the United States remains at a very low level, and now with
the entry of all states in the program, the potential for early eradication is great.

Slide 13

West Virginia attained Validated Brucellosis-Free status (Stage III) during the year, bringing to 33 the number of states plus Puerto Rico and the Virgin Islands that have achieved this goal. West Virginia’s advancement from “Stage I” to validated status is credited to its new pseudorabies program which led to statewide testing of all breeding herds. The great majority of the nation’s swine are located in states that are Validated Brucellosis-Free.

In addition to West Virginia, the states of Alaska, Arizona, California, Colorado, Connecticut, Delaware, Idaho, Indiana, Iowa, Nebraska, Maine, Maryland, Minnesota, Montana, Nevada, New Hampshire, New Jersey, New York, North Dakota, Illinois, Ohio, Oregon, Pennsylvania, Rhode Island, South Dakota, Tennessee, Utah, Vermont, Virginia, Washington, Wisconsin, and Wyoming, as well as Puerto Rico and the Virgin Islands, held Validated Brucellosis-Free status at the end of FY 1989. Seven states were in Stage II — Alabama, Arkansas, Georgia, Hawaii, Louisiana, New Mexico, and Oklahoma. Ten states were in Stage I — Florida, Mississippi, North Carolina, South Carolina, Kentucky, Massachusetts, Michigan, Kansas, Missouri, and Texas. With the admission of Mississippi, New Mexico, and Texas in FY 1989, all states are now officially enrolled in the program.

Slide 14

The reactor rate for all surveillance tests of 0.04 percent did not change from FY 1988. The on-farm testing reactor rate diminished from 0.04 percent in FY 1988 to 0.02 percent in FY 1989. This dramatic decrease in the on-the-farm reactor rate reflects the success of swine brucellosis eradication in states where the disease was once epidemic. Increased surveillance and on-the-farm testing associated with pseudorabies eradication has also substantially improved the identification and elimination of foci of swine brucellosis infection.

A total of 59 infected herds were detected in FY 1989. For the most part, these were small herds in states with endemic areas (Alabama, Florida, Georgia, and Oklahoma). The total number of MST reactors was 531 and this is a reduction from the 787 reactors in FY 1988.
Cattle Brucellosis
State Classification

<table>
<thead>
<tr>
<th>Number*</th>
<th>27</th>
<th>17</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>Herd Infection Rate</td>
<td>0</td>
<td>&lt;0.025%</td>
<td>&lt;1.5%</td>
</tr>
<tr>
<td>Adjusted MCI Rate</td>
<td>&lt;0.05%</td>
<td>&lt;0.1%</td>
<td>&lt;0.3%</td>
</tr>
</tbody>
</table>

* Not Included:
District of Columbia - Free
Virgin Islands - Free
Puerto Rico - A
Yellowstone National Park, WY - Not Classified

September 1989
Brucellosis
Distribution of Beef Cattle by Brucellosis Status

Class A
48%

Free
20%

Class B
32%

September 1989
Brucellosis
Distribution of Dairy Cattle by Brucellosis Status

Free 60%
Class B 9%
Class A 31%

September 1989
Brucellosis
Distribution of All Cattle by Brucellosis Status

Class A 44%
Free 30%
Class B 26%

September 1989
### Brucellosis Eradication

**Number of Reactor Herds Found (According to State Classification)**

<table>
<thead>
<tr>
<th>Year</th>
<th>Class Free</th>
<th>Class A</th>
<th>Class B</th>
<th>Class C</th>
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<td>32</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1983</td>
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<td>20</td>
<td>19</td>
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<td>5</td>
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<td>1985</td>
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<td>19</td>
<td>8</td>
<td>4</td>
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<td>1986</td>
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<td>18</td>
<td>7</td>
<td>4</td>
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<td>1987</td>
<td>25</td>
<td>19</td>
<td>6</td>
<td>2</td>
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<tr>
<td>1988</td>
<td>27</td>
<td>16</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>1989</td>
<td>27</td>
<td>17</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

**New State Classification (Effective May 1, 1982)**

- **Class A**
- **Class B**
- **Class C**

**Classifications:**
- Certified-Free
- Modified Certified

**States with dual status:**
- Wyoming - Class Free and Class A
- Texas and Florida - Class B and Class C
- Montana - Class Free and Class A
- Texas and Florida - Class B and Class C
- Arizona - Class B and Class C
- Texas and Florida - Class B and Class C
- Florida - Class B and Class C

(Estimated Fiscal Year)

---

**Number of States in Each Classification**

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Certified Free</th>
<th>Modified Certified</th>
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</thead>
<tbody>
<tr>
<td>1982</td>
<td>32</td>
<td>18</td>
</tr>
</tbody>
</table>

---

**Graphs and Charts:**

- Bar graphs showing the number of reactor herds found each year.
- Line graphs showing the trend over the years.

---

**Footnotes:**

*Estimated Fiscal Year

---

**Table:**

<table>
<thead>
<tr>
<th>Year</th>
<th>Class Free</th>
<th>Class A</th>
<th>Class B</th>
<th>Class C</th>
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<td>1986</td>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>1989</td>
<td>27</td>
<td>17</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>
Brucellosis Eradication

Percent of Total Reactor Herds Found

*Fiscal year 1989
Total herds: 2,502

41.5%
States: 1
Herds: > 1,000
Total reactor herds = 1,037

4.4%
States: 40
Herds: < 30
Total reactor herds = 110

26.6%
States: 7
Herds: 30 < 200
Total reactor herds = 666

27.5%
States: 3
Herds: 200 < 1,000
Total reactor herds = 689

* Estimated
Brucellosis Eradication

Milk Ring Test Results (BRT)

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Total Suspicious BRT Tests</th>
<th>Follow-up Herd Blood Tests</th>
<th>Infected Herds Found</th>
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<tbody>
<tr>
<td>1979</td>
<td>2,177</td>
<td>350</td>
<td>1944</td>
</tr>
<tr>
<td>1980</td>
<td>3,091</td>
<td>317</td>
<td>2,553</td>
</tr>
<tr>
<td>1981</td>
<td>4,771</td>
<td>2,733</td>
<td>2,096</td>
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<tr>
<td>1982</td>
<td>3,607</td>
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<tr>
<td>1989*</td>
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<td>1,136</td>
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*Estimated
Brucellosis Eradication

Market Cattle Testing Program

<table>
<thead>
<tr>
<th>Fiscal year</th>
<th>At packing plants</th>
<th>Other</th>
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<tbody>
<tr>
<td>1979</td>
<td>54.8%</td>
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<td>1980</td>
<td>41.7%</td>
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<td>1986</td>
<td>46.1%</td>
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<td>52.8%</td>
</tr>
<tr>
<td>1988</td>
<td>49.0%</td>
<td>51.0%</td>
</tr>
<tr>
<td>*1989</td>
<td>51.5%</td>
<td>48.5%</td>
</tr>
</tbody>
</table>

*Estimated

Millions of cattle blood tested
Brucellosis Eradication

Blood Testing: Cattle

Millions cattle tested

Thous. reactors found

Fiscal year

*Estimated
Brucellosis Eradication
Calves Vaccinated

*Estimated
STATE/FEDERAL BRUCELLOSIS ERADICATION PROGRAM

1. Status in Program of States
33 states, Puerto Rico, and Virgin Islands — Validated Free.
7 states in Stage II.
10 states in Stage I.
3 states... Mississippi, New Mexico, and Texas entered program in FY 1989.

2. Validated Free Herds — 3,445
This is an 11% increase from last year (3,104).

3. Number of swine tested in FY 1989
First point 78,096 1,085,379 MST (1.9M)
Slaughter 1,007,283 down 42 percent
Area and Other 780,035 up 3 percent (754,000)
Total 1,865,414 decrease of 26.9 percent (2.6M)

4. Number of brucellosis reactors in FY 1989
First point (0.28%) 220
Slaughter (0.03%) 311 531 (0.05 percent) (0.04%)
Area and Other 160 0.02 percent (0.04%)
Total 691 0.04 percent (0.04%)

5. Unidentified swine tested — MST
Number Reactors Percent
FY 1989 881,605 256 (37% all reactors) 0.03
FY 1988 650,000 ... ... ...

6. States (9) Reporting New Infected Herds in FY 1989
FY 1988 — 8 States

Alabama 22 22
Georgia 13 15
Florida 9 18
Oklahoma 7 0
North Carolina 3 1
Louisiana 2 0
Arkansas 1 0
South Carolina 1 5
Virginia 1 1
Missouri 1
Massachusetts 1

TOTAL 59 TOTAL 64
Three states (Kentucky, Massachusetts, and Missouri) reported one infected herd each last year and none this year. Four states (Arkansas, Oklahoma, Louisiana, Virginia) reported infected herds in FY 1989 after reporting none in FY 1988.

7. Swine Brucellosis/Pseudorabies Surveillance Task Force

8. Developed docket postponing PCFIA as an official test for Swine Brucellosis.

9. Developed proposed changes to the UM&R.

10. Developed a plan and budget for an accelerated 3 year eradication program.

11. Conducted a national survey of swine slaughter plants.

12. Developed a quarterly reporting form for swine brucellosis eradication activities.

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Swine Brucellosis
Program Stages - October 1, 1989
Swine Brucellosis
Validated Herds FY 1989 (Sept. '89)

Total Herds: 3,445
## Swine Brucellosis

### Number of Swine Tested

<table>
<thead>
<tr>
<th></th>
<th>FY 1989</th>
<th>FY 1988</th>
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<tbody>
<tr>
<td>MST</td>
<td>1,085,379</td>
<td>1,900,000</td>
</tr>
<tr>
<td>Area &amp; Other</td>
<td>780,035</td>
<td>754,000</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>1,865,414</strong></td>
<td><strong>2,654,000</strong></td>
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## Swine Brucellosis Reactors

### FY 1989

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>MST</td>
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<td>0.05</td>
</tr>
<tr>
<td>Area &amp; Other</td>
<td>160</td>
<td>0.02</td>
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<tr>
<td><strong>Total</strong></td>
<td>691</td>
<td>0.04</td>
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<tr>
<td></td>
<td>FY 1989</td>
<td>FY 1988</td>
</tr>
<tr>
<td>----------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Number</td>
<td>881,605</td>
<td>650,000</td>
</tr>
<tr>
<td>Reactors</td>
<td>256</td>
<td>- - -</td>
</tr>
<tr>
<td>Percent</td>
<td>0.03</td>
<td>- - -</td>
</tr>
<tr>
<td>State</td>
<td>Cases</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Alabama</td>
<td>22</td>
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<tr>
<td>Georgia</td>
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<td>Oklahoma</td>
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<tr>
<td>North Carolina</td>
<td>3</td>
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<tr>
<td>Louisiana</td>
<td>2</td>
<td></td>
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<tr>
<td>Arkansas</td>
<td>1</td>
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<tr>
<td>South Carolina</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Total - 59
Swine Brucellosis
Infection Rate

Percent

0.5

0.4

0.3

0.2

0.1

0


Fiscal Year

* Estimated

Total tests  On farm  MST
Swine Brucellosis
Animals Blood Tested

Percent

Fiscal Year


* Estimated

Total tests  On farm  MST

HUBER, KOPEC, STEWART
REPORT OF THE COMMITTEE ON BRUCELLOSIS
LAS VEGAS, NEVADA
OCTOBER 30-31, 1989

Chairman: Mr. J. B. Armstrong, Kingsville, TX

Vice Chairman: Dr. T. H. Woods, Little Rock, AR

J. Adams, VA; J. L. Alley, AL; L. A. Anderson, IA; J. F. Badger, MO; C. E. Barton, TN; P. Becton, FL; J. S. Cargile, TX; D. B. Childs, FL; J. A. Cobb, GA; T. Conger, AR; T. M. Cook, DC; A. M. Creswell, TN; M. L. Dierks, NE; F. J. Drazek, NY; H. F. Embry, IL; S. R. England, NM; B. H. Espe, OK; W. B. Fairchild, LA; G. H. Frye, MD; B. Gallagher, SD; P. C. Genho, FL; F. D. Gregerson, CO; J. H. Hagler, TX; G. A. Hall, OK; W. T. Harrer, MT; R. L. Hartin, OK; E. R. Hinshaw, AZ; J. W. Holcombe, TX; J. D. Huber, MD; F. S. Idtse, WI; E. Jeffers, NM; A. W. Keating, IL; J. D. Kopec, MD; H. F. McCrory, MS; R. E. Nelson, VT; W. G. Nelson, ID; D. L. Notter, KY; D. W. Parr, TX; J. O. Pearce, Jr., FL; W. D. Prichard, OR; P. K. Saini, MD; J. E. Slauter, MO; W. E. Stemler, IL; N. Stirling, SD; N. R. Swanson, WY; L. P. Thomas, WV; E. T. Thorne, WY; D. K. Thorpe, SD; K. J. Throlson, ND; L. C. Vanderwagen, CA; D. U. Walker, VT; J. M. Williams, NE.

(35 members were present during the first day, and 33 during the second day.)

Chairman John B. Armstrong called the meeting to order. He indicated that he was gratified by the continued progress in the program in terms of the continued reduction in the number of infected herds that has been made in the past year. He emphasized that the positive momentum that has been established must be maintained, despite the tight budgetary constraints, which may delay the proposed target dates in the Rapid Completion Plan.

Dr. John Kopec gave an update on the status of the national brucellosis program. At the end of fiscal year 1989, the United States had 1,411 known infected herds, which was a 39% reduction from what it was the previous year, at 2,345 herds. He further stated that 95.6% of the reactor herds were located in 11 states. Of the total cattle population, 30% of the cattle were located in Free States (27), 44% in Class A States (17), and 26% in Class B States (6). In FY 89, 2,502 reactor herds were disclosed, compared with 3,514 in FY 88. In FY 89, 9.2 million calves were vaccinated, which was an 8% reduction from the record number of 10 million which were vaccinated in FY 1988, even though the number of calfhood vaccination eligible heifers went up by 2% in FY 89.

The eradication of swine brucellosis continued to show headway. The majority of swine are now located in the 35 validated Free (Stage 3) States. There are ten states that are in Stage 1, and seven States in Stage 2, in the national swine brucellosis eradication program. There were only 59 infected herds detected in FY 89.

Dr. Scott Reynolds gave a report on the comparison of the card test with
the Concentration Immunoassay Technology (CITE), Particle Concentration Fluorescence Immunoassay (PCFIA), and the Automated Complement Fixation (ACF) Tests in the diagnosis of brucellosis infection. He brought out the point that the card test has outlived its usefulness in the highly vaccinated population that we are dealing with now. In this trial, he cultured lymph nodal tissues out of 132 card positive, CITE negative, PCFIA negative, and ACF negative cows, without isolating a Brucella abortus organism out of any of them. He concluded that animals moving through auction markets should be screened on the card test, but not be classified as reactors if they are negative on the CITE test. (See Appendix A)

Dr. Reynolds also gave a report on his retrospective study of the use of the PCFIA as a diagnostic test for brucellosis infection in cattle. He compared the Texas Plan with the Federal Plan for classifying animals in the MCI program.

In the Texas Plan:
1. All cattle would be screened on the PCFIA.
2. All sera showing PCFIA values of 0.60 or less would be run on the ACF.
3. The classification scheme follows:

<table>
<thead>
<tr>
<th></th>
<th>PCFIA</th>
<th>ACF</th>
<th>CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cattle</td>
<td>0.30 or less</td>
<td>Not Applicable</td>
<td>Reactor</td>
</tr>
<tr>
<td>Nonvaccinates</td>
<td>0.31 or more</td>
<td>11 or more</td>
<td>Reactor</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>0.31 or more</td>
<td>21 or more</td>
<td>Reactor</td>
</tr>
</tbody>
</table>

However, the Federal Plan would classify as reactors all animals with PCFIA results of 0.50 or less, regardless of what ACF results are. Dr. Reynolds concluded that animals which are ACF negative and show PCFIA values between 0.31 and 0.50 should not be classified as reactors.

Dr. C. Dix Harrell gave a report on the use of the Fluorescent Concentration Assay (FCA) test in the diagnosis of Brucella abortus infection in range cattle, and the comparison with the PCFIA and ACF tests. In large adult vaccinated range cattle herds, it is often difficult to hold the cattle until test results are received back from the lab so that reactor and suspect animals may be identified and separated from the herd. In one particular herd, consisting of 2,543 animals, 40 reactors were removed on the basis of the FCA test results. The other two tests (PCFIA and ACF) were in agreement on 37 of the 40 reactors. Only four cows showed false positive on the FCA (or a 0.15% overcondemnation rate). The FCA showed a 95% sensitivity rate (38 out of 40), and a 99.8% accuracy and specificity rate, as compared with the other tests. (See Appendix B)

Dr. L. Garry Adams gave an evaluation of the competitive ELISA (cELISA) as a diagnostic test for brucellosis. He demonstrated that the cELISA has shown 100% specificity on differentiating vaccination titers
BRUCELLOSIS

from infection in heifers as early as three weeks post vaccination. In the group of animals that had been challenged with Brucella abortus 2308, it showed 100% sensitivity in picking up infections at 11 weeks after challenge.

Dr. Adams also gave a report on the efficacy of his killed sub unit vaccine composed of the outer membrane-peptidoglycan portion of the cell wall of Brucella abortus. He concluded that even though it reduced fetal mortality significantly, like Strain 19, offered no protection against infection under experimental conditions. It would require two injections 60 days apart, plus an annual booster in order to achieve a level of effectiveness against fetal death comparable with Strain 19. This killed sub unit vaccine unlike Strain 19, does not cause any cross-reactivity (false positives) following vaccination.

Dr. John Cobb gave a report on the role of calfhood vaccination in the final eradication of brucellosis. He mentioned that Brucella abortus Strain 19 vaccine involved both a miracle (concerning its discovery) and a tragedy (referring to how it was misused and abused through the years). It has made a major contribution in reducing the levels of brucellosis infection in higher incidence areas, but it now was time to shift from quantity (the numbers of calves vaccinated) to quality (using it with discretion) in its utilization. The cost of calfhood vaccination in 1988 was estimated to be $25 million, which does not include program costs in tracing animals with residual titers, and in testing herds because of vaccination titers. He stated that, as we approach eradication, vaccination should be restricted to dairy, registered, and high-risk breeding heifers, rather than high volume, low-quality vaccination often resulting from legally mandated vaccination. He proposed a resolution that all mandated vaccination requirements be stricken from the UM&R and CFR, as well as from state regulations. (See Appendix C)

Mr. John S. Cargile gave a report on the Rapid Completion Plan, which has been developed by APHIS at the request of industry representatives. He outlined the objectives, timetable, and cost of implementing the plan. In the long run, he said, it would be the most cost-effective way of eradicating brucellosis. He presented a resolution that the USAHA support the implementation of the Rapid Completion Plan. (See Appendix D)

Mr. Cargile also offered proposed changes in the UM&R standards which correspond with the recommendations made earlier by Dr. Reynolds. He redefined the definition of MCI reactors (with reference to PCFIA and ACF test results). His proposal also included designating the card test as a presumptive test at the market, if the CITE test is being used. CITE-negative animals could then be classified as “negative,” and be allowed to move without restriction.

He also offered two other resolutions in support of the cELISA test, and the sub unit killed vaccine, both of which were discussed by Dr. Adams in his presentation.
He proposed that the cELISA be accepted as a supplemental test to aid in differentiating between vaccination and infection, as interpreted by the designated epidemiologist. Concerning the sub unit killed vaccine against brucellosis, he proposed that it be evaluated in cattle under actual field conditions under the supervision of USDA, APHIS, Veterinary Services designated epidemiologists.

Dr. Jan Huber offered several motions concerning changes in UM&R standards and definitions regarding test-eligible animals and the movement of calves out of affected herds.

Dr. Frank Drazek gave a detailed report on the calfhood vaccination of calves in his state. He indicated that calfhood vaccination eligible ages differ from UM&R standards (4 to 12 months), New York (3 to 8 months), and Canada (2 to 8 months). He cited a report by Sanders and Pickerill which indicated that a reduced dose given to heifers down to two months of age gave a protection comparable to that of a standard dose given between ages of four and six months. He made a motion that calves vaccinated with the reduced dose of Brucella abortus Strain 19 between three and 12 months of age be considered as official vaccinates according to the UM&R but emphasized that the final determination be left up to the individual states.

Mr. Jim Horne gave the report of the subcommittee on brucellosis education. Committee members were highly supportive of the Rapid Completion Plan but felt that heavy emphasis should continue to be placed on education and information programs. The committee believes that practitioner and producer involvement in the program are critical. The committee submitted a resolution that funding for the development and dissemination of information and education be continued.

Dr. Paul Doby gave the report for the Advisory Committee on Swine Brucellosis. Dr. Doby said that there were 59 infected swine herds located in nine states (disclosed in FY 89 as opposed to 65 in FY 88). The four states showing the greatest amount of infection were Alabama (22), Georgia (13), Florida (9), and Oklahoma (7). He stressed the role of feral swine in perpetuating both brucellosis and pseudorabies infection. He quoted Dr. Frank Mulhern on the proposed three-year plan, which would lead to the complete eradication of swine brucellosis at a cost of $10 million. He emphasized that the pork producers of the nation are in favor of doing what needs to be done to complete the job. (See Appendix E)

Dr. Ray Hinshaw, representing the Western USAHA, indicated that the wildlife of the Yellowstone National Park in Wyoming pose a risk to the surrounding farms and ranches because the buffalo and elk are infected with brucellosis. He presented a resolution to require the federal agencies managing those wildlife to compensate those livestock owners (and other damaged entities) for losses brought about by the transmission of diseases from those wildlife.
BRUCELLOSIS

Dr. Taylor H. Woods reported on the special subcommittee which met in Little Rock, Arkansas, on January 18, 1989. He mentioned that six items were considered during that meeting:

1. Proposed UM&R changes concerning the qualification for, and maintenance of, status classifications;
2. Test requirements for the entry of cattle into quarantined feedlots for surveillance purposes;
3. Changes in the UM&R standards in calculating rates and statistics for the classification and assignment of area status;
4. The reduction of the number of BRT rounds in Class-Free areas to two rounds per year;
5. Further reducing the dosage of *Brucella abortus* Strain 19 vaccine;
6. Testing requirements of bulls in certified free herds.

Dr. Greg Nelson said that Class-Free areas should be allowed the option to have quarantined feedlots within their borders because suspect animals and S-branded cattle need a place to be fed out. He guaranteed that quarantined feedlots in Idaho had never lost any cattle, nor had there ever been any documented spread of the disease from those feedlots. He introduced a resolution to allow quarantined feedlots within Class-Free areas.

Dr. Tom Thorne presented a resolution which had gone through the Wildlife Diseases Committee. He emphasized that *Brucella abortus* in the wildlife populations of the Greater Yellowstone Ecosystem in Montana, Wyoming, and Idaho was being neglected by the state and federal agencies involved with the management of those areas. His resolution encouraged a coordinated effort among all agencies which would focus on the control and/or eradication of *Brucella abortus* from that habitat.

Reports were heard from the state officials from the six higher incidence states:

1. Arkansas, Dr. Taylor H. Woods.

Dr. Woods gave the sequence of events which occurred from the time that Arkansas was reclassified from a “B” to a “C” state in August of 1983. He said that the program had to be completely overhauled in order to begin to eradicate, rather than propagate, the disease. The key element in the success of the program was the legislative support (in terms of giving the livestock and poultry commission the authority to promulgate and enforce regulations, as well as appropriating the funds necessary to hire a staff), and the education and orientation of the producers in the state. Arkansas had a high of 691 infected herds in September of 1984. On October 1, 1989, there were 58 known infected herds in the state.

2. Florida, Dr. Paul Becton.
REPORT OF THE COMMITTEE

Dr. Becton outlined the progress which had been made in Florida over the past ten years:

1978–80 — 28,378 reactor animals disclosed from among 1,135 infected herds.
1988–89 — 2,064 reactor animals disclosed from among 248 infected herds.

In FY 86, there was a total of 439 infected herds disclosed in the 14 higher prevalence counties. In those same counties in FY 88, only 146 infected herds were disclosed. The projected date for qualifying for Class “A” status is September 1990. Emphasis would continue to be on whole herd vaccination, and motivating and educating both regulatory personnel and livestock producers.

3. Louisiana, Dr. William Fairchild.

Nine years ago, there were 150 infected herds in Cameron Parish. Today, there are only 150 herds in the entire state. The secret to the success in Louisiana lies in the use of professional judgment and flexibility. On September 1, 1988, the herd infection rate was 1.48% with an adjusted MCI reactor rate of 0.25%. On September 1, 1989, the herd infection rate was down to 0.86% with an adjusted MCI reactor rate of 0.13%. Dr. Fairchild indicated that continuing to make progress was very important because it would be impossible to reestablish positive momentum if we began to “go backward” again.

4. Oklahoma, Dr. Robert Hartin.

The accumulated herd infection rate over the past 12 months has been 0.39%, with an adjusted MCI reactor rate of 0.05%. It is projected that Oklahoma will be able to qualify for Class “A” by October 1, 1990. The governor has appointed a Brucellosis Advisory Committee, consisting of representatives from all segments of the industry. That committee made many positive recommendations (including the mandatory adult vaccination or depopulation of infected herds) which will help to expedite the task of eradicating brucellosis.

5. Mississippi, Dr. Fred McCrory.

On October 1, 1989, there were 58 infected herds out of the 32,000 herds in the state. The success in Mississippi has been attributed to the adherence to UM&R standards, area and county testing, epidemiological follow-up, testing for a mile radius around infected herds, whole herd vaccination, depopulation, and calfhood vaccination.

6. Texas, Dr. John Holcombe.

In April 1987, Texas achieved Class “B” status. This achievement required the remaining 12 livestock markets to participate in the first point testing program. This was a positive step for the brucellosis program, even though the inclusion of these 12 markets resulted in an increase in the number of quarantined herds from 915 in April 1987, to almost 1,100 by
BRUCELLOSIS

June 1988. Once this peak was reached, the number of quarantined herds has steadily declined. In August 1989, the state had 715 known infected herds; that number shrunk to 677 by October. Of the infected herds that have been found in the last month, 50% were through the market testing program, 32% through adjacent herd testing, 6% through slaughter surveillance, and 11% through other means (area testing, diagnostic tests, or private tests). The eradication efforts are now focused on the 55 higher incidence counties in the eastern and gulf coast part of the state. By Spring of 1991, it has been projected that Texas will qualify for Class “A” status.

Dr. Jim Alexander gave the report of the Scientific Advisory Committee.

Two members of the committee (Dr. Garry Adams and Dr. Alexander) convened to discuss the proposed resolutions. They supported many of the proposals, making minor recommendations on each of them, except for the lowering of the calfhood vaccination eligible age down to three months of age. Dr. Frank Drazek questioned Dr. Alexander about how the United States can accept Canadian heifers (which are vaccinated down to two months of age and yet refuse to lower the lower age limit for our heifers. Dr. Alexander indicated that he couldn’t address the import requirements, but that research indicated that maternal antibodies may interfere with acquired immunity up until four months of age. (See Appendix F)

Dr. John Cobb’s resolution to remove all reference to legally mandated calfhood vaccination from the UM&R was brought to the floor for a vote, and it failed to pass.

Mr. John Cargile’s resolution to support the Rapid Completion Plan passed.

Mr. Cargile’s motion to change the definition of MCI reactors, and the role of the card test in the classification of MCI reactors in the UM&R was amended. It passed the floor vote in its amended form. (See Appendix G)

The resolutions presented by Mr. Cargile to support the cELISA test, and the killed sub unit *Brucella abortus* vaccine, both were adopted.

Dr. Frank Drazek’s resolution to reduce the lower age limit for calfhood vaccination to three months failed to pass.

The resolution of Drs. Terry Conger and Brian Espe to approve the CITE test for nonvaccinates passed.

Dr. W. G. Nelson’s resolution to amend CFR and UM&R standards to allow quarantined feedlots in Class-Free areas was hotly debated, but it passed.

The resolution presented by the subcommittee on Education to continue the funding and efforts for education passed the vote on the floor.

The two resolutions of Drs. Thorne and Hinshaw addressing the Yellowstone National Park brucellosis problem both were passed.

Dr. Jan Huber went over several proposed changes in the UM&R
REPORT OF THE COMMITTEE

standards; all were approved by the committee. (See Appendix H)

Many issues were discussed, debated, and resolved during this annual meeting of the Brucellosis Committee. The group believed a concerted effort needs to be made to continue progress, despite whatever budgetary and political problems may loom on the horizon.

APPENDIX A

A COMPARISON OF THE CARD TEST WITH THE
CONCENTRATION IMMUNOASSAY TECHNOLOGY
PARTICLE CONCENTRATION FLUORESCENCE
IMMUNOASSAY AND AUTOMATED COMPLEMENT
FIXATION TESTS IN THE IDENTIFICATION OF SALE BARN
BRUCELLOSIS REACTORS

Scott L. Reynolds, M.S., D.V.M., Dipl. A.C.V.P.M.
Vicki Swann, B.S.

INTRODUCTION

The use of the Card Test as a definitive diagnostic tool for brucellosis has hurt the program in Texas. The Card Test has been shown to be a fair indicator of disease in nonvaccinated animals. Conversely, its effectiveness in vaccinated animals is poor. For this reason, many ranchers in Texas refuse to vaccinate cows or even calves because of the problems related to false diagnosis. At the present time, a card positive cow that is negative on the Particle Concentration Fluorescence Immunoassay (PCFIA) and Automated Complement Fixation (ACF) test places a stigma on the cow and prevents her from moving in commerce. In most cases, the animal is needlessly shipped to slaughter. The purpose of this study is to evaluate the efficacy of the card test in the diagnosis of brucellosis.

STUDY DESIGN

Animals:

Cows were selected for the study based solely on our ability to harvest lymph nodes. Therefore, the cows to be evaluated were selected from sale barns where animals could easily be traced to slaughter houses.

Animal Management:

All card positive animals, regardless of vaccination history, were subject to the Concentration Immunoassay Technology (CITE) test. Blood from CITE negative cows destined for slaughter and available for the harvest of lymph nodes was utilized for the study. The Card and CITE tests were repeated in the State-Federal laboratory. The PCFIA and ACF tests were run on all sera. Only those card positive cows that were confirmed in the
laboratory to be CITE, PCFIA and ACF negative had lymph nodes submitted for culture assay. A total of 132 cows met the study design criteria. Of these, 109 were vaccinated and 23 were unvaccinated.

**CULTURE TECHNIQUE**

The supra pharyngeal, mandibular and supra mammary lymph nodes were chosen for harvesting. All tissues were packed in whirlpacks and frozen while waiting for final laboratory results. At the laboratory, lymph nodes were removed from surrounding fat, dipped in alcohol and flamed and sliced. The cut surface was minced and rubbed over the surface of the medium. All tissues were assayed according to the procedures utilized by the National Veterinary Services in Ames, Iowa, except Farrels media was substituted for W media.

**AUTOMATED COMPLEMENT FIXATION TEST**

The Technicon Auto Analyzer II, located at the State-Federal Laboratory, Austin, Texas was utilized for the ACF test. A detailed description of reagents may be found in the Technicon instruction manual on “Automated Complement Fixation Testing.” Diagrams of equipment and flow of serum and reagents have been pictured in detail.

**PARTICLE CONCENTRATION FLUORESCENCE IMMUNOASSAY TEST**

The PCFIA was performed by the State-Federal Laboratory, Austin, Texas. Reagents and equipment were provided by IDEXX Corporation, Portland, Maine.

*Procedure and Principles*

PCFIA is a fluorescence immunoassay technique which utilizes submicron polystyrene particles as the solid phase onto which *B. abortus* antigens are attached. The conjugate is Fluorescein Isothiocynate labeled antibody to *B. abortus* in phosphate buffered saline.

The test serum is pipetted into a specially designed 96 well plate. The plate is inserted into the AgriChek Screen Machine. A total of ten plates may be run simultaneously. All steps below are performed by the screen machine. The read out is performed by a computer.

In the assay, the test sample and coated particles are initially mixed and incubated in the well plate. During this initial incubation period, conjugate is added, allowed to react and the reaction mixture is then filtered through the membrane at the bottom of each well. The particles, being too large to pass through the membrane, are retained on the surface of the membrane. Each well is washed to remove unbound conjugate and antibody and then moved into position below a front-surface fluorimetric read system. The amount of particle-bound fluorescence is measured as counts from the
The system reads fluorescence in two channels, a sample channel (A) and a reference channel (B). The ratio of these counts in each channel normalizes variation due to pipetting.

Anti-\textit{B. abortus} PCFIA is a competitive immunoassay for antigen-specific antibodies. During the reaction, specific antibodies in the sample compete with the conjugate for antigen binding sites on the solid phase. The amount of conjugate bound to the solid phase will decrease as the specific antibody concentration increases in the sample, thus providing an inverse measurement of specific antibody. Binding of non-specific antibody to the solid phase does not affect the competition between specific antibody in the sample and the conjugate, and thus, does not affect the resulting signal. Non-specific binding to the antigen on the solid phase is displaced by either the higher affinity conjugate or specific antibody in the sample and, again, does not affect the resulting signal.

\textbf{Reagents}

1) \textit{B. abortus} Coated Particles. Antigen coated particles in Phosphate Buffered Saline with protein stabilizers. Preserved with Sodium Azide and Thimerosal.

2) Anti-\textit{B. abortus} : FITC Conjugate. Fluorescein isothiocyanate labeled antibody to \textit{B. abortus} in Phosphate Buffered Saline with protein stabilizers. Preserved with Sodium Azide and Thimerosal.

3) \textit{B. abortus} Strong Positive Control. Bovine anti-\textit{B. abortus} serum, diluted in sample diluent. Preserved with Sodium Azide and Thimerosal.

4) \textit{B. abortus} Weak Positive Control. Bovine anti-\textit{B. abortus} serum, diluted in sample diluent. Preserved with Sodium Azide and Thimerosal.

5) Native Control Bovine serum nonreactive to \textit{B. abortus}, diluted in sample diluent. Preserved with sodium Azide and Thimerosal.

6) Sample Diluent. Phosphate Buffered Saline with protein stabilizers. Preserved with Sodium Azide and Thimerosal.

7) Wash Concentrate (10x). Phosphate Buffered Saline, 10x Preserved with Sodium Azide and Thimerosal.

\textbf{CITE TEST}

The CITE test was performed by personnel of the Texas Animal Health Commission at the sale barn and the State-Federal Diagnostic Laboratory, Austin, Texas. Reagents and assay devices were provided by IDEXX Corporation, Portland, Maine.

\textit{Description—Principles.}

\textit{CITE \textit{B. abortus} Antibody Test} is an enzyme immunoassay designed to detect the presence of antibodies to \textit{B. abortus} in bovine serum or plasma. The test incorporates advanced immunoassay device technology providing a unique format for a solid phase immunoassay for \textit{B. abortus} antibodies.
BRUCELLOSIS

In the CITE format, *B. abortus* antigens and bovine immunoglobulin calibrator have been spotted separately onto a fiber membrane in the device. Sample and reagents are applied onto the surface of the membrane and flow through the bioactive spots.

Antibodies to *B. abortus*, if present are captured by the immobilized *B. abortus* antigens on the sample spot. Enzyme conjugated anti-bovine antibodies are then added, and bind to the captured *B. abortus* immunoglobulins forming an antigen-antibody sandwich. After washing away unbound material from the membrane, an enzyme substrate solution is added. Subsequent color development is proportional to the concentration of *B. abortus* antibodies captured.

Color will also develop in the calibration spots. Test interpretation, by comparison of color in the sample and calibration spots, correlates to the AgriChek® *B. abortus* Particle Concentration Fluorescence Immunoassay (PCFIA) System test results. The low calibration spot is standardized to the PCFIA positive-negative cutoff. The high calibration spot is standardized to the PCFIA reactor cutoff. The mid-range calibration spot has been included to provide an additional comparison for a sample in the suspect category. In addition to their calibration function, these spots indicate that the assay reagents are functional.

RESULTS

None of the 132 supplemental negative cows exhibited culture positive isolates of *Brucella abortus*. (See Appendix “A”).

DISCUSSION

This study strongly suggests that the Card test is not an accurate indicator of disease and its further use as a definitive diagnostic tool will hinder the Brucellosis Program.

In closing, CITE negative vaccinated cows should be classified as negative for brucellosis and be allowed to move freely in the markets.

Even though only 23 nonvaccinated animals were available for the study the lack of culture positive isolates in all 23 animals suggests that card positive, CITE negative, nonvaccinated cows were not infected.
**REPORT OF THE COMMITTEE**

## Test Animals

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Test Date</th>
<th>Vaccinated</th>
<th>Culture Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>74CUJ3755</td>
<td>6/12/89</td>
<td>No</td>
<td>Nonisolate</td>
</tr>
<tr>
<td>74CUM6323</td>
<td>7/14/89</td>
<td>&quot;</td>
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</tr>
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INTRODUCTION

The elimination of *Brucella abortus* infection from large range cattle herds is complicated by difficulties due to lack of holding facilities while awaiting laboratory results. Several days may elapse before the identity of suspect/reactor cattle is known and thereby leading to these reactors remaining in the herd until they are removed on subsequent testing.

Until the development of the Fluorescent Concentration Assay (FCA), the card test was the only available test routinely used to segregate test positive cattle while awaiting the completion of laboratory testing. In vaccinated herds, the card test often results in excessive numbers of cattle requiring segregation pending laboratory testing. Furthermore, pairing calves with their dams increases the amount of time and labor required to segregate the cattle.

The need for an accurate field test for the serodiagnosis of *B. abortus* in large herds of vaccinated range cattle is evident. This paper reports the results of the accuracy of the FCA in the field diagnosis of *B. abortus* infection in a herd unable to segregate cattle pending confirmatory laboratory testing.

STUDY DESIGN

A commercial beef herd of approximately 14,000 cattle adult vaccinated with 300 million colony forming units (cfu) liquid strain 19 vaccine 12 months earlier was used for this study. Blood samples were collected and tested by the FCA at the premises using a van and portable electric generator. Testing was conducted over a period of several months under a variety of environmental conditions (hot/cool, dry/wet, humid, dusty, etc.). The FCA was used as a positive or negative test. Animals testing positive on the FCA were branded and shipped to slaughter. Serum was sent to the State/Federal laboratory in Austin, Texas for confirmatory testing with the Particle Concentration Fluorescence Immunoassay (PCFIA) and Automated Complement Fixation (ACF) tests.

THE AUTOMATED COMPLEMENT FIXATION TEST (ACF)

The Technicon Auto Analyzer II, located at the State/Federal Laboratory, Austin, Texas, was utilized for the ACF test. A detailed description of
REPORT OF THE COMMITTEE

reagents may be found in the Technicon instruction manual on "Automated Complement Fixation Testing". Diagrams of equipment and the flow of serum and reagents have been pictured in detail.

PARTICLE CONCENTRATION FLUORESCENCE IMMUNOASSAY (PCFIA)

The PCFIA was performed by the State/Federal laboratory, Austin, Texas. Reagents and equipment are supplied by IDEXX Corporation, Portland, Maine.

The PCFIA is a fluorescent immunoassay technique which utilizes submicron polystyrene particles as a solid phase onto which antigens from B. abortus are attached. The conjugate is fluorescein Isothiocyanate labeled antibody to B. abortus in Phosphate Buffered Saline.

The test serum is pipetted into a specially designed 96 well plate and the plate is then inserted into the Agrichek Screen machine. A total of ten plates may be run simultaneously. The steps below are performed by the screen machine and calculations of the results are performed by a computer.

Once the 96 well plates are placed into the screen machine, the serum samples and coated particles are mixed and incubated. During the initial incubation, conjugate is added and allowed to react. The reaction mixture is then filtered through the membrane at the bottom of each well retaining the polystyrene particles which are too large to pass through the membrane. Each well is washed to remove unbound conjugate and antibody and then moved into position below a front surface fluorimetric read system. The amount of particle bound fluorescence is measured in two channels, a sample channel A and a reference channel B. The ratio of the measurements in each channel normalizes variation due to pipetting.

Anti-B. abortus PCFIA is a competitive immunoassay. During the reaction, anti-Brucella serum antibodies compete with the conjugate for antigen binding sites on polystyrene particles. As the anti-Brucella serum antibody concentration increases in the test sample, the amount of conjugate binding to the polystyrene particles decreases, thus providing an inverse measurement of the amount of anti-Brucella antibody in the test serum. The binding of nonspecific antibody to the polystyrene particles is displaced either by the higher affinity conjugate or specific antibody in the serum sample. Thus the competition between specific serum antibodies and the conjugate and the resulting signal being measured is not affected by low affinity nonspecific antibody.

REAGENTS

1. B. abortus coated particles. Antigen coated polystyrene particles in Phosphate Buffered Saline with protein stabilizers. Preserved with Sodium Azide and Thimerosol.
2. Anti- *B. abortus* FITC Conjugate. Fluorescein Isothiocyanate labeled antibody to *B. abortus* in Phosphate Buffered Saline with protein stabilizers. Preserved with Sodium Azide and Thimerosal.


**FLUORESCENCE CONCENTRATION ASSAY (FCA)**

The FCA is a manual version of the PCFIA performed on the Fluorescence Concentration Analyzer utilizing the same reagents. Samples are diluted in sample diluent (2 μl serum sample to 50 μl diluent) in a 96 well plate designed with a 0.22 micron filter attached to the bottom of each well. After diluting the sample, 20 μl of *B. abortus* coated polystyrene particles are added and the reaction is incubated for 14 minutes. Following this initial incubation, 20 μl of Anti-*B. abortus* FITC Conjugate is added and the mixture is incubated an additional 9 minutes before a vacuum is applied. The plate is sealed such that when a vacuum is applied, the excess solutions are drawn through the filter leaving the particles layered on the top of the filter.

Following removal of the reactants, the particles are washed with 50 μl of wash solution and the evacuation procedure is repeated. Following evacuation, the wells are placed under a front surface fluorometer and the amount of particle bound fluorescence is measured. The resulting signal is related to the signal for the negative control and the sample signal reduction is determined (the serum to negative control ratio—S/N). The S/N ratio decreases as the amount of specific antibody increases, giving an inverse measurement of the anti-*B. abortus* antibody in the test sample.

**DATA ANALYSIS**

Data analysis comparisons were made to assess the accuracy of the FCA as a field diagnostic test. For this study, animals with PCFIA titers ≤ 0.20 and/or ACF titers ≥ 21 were classified as brucellosis reactors. Animals were classified as suspects if they were positive at the 1:10 dilution on the ACF test and/or their PCFIA values ranged from 0.21-0.30. The FCA was considered to be positive at values ≥ 0.30. For the purpose of data analysis the three tests were considered to be in agreement when the PCFIA and FCA were ≤ 0.30 and the ACF was ≥ 21.

**RESULTS**

Table 1 summarizes the results of testing the first 2608 cattle of the 14,000 cow herd. A total of 2543 (97.51%) cows were classified as negative on all tests utilized, while 25 suspects and 40 reactors were disclosed. The herd reactor rate was calculated to be 1.53% (40 reactors out of 2608 tested). The FCA, PCFIA, and ACF agreed in 37 (92.5%) of the 40 reactors.
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Table 2 summarizes those cattle that were falsely positive on the FCA test. A total of 4 FCA positive cows did not meet our criteria for reactors following supplemental testing. As can be seen, 3 of the 4 FCA positives were suspects on the ACF test (1:10 titer) while one FCA positive was negative on ACF (<1:10 titer).

Table 3 summarizes those cattle that were negative on the FCA but reactors on the PCFIA and ACF tests. As can be seen, two cows were falsely negative on the FCA test. You will note that both reactors are strongly positive on the PCFIA and have ACF titers of 161. The PCFIA results strongly indicate some type of laboratory error occurred while conducting the FCA test in the field since the FCA and PCFIA utilize the same methodology, only the PCFIA is an automated test which virtually eliminates human error. For this study, however, 2 of 40 reactors (5.0%) were falsely negative on the FCA.

Table 4 categorizes FCA test results with the final classification for reactors and nonreactors. For this study, the FCA detected 38 of 40 reactors resulting in a sensitivity of 95.0%. The FCA correctly determined 2564 of 2568 cows as negative for brucellosis, a specificity of 99.8%. The accuracy of the FCA, defined as the number of reactors plus the number of nonreactors correctly identified by the FCA divided by the total cows tested, was 99.8%. The FCA overcondemned 4 (0.15%) cows and failed to detect 2 (0.08%) reactors out of 2608 cows tested.

DISCUSSION

The results of this study indicate the use of the FCA in the field diagnosis of *B. abortus* infection in range cattle is a viable alternative in herds unable to segregate cattle pending laboratory confirmation and supplemental testing. It was the authors intent to remove all cows having ACF titers of 21 or greater and/or PCFIA titers of 0.20 or less as brucellosis reactors. In this regard, the FCA performed very well. Only two reactors were falsely negative on the FCA and this was most probably due to laboratory error since the PCFIA was strongly positive in these cows.

Conversely, only four cows were falsely positive on the FCA. Three of these were suspects with ACF titers of 11 while one cow was negative on ACF. Additional testing of these suspects, had it been feasible, may have disclosed one or more of them to be incubating reactors. A total of 40 reactors were found in the cattle tested and 38 were identified by the FCA. With further experience to reduce human error, the authors feel this percentage can approach 100%.

This study indicates the FCA compares favorably with the ACF and PCFIA tests. Based on the authors experience, the FCA would be the preferred test to run in lieu of the card test in adult vaccinated herds unable to segregate and hold cattle pending laboratory results. The producers time and labor of working the herd is greatly reduced because reactors can be
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removed the same day the herd is tested. The accuracy of the FCA can be an important test available for the field diagnosis of brucellosis infection in large vaccinated beef herds.

ACKNOWLEDGEMENTS

The authors are grateful to Rick Nabors, Steve Melton and Steve Rodriguez, State/Federal Laboratory, Austin, Texas for their laboratory support. Special thanks to Chet Crum and Barbara Obrien of IDEXX Corporation who generously furnished the FCA equipment and reagents, and to Jo Anne Conner and Dee Robinson for preparation of the manuscript.

Table 1

Results of Testing the First 2608 Cattle

<table>
<thead>
<tr>
<th>Negative</th>
<th>Suspect</th>
<th>Reactor</th>
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<tbody>
<tr>
<td>2543 (97.51%)</td>
<td>25 (0.96%)</td>
<td>40 (1.53%)</td>
</tr>
</tbody>
</table>

Negative = PCFIA > 0.30; FCA > 0.30; ACF < 11
All three tests in agreement = 37 or 40 reactors (92.5%) (PCFIA and FCA ≤ 0.30 and ACF > 21)

Table 2

Cows Falsely Positive on the FCA Test

<table>
<thead>
<tr>
<th>Cow #</th>
<th>PCFIA</th>
<th>FCA</th>
<th>ACF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNZ6199</td>
<td>0.39</td>
<td>0.26</td>
<td>11</td>
</tr>
<tr>
<td>CNP5518</td>
<td>0.28</td>
<td>0.25</td>
<td>11</td>
</tr>
<tr>
<td>CNP5581</td>
<td>0.21</td>
<td>0.24</td>
<td>11</td>
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<tr>
<td>CNP5047</td>
<td>0.33</td>
<td>0.22</td>
<td>01</td>
</tr>
</tbody>
</table>

Table 3

Cows Falsely Negative on the FCA Test

<table>
<thead>
<tr>
<th>Cow #</th>
<th>PCFIA</th>
<th>FCA</th>
<th>ACF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRQ4202</td>
<td>0.10</td>
<td>N</td>
<td>161</td>
</tr>
<tr>
<td>CRQ4203</td>
<td>0.12</td>
<td>N</td>
<td>161</td>
</tr>
</tbody>
</table>

Two of forty (5.0%) reactors were falsely negative on the FCA.
This presentation might well be captioned, “The Miracle and Tragedy of Strain 19”. The miracle, of course, is the accidental discovery of Strain 19 by Dr. Buck in 1923, and its rather precise balance of antigenicity and pathogenicity that has made it a most useful tool in producing resistance against brucellosis in cattle. Further, is the fact that Strain 19 has remained essentially unchanged for two-thirds of a century in spite of being maintained for the most part under artificial conditions. Conversely, the tragedy is the misuse and abuse of Strain 19 since its introduction.

It was introduced in 1941, some 13 years prior to the beginning of the national brucellosis eradication program. From 1941 to 1954, its use was largely unregulated. However, in 1946, Mingle identified the two main drawbacks to the use of Strain 19. These were residual vaccine titers and persistent Strain 19 infection.

The first Strain 19 vaccines were liquid products. In order to maintain viability and a reasonable shelf-life, it was necessary to include large numbers of viable organisms in the product. As lyophilization technology developed, a longer shelf-life could be achieved. However, it was still necessary to include large numbers of organisms because the lyophilizing process destroyed up to 50% of those originally present. The phenomenon of endotoxic shock is thought to be due to the large numbers of dead organisms resulting from lyophilization and viability decay.

The dose of Strain 19 for calfhood vaccination remained essentially unchanged at 25–90 billion organisms until 1981. However, the problems of vaccine titers, persistent Strain 19 infection, and endotoxic shock persisted. The need for a less troublesome vaccine resulted in the discovery that a dose containing far fewer organisms produced essentially comparable resistance when administered at recommended ages. Advances in production technology, transportation and storage made possible a marked reduction in the number of organisms per vaccine dose. Introduced in 1981, the reduced-dose vaccine containing 3–10 billion viable organisms, is the product currently recommended and used today.

Let’s review for a moment the pluses and minuses of calfhood vaccination. On the plus side when properly administered:

1. It stimulates substantial resistance against brucellosis.
2. It decreases the risk of vaccinated herds becoming infected.
3. It reduces the number of reactors, the number of abortions, and thus, the rate of spread should vaccinated herds become exposed and infected.

Just how good is Strain 19 calfhood vaccination? A recent summary by
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Huber of all challenge calfhood vaccination trials conducted since 1979 with the reduced dose, showed that 26% of the vaccinates became infected when challenged. Conversely, this study indicated that 74% were either protected by vaccination or were naturally resistant. A summary by Manthae of studies prior to 1979 with the standard dose, showed results ranging from 65–75% efficacy. It appears then that the oft cited efficacy rate of 65–75% is still valid today.

The minuses of calfhood vaccination fall into two categories. First are the long recognized problems with residual antibody titers and persistent Strain 19 infection.

The second category involves the misuse and abuse of Strain 19 vaccination. Some specific examples are:

1. Overage vaccination.
2. Underage vaccination.
3. Improper storage of vaccine.
4. Incorrect dosage.
5. Administration of vaccine to calves receiving concurrent antibiotic therapy.
6. Failure to properly identify vaccinates.
7. Failure to report vaccinations.
8. Identifying calves as vaccinates without vaccination.

So, what are the consequences of these minuses:

1. Overage vaccination or overdosing results in a higher rate of residual titers and persistent Strain 19 infection. Another finding from Huber’s analysis of the reduced-dose studies was that residual titers and persistent Strain 19 infection are both age and dose related. Therefore, much of the advantage gained from reducing the dose is lost when calves are vaccinated overage.

2. The administration of less than immunizing doses of Strain 19 will result in vaccinates with little or no immunity, whether it be due to non-viability, incorrect dosage, interference from antibiotics, or outright failure to inject the vaccine. Yet, because these animals are identified as official vaccinates, they enjoy the inherent benefits of movement and increased age at which routine brucellosis testing is required, thus, delaying and confusing the diagnosis of brucellosis in many cases.

3. Further confusion in diagnosis and infected herd management results with calfhood vaccinates that are improperly identified and reported.

Let’s look at some of the factors that have contributed to the misuse and abuse of calfhood vaccination.

1. There has been a disproportionate emphasis on quantity rather than quality of calfhood vaccination. Statistics on calfhood vaccination invari-
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ably state total numbers and disregard the intended utility of the vacci-
nates. For example, the 1988 national brucellosis statistics showed
nearly 10 million heifer calves, the largest number in the history of the
brucellosis program, as having been calfhood vaccinated. It failed,
however, to show how many of these were feeder heifers destined to
feedlots, never to be used as breeders, and whose vaccination contributed
nothing to the level of resistance of the national cattle herd.

2. Another contributing factor is the overestimation of the relative value
of calfhood vaccination in the total eradication effort. Considering the
efficacy of Strain 19 vaccine, it is generally agreed that 80–85% of re-
placement heifers must be vaccinated in order for it to have a significant
effect toward total eradication. No State has ever been able to sustain this
level of vaccination.

3. A third factor is mandatory vaccination laws and regulations. Specifi-
cally, these require calfhood vaccination for change of ownership, inter-
state movement, or importation into a specific State. Obviously, the
intent is to increase the resistance of certain cattle to brucellosis.

However, the benefits of mandatory calfhood vaccination are a mixed bag
at best. In the first place, when calfhood vaccination is a requirement for
sale or movement, then it will be done, oftentimes with little regard for age
or quality of vaccination. A high percentage of calves are vaccinated just
prior to shipment at a time when they are under great stress. Frequently,
Strain 19 is administered concurrently with broad spectrum antibiotics. All
these things converge to severely compromise the efficacy of vaccination.

It is generally agreed that calfhood vaccination of an animal already
exposed to an infective dose has no effect on the development of the disease.
Therefore, vaccination offers very little, if any, protection against importing
brucellosis in young feeder age heifers. Considering the relatively low
number of feeder animals that get out of feedlot channels, the cost of
mandatorily vaccinating feeder heifers seems inordinately high compared
to the benefits derived.

Contrary to some popular thinking, calfhood vaccination is not free. Let’s
look at some of the costs:

1. Cost of vaccine:
   a. Single dose — $.50/dose
   b. Five dose — $.138/dose
   c. Twenty-five dose — $.114/dose

At an average cost of 25 cents per dose, the cost of vaccine in the United
States last year can be closely estimated at 2-1/2 million dollars.

2. Administration of vaccine runs from $2.00/head up. At this rate, the
total tab for calfhood vaccination was at least 20 to 25 million dollars in
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3. A hidden, but real cost is that involved in tracing and testing herds from which MCI reactors originate that are the result of overage vaccination or failure to properly identify vaccinates. In many States, this very likely constitutes the majority of total program costs.

4. Another hidden, and unmeasurable cost is that of vaccination failure. The absence of acquired resistance where it is expected and needed in vaccinates can be terribly expensive.

5. A fifth cost is the ongoing search to find increasingly expensive diagnostic tests to differentiate residual vaccine titers from those caused by field strains. As you know, there is no test that consistently does this at present.

6. Loss of export markets. This is a mixed bag because some countries won’t accept calfhood vaccinates while others require it.

For the past three years, there has been a dramatic drop in the national monthly prevalence of brucellosis infected herds. As of July 31, 1989, there were only 1539 known infected herds in the entire country. The risk of exposure is quite low and declining rapidly every day. For the first time, the national cooperative brucellosis program is in a position whereby the disease can be virtually eradicated within a short time.

So, what should be the role of calfhood vaccination in the final phases of the eradication effort? Obviously, there is no single answer to this question. However, considering the current status and direction of the brucellosis program there are several issues concerning calfhood vaccination that need the judicious consideration of regulatory leaders throughout the country.

1. First, is the issue that it will be necessary to stop calfhood vaccination altogether before complete eradication can be achieved. This philosophy was developed in some other countries 25 years or so ago. It was developed at a time when the standard dose vaccine was used and before some of our better supplemental diagnostic tests were developed. With the reduced dose vaccine and current diagnostic tests, it very well may not be necessary to stop calfhood vaccination altogether. On the other hand, if consistent high quality calfhood vaccination cannot be achieved, then it is very likely that it may have to be discontinued before eradication can be accomplished.

2. Along this same line, it is time to start dispelling the widespread theory that the dramatic increase in brucellosis during the early 1970's was due solely to the de-emphasis of calfhood vaccination. No doubt, the degree to which the increase occurred was influenced by the reduction in vaccination. More importantly however, was the redirection of both fiscal and human resources away from brucellosis to other programs under the misguided conclusion that the remaining brucellosis would go away with a minimum amount of effort.

3. The overselling of calfhood vaccination should be discontinued. There
is little doubt that there is and will be a need for some time for good calfhood vaccination. However, the real need for calfhood vaccination is concentrated in relatively few segments of the cattle industry. In view of the current fiscal problems in the brucellosis program, calfhood vaccination certainly appears to be one cost that could be shifted to the segments of the industry that use and need it. It is a common observation that a much higher quality vaccination is achieved when done at the request of the cattle owner than when done by regulatory mandate.

4. Remove mandatory calfhood vaccination requirements at all levels. It has been amply demonstrated that when calfhood vaccination becomes a requirement for change of ownership or movement, then it will somehow be done for those reasons. The primary purpose of stimulating uncomplicated resistance against brucellosis becomes a secondary consideration. It is at this point that much of the abuse and misuse of calfhood vaccination occurs.

5. As regulatory agencies recognize that there are areas of need for calfhood vaccination that will likely continue for a while, but move quickly to a position of selected emphasis. The emphasis should be directed toward dairy replacement and purebred beef heifers, as well as commercial beef replacement breeding heifers that are likely to experience significantly increased risk.

Further, the emphasis should be high quality, rather than quantity, calfhood vaccination. Where infected herds occur, adult vaccination should be quickly employed to bring the outbreak under control.

6. Consider reducing the official age for calfhood vaccination to 4–10 months, or even 4–months.

7. Remember, it will be two to three years before affects of changes in calfhood vaccination are noticeable.

Our remarks today were, in no way, intended to belittle the value of good calfhood vaccination. Rather, they were given to address some of the current problems with calfhood vaccination and to stimulate consideration of how the procedure might best be used in the context of complete eradication.
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APPENDIX D

BRUCELLOSIS ERADICATION
THE PROPOSED RAPID COMPLETION PLAN

Introduction

In February 1989, the National Cattlemen's Association requested that the Animal and Plant Health Inspection Service (APHIS) develop and present an alternative plan for the eradication of brucellosis. In response to that request, in March 1989, APHIS developed the "Plan for the Rapid Completion of Brucellosis Eradication." The plan was provided to stimulate discussion of possible alternatives to the current U.S. Department of Agriculture (USDA) policy on brucellosis eradication, and does not represent USDA policy for this program.

During review of the Rapid Completion Plan, representatives of the livestock industry and the animal health profession expressed concern about the rigidity of the plan's depopulation component. This document, reflecting industry suggestions, presents further clarification and revision of the conditions under which depopulation and whole herd vaccination would be conducted.

Overview of the Plan

In developing an alternative plan for the eradication of brucellosis, it is recognized that with long-established animal diseases the last vestiges of infection are the most difficult and costly to eradicate. A concerted effort is required to locate unobtrusive pockets of infection and provide assurances to stakeholders that full eradication is preferable to an approach that only controls the disease at a low prevalence level.

Objectives

The first objective of the Rapid Completion Plan, to have no brucellosis infected herds under quarantine in 2 years, may not be completely achievable and could conflict with other recommended modifications of the plan. The final objective of all States Class Free or in the qualifying process for Class Free classification in 5 years was considered to be attainable and realistic if the necessary resources can be made available to implement the entire plan. It would require adequate funding, producer cooperation, and aggressive administration at the local, State, and national level. Intermediate objectives to replace the Rapid Completion Plan's first objective are as follows:

1. In 1 year, all identified infected herds in the United States will be on a herd plan which requires whole herd vaccination.
2. In 2 years, all identified infected herds in the current Class A States would be depopulated or whole herd vaccinated.
3. In 3 years, all current Class B and Class C areas would be Class A or Class Free or in the qualifying process for Class Free classification.

4. In 4 years, all States except the current Class B and Class C States, would be Class Free or in the qualifying process for Class Free classification.

5. In 5 years, all States would be Class Free or in the qualifying process for Class Free classification.

Technical Approach

Program experience as well as epidemiologic studies have shown that the following key program elements are essential for achieving rapid eradication in an efficient manner:

1. A surveillance system which is adequate in extent of coverage, sensitivity for detecting, and tracing back to herd of origin, all reactor animals.

2. A whole herd depopulation strategy that is consistently applied and sensitive to fluctuations in market prices.

3. Use of whole herd vaccination to control spread of infection within herds while the infection is being eradicated.

4. Full compliance with standards of the Uniform Methods and Rules (UM&R) for brucellosis eradication.

To provide for the above key elements and achieve the eradication in as short a time as possible, acceleration of selected critical activities within the current brucellosis program operational structure will be required. These accelerated activities include:

- State program technical reviews.
- Enhanced First Point Testing.
- Increased adjacent and contact herd testing.
- Increased epidemiology testing.
- Mandatory whole herd vaccination.
- Increased depopulation.
- Use of local task forces for area testing where required.
- Increased training in high incidence States.

These accelerated activities must be implemented on a national scale so that problems associated with the movement of animals from adjacent areas or States that harbor infection are addressed at the same time. The operational logic of a rapid eradication program would be as follows:

1. Development of State and national program goals to guide and motivate APHIS, State, and industry personnel;
2. Rigorous technical reviews of individual State programs;
3. Immediate follow-up/reinforcement of State technical review recommendations (e.g. with personnel, training, depopulation funding, equipment) as required;

4. Continual monitoring of program progress through an enhanced surveillance system.

A rapid eradication program will require that, (a) funding and staffing levels are adequate to carry out the depopulation strategy, enhanced surveillance, and other accelerated activities, and (b) the goals of such an effort will provide a credible vehicle for organizing all stakeholders, especially in the high incidence States.

**Detailed Discussion of Depopulation and Whole Herd Vaccination Activities**

A depopulation based program is the most cost-effective approach to complete eradication of brucellosis and the only way to accomplish that in a short period of time. There are, however, situations where depopulation may not be the most cost-effective approach either for the herd owner or the brucellosis program as a whole, such as in the case of very large herds, valuable purebred herds, or where adequate separation of an infected herd from uninfected herds exists. In cases like these, use of whole herd vaccination, periodic retesting of infected herds, and culling of reactors may be the preferred management approach.

The discussion which follows presents in greater detail the guidelines for determining when depopulation and whole herd vaccination would be conducted.

**Increased Depopulation**

1. Increase herd depopulation in all areas of the U.S. with mandatory depopulation in Class Free states.

2. All currently known infected herds may be offered herd depopulation following any subsequent herd retest with reactors.

3. All new infected herds found in which more than 10% of the herd is found to be infected may be offered herd depopulation as soon as field strain infection is confirmed by culture or other epidemiological evidence.

4. Large herds or valuable purebred herds may be offered whole herd vaccination instead of depopulation indemnity if this is agreed to by the State animal health officials, VS Area Veterinarian in Charge, designated epidemiologist in the State, and the regional epidemiologist. The following conditions will be considered in making this decision:

   a. There is a reasonable expectation that the herd will be free of field strain infection within 1 year and/or be released from quarantine within 2 years.
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b. An economic analysis shows the costs of testing the herd (including costs to the farmer or rancher) will be substantially less than the costs of depopulation. If continued maintenance of the herd will cause delay in advancement of the State to Class Free status, then the cost to the rest of the livestock industry in the State during the period of expected delay must also be considered.

c. There is adequate separation from other herds so that there is reasonable assurance that the infection will not spread to other herds.

d. The herd owner agrees to adopt herd management practices in a herd plan recommended by their practicing veterinarian, the State or Federal VMO, and the designated epidemiologist in the State, to prevent spread of disease within the herd.

e. The herd owner agrees not to add new breeding animals to the infected herd unit until it has had at least one complete negative herd test.

5. Herd owners may be offered whole herd depopulation with incentive indemnity for exposed animals following any subsequent test in which reactors are disclosed even if they have previously declined depopulation.

Mandatory Whole Herd Vaccination

1. Whole herd vaccination will be required in all infected herds that are not depopulated unless vaccinating the adult cattle in the herd would create an economic hardship. In such a case a herd plan must be agreed to by the State animal health officials, VS Area Veterinarian in Charge, designated epidemiologist in the State, and the regional epidemiologist. The following conditions will be considered in making this decision:

a. There is a reasonable expectation that the herd will be free of field strain infection within 1 year and/or be released from quarantine within 2 years.

b. The herd is a valuable purebred herd with potential international export market which would be lost by vaccination of cattle in the herd.

c. There is adequate separation from other herds so that there is reasonable assurance that the infection will not spread to other herds.

d. The herd owner agrees to adopt herd management practices in a herd plan recommended by their practicing veterinarian, the State or Federal VMO, and the designated epidemiologist in the State, to prevent spread of disease within the herd.

2. Owners of infected herds who do not vaccinate their whole herd will not
receive any indemnity for either initial or subsequent reactors found.

3. Herd owners who own large herds or herds not recommended for de-
opulation will be expected to vaccinate their whole herd as soon as
possible after field strain infection is confirmed in their herd by culture
or other epidemiological evidence.

4. Herd owners of herds where initial reactors were not found to be field
strain infected by bacteriological culture and other epidemiological
evidence may receive reactor indemnity for initial or any subsequent
reactors found without whole herd vaccination of their herds.

5. Infected cattle herds may be whole herd vaccinated as an alternative
to herd depopulation at either the owner's option when depopulation
has been offered or as a State and APHIS-Veterinary Services (VS)
option if depopulation is not considered cost effective.
   a. Owner option. Herd owners who choose to vaccinate their herd
      rather than depopulate their herds when the depopulation option
      has been offered.
      • Will be expected to vaccinate their whole herd as soon as possible
        to be eligible for any indemnity on reactors.
      • May receive reactor indemnity for any initial or subsequent
        reactors found in the herd.
   b. State-VS Option. Herd owners who vaccinate instead of depopulat-
ing their herd at the recommendation of the State and VS represen-
tatives.
      • Will be expected to vaccinate their whole herd as soon as possible
        to be eligible for any indemnity on initial or subsequent reactors.
      • May receive reactor indemnity for any reactors found on an
        initial herd test and may receive incentive indemnity for any
        subsequent reactors found.

   a. Testing Whole Herd Vaccinated herds.
      • After whole herd vaccination it is recommended that the herd be
        retested 60 days after vaccination to establish baseline titers on
        all cattle in the herd.
        — Use of tests such as the PCFIA with increased sensitivity may
          be used to identify cattle with potential infection.
        — Within herd segregation of cattle to separate suspicious cattle
          may be used in large herds.
      • Herds must be retested 180 days after whole herd vaccination.
   b. Time limits for clearing whole herd vaccinated herds.
      • Any herd which has not been cleaned up within 2 years
        following whole herd vaccination will be offered depopulation.
Costs

The Rapid Completion Plan has the lowest amount of average annual cost both to the taxpayers of the various States, the nation, and to the cattle industry, compared to other alternatives examined. Revisions suggested by the industry would not change the essential character of the plan, i.e. achieving complete eradication of the disease in a short period of time, and should result in comparable costs. Lower depopulation costs would be offset, at least in part, by higher testing costs, perhaps over a longer period of time. Precise estimates depend on the management options chosen, aggressive implementation, and other conditions placed on the program.

April 28, 1989

Questions & Answers

BRUCELLOSIS RAPID COMPLETION PLAN

1) What is the Plan?  
RESPONSE: An acceleration of U.S. brucellosis eradication efforts to locate and eliminate last pockets of infection.

2) What is the Objective of the Plan?  
RESPONSE: To have all States Class Free or in the qualifying process for Class Free classification in 5 years. Currently, the highest brucellosis infection levels are concentrated in 7 Southern States, (Arkansas, Florida, Kentucky, Louisiana, Mississippi, Oklahoma and Texas).

3) Why is the Plan Necessary?  
RESPONSE: Unless the plan is implemented, USDA will phase out their involvement beginning in FY 1991 leaving only a "residual program" with funding at approximately $35 million per year to cover only disease surveillance, reporting and enforcement of interstate regulations. Unless the accelerated program is adequately funded, key elements (depopulation, adult vaccination, secondary surveillance, and calfhood vaccination) for achieving eradication will be lost. Funding will be reduced from $55.8 million to approximately $35.9 million in FY 1991. Such a cut will be in addition to the $10 million cut this past year which has had an adverse impact on program operations. Progress in eradicating brucellosis will suffer, due to a lack of funding.
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4) What is needed? RESPONSE: Congress is being asked to fund the Rapid Completion Plan which will require funding for both a “core” program of slaughter, Brucellosis Ring Test (BRT) surveillance and compliance. Accelerated activities will focus on increased depopulation, state program technical reviews, enhanced first point testing, increased adjacent and contact herd testing, epidemiology and training in high incidence states.

(See Budget Plan Attached)

5) Why is it important for every state to support this effort? RESPONSE: The accelerated activities must be implemented on a national scale at the same time to prevent movement of animals from adjacent areas or States that harbor infection. Currently, there are 32 large infected dairy herds in the Chino Basis of Southern California. Unless the Rapid Completion Program is adequately funded, infection from higher incidence states will spread and reinf ect both Class A and Free States.

BUDGET PLAN

In this section, budget breakouts of the plan for the Rapid Completion of the Brucellosis Eradication Program are provided. The Rapid Completion Plan will require funding for both a “core” program that maintains a basic brucellosis program infrastructure of surveillance, prevention, and eradication (e.g. personnel, regular indemnity, testing); and accelerated activities that focus on locating and eliminating infection.
### Plan for the Rapid Completion of the Brucellosis Eradication Program Budget by Accelerated and Core Activity

($ in million)

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<td><strong>$11.60</strong></td>
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<td><strong>$85.24</strong></td>
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A major goal of the rapid completion plan is to have all identified brucellosis infected herds in the current Class A states depopulated or on whole herd vaccination programs. This goal is projected to be achieved in two years from beginning of program. Projections of new reactor herd detections indicated that within 3 years, the core program can begin to focus on surveillance activities — with a corresponding decrease in cost. Funding for selected accelerated activities will be required after FY 1991 as “residual” infected herds are found and depopulated.

**PROPOSED UM&R CHANGES**

(See Chapter 2, Part II, Section C, Subsection 3)
(See Chapter 2, Part III, Section C, Subsection 3)
(See Chapter 2, Part IV, Section C, Subsection 3)

**Prevalence rate of MCI reactors**

MCI reactors are defined as:

A. Nonvaccinated cattle that are positive on the standard card test; or,

B. Vaccinated cattle that are positive on the rivanol at 1:25 or higher; or,

C. Cattle that are 0.30 or less on the PCFIA test; or,
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D. Nonvaccinated cattle with CF test results of 2+ 1:20, or higher, Technicon Test results of +1:10 or higher and vaccinated cattle with CF test results of 1+ 1:40, or higher, Technicon Test results +1:20 or higher.

(+ = fixation of complement observed.)

Calculation of the adjusted MCI reactor prevalence rate is determined by deleting from the gross number of MCI reactors:

(1) reactors that originated from known reactor herds,

(2) reactors that are traced to out-of-state origins,

and

(3) reactors that originated from herds of origin in which the subsequent herd blood test was negative.

(See Chapter 1, Part II, Section 4, Subsection C, 1. c.)

c. Standard Card Test — Standard card test results are recorded as either negative or positive. The card test is to be used as an official test to classify cattle and bison as reactors only under the following circumstances:

1) When conditions and time are such that no other test is available; or

2) When the owner and/or his agent request it because of time or situation constraints; or

3) In stockyards, when the State animal health official specifically designates the card test as the only official test in stockyards. No other test except the BAPA is to be used in stockyards as an official test in those States where the card test has been given the only official test status in stockyards; or

4) In stockyards when the State animal health official designates the card test as the official test, the card test will be the only official diagnostic test for nonvaccinated cattle or bison. The card test may be used in conjunction with the Standard Plate test (SPT), Rivanol Test or CITE® test as supplemental tests on vaccinated cattle or bison. Vaccinated cattle or bison which are positive to the card test, must be classified as suspects if all supplemental tests done are negative or suspicious. If any one of the supplemental tests done has a reactor test results, the card test positive animal must be classified as a reactor.

5) To classify cattle and bison as negative on surveillance samples collected at slaughter, on routine samples collected on farms, and on tests of suspicious and affected herds; or

6) To test Market Cattle Identification (MCI) test samples. Cattle and bison that are tested under the MCI program using an approved
REPORT OF THE COMMITTEE

presumptive test (BAPA or RST) and that are found to be positive on the presumptive test must be retested using the standard card test and the standard plate or tube agglutination test. If there is no further testing, all samples that are positive on the standard card test or standard plate or tube agglutination test will be reported as MCI reactors and traced to the herd of origin. Nonvaccinated cattle that are positive on the standard card test must be reported positive on the standard card test must be reported as MCI reactors for the purposes of State or Area classification.

The card test will not be used to classify cattle and bison as reactors under the following circumstances:

1) In stockyards when the state animal health official designates the card test as a presumptive test, animals that are card positive and negative to the CITE test will be classified as brucellosis negative.

2) All Market Cattle Identification (MCI) test samples will be screened with the PCFIA test. Animals will be classified as reactors if they are 0.30 or less on the PCFIA test. Sera with a value of 0.60 or less will be subjected to the Complement Fixation (CF) test. The following animals will be classified as reactors: nonvaccinated animals with CF test results of 2+ 1:20 or higher, (Technicon Test results of +1:10 or higher); vaccinated animals with CF test results of 1+ 1:40 or higher, (Technicon Test results of +1:20 or higher.)

Results of the standard card test may be used to supplement the results of other tests conducted in the cooperative State-Federal brucellosis laboratory to give the designated epidemiologist additional information when classifying cattle and bison.

(See Chapter 1, Part II, Section 4, Subsection C, 1. h)

h) PCFIA® (Particle Concentration Fluorescence Immunoassay) may be used as presumptive or diagnostic test with the following diagnostic criteria:

1) Negative — S/N values greater than 0.60 [0.70].

2) Suspect — S/N values less than or equal to 0.60 [0.70] and greater than 0.30 [0.25]

3) Reactor — S/N values less than or equal to 0.30 [0.25].

4) S/N values may be interpreted differently if done by a designated epidemiologist.
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APPENDIX E

SWINE BRUCELLOSIS SUB-COMMITTEE REPORT
MINUTES OF MEETING NOV. 30, 1989, LAS VEGAS, NV

Paul Doby opened the meeting with 11 members of the sub-committee present, plus a number of guests.

Dr. W. C. Stewart, APHIS-VS, reported on the national swine brucellosis eradication program as follows:

Testing during the last two federal fiscal years —

<table>
<thead>
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<th>FY 89</th>
<th>FY 88</th>
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<tr>
<td>MST</td>
<td>1,085,379</td>
<td>1,900,000</td>
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<tr>
<td>Area and other</td>
<td>780,035</td>
<td>754,000</td>
</tr>
<tr>
<td>Total</td>
<td>1,865,414</td>
<td>2,654,000</td>
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The drop of 27% in the number of tests during the year from the previous year was in large part a result of requests by this committee and others that MST testing be concentrated in that area of the country (the south and southeast) where the disease is present. This has meant less testing in the concentrated hog areas of the country which have been free for some time.

The number of reactors and the reactor rate for the last two years—

<table>
<thead>
<tr>
<th></th>
<th># of Reactors</th>
<th>Rate</th>
</tr>
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<tbody>
<tr>
<td>MST</td>
<td>531</td>
<td>0.05%</td>
</tr>
<tr>
<td>Area and other</td>
<td>160</td>
<td>0.02%</td>
</tr>
<tr>
<td>Total</td>
<td>691</td>
<td>0.04%</td>
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Asked whether all these reactors were confirmed on rivanol, Stewart replied that information in reports was not adequate to determine, but that a new reporting form has been prepared and in the future that information will be available. The information was also inadequate to provide a breakdown on the areas from which the sows and boars came in the MST testing program.

He noted that fewer reactors are being found in on-farm testing than in the past and that the MST rate has remained about the same in recent years.

In FY '89 a total of 881,605 unidentified sows were tested and 256 reactors were found, a reactor rate of 0.03%. Next year the intention is to sample only identified sows.

The number of validated herds at 3,445, is an increase of 11% over the previous year.

Infected herds found during the year were as follows:
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Alabama 22 (mostly multi-owner community herds)
Georgia 13 (all have been depopulated)
Florida 9
Oklahoma 7 (6 have been depopulated)
North Carolina 3
Louisiana 2
Arkansas 1
South Carolina 1 (has been depopulated)
Virginia 1

The 59 new infected herds disclosed in '89 compared with 65 in '88. Three of the states which found new cases last year had reported no cases the previous year (OK, LA and VA).

Program status—34 states are now validated free, including West Virginia, which qualified on Oct. 1 and Massachusetts even more recently. Puerto Rico and the Virgin Islands are also free. Seven states are in Stage II and nine are in Stage I. This represents a milestone for the program since it is the first time that all states have been recorded as being in some stage of the program.

Two of the recommendations of the task force requested by USAHA which studied the MST program have been implemented: (1) combining of the swine brucellosis and pseudorabies sampling program and (2) testing only identified sows/boars. Others will be implemented as soon as possible.

The recommendation of the sub-committee that the PCFIA test be approved as an official test is in process and a proposed revision of the UM&R has been developed.

A three-year plan leading to final eradication has been developed at the request of the industry and a survey has been conducted to identify where sows and boars are slaughtered.

It is hoped that the new quarterly reporting form will provide better information on individual infected herds.

Plans for FY '90 are to test all sows sold for slaughter in the infected states, to increase indemnity payment rates and to add a full time epidemiologist working in the infected states.

One of the recommendations of the task force which studied the MST program was that APHIS-VS acquire the authority to require the collection of samples in packing plants. This has not yet been done but negotiations are underway with FSIS for that agency to require collection of the samples as has been done in the cattle brucellosis program. The sub-committee indicated its support for these negotiations with FSIS and approved a resolution on this subject.

Neal Black moved, seconded by Bret Marsh, that the sub-committee commend USDA-VS for (1) its response to a request from a number of USAHA committee chairmen to appoint a task force to study the collection of samples and testing of those samples in the swine brucellosis MST
BRUCELLOSIS

program and the response of APHIS to the recommendations of that task force which will result in a greatly enhanced MST program for both swine brucellosis and pseudorabies, and (2) the development of a policy for the MST program that results in collecting of samples from identified sows only, which should also increase the efficiency of that program. The motion carried.

Neal Black then reported on a symposium on feral swine which had been requested by this sub-committee. The meeting, last April in Orlando, FL, was attended by about 75 regulatory officials, scientists and livestock producers. It was quickly apparent to those attending that feral swine are a resource which is managed by states like Florida for hunting and trapping and that elimination of the feral swine is not an option for solving any disease problems. While the seminar did not officially reach any conclusions, these seemed obvious:

1. Feral swine are infected with diseases (PRV and swine brucellosis) which they can transmit to domestic cattle and swine.

2. Feral pigs are important to states from an economic standpoint as a result of the interest in hunting and trapping.

3. Elimination of the feral pig population is not a practical solution. It probably would not be possible even if the funds were available, and opposition from segments of society interested in maintaining the population would probably make elimination a political impossibility.

4. Reduction of the population in certain areas to reduce chances of transmission of disease might be possible.

5. Fencing the wild pigs would not be practical, but confining domestic swine to prevent exposure to wild pigs might be practical.

6. Wild pigs vary in type from obviously feral pigs to those which might pass as domestic feeder pigs, compounding the problem if they are introduced into normal market channels.

7. More knowledge is needed about the population dynamics of feral pigs.

8. Movements of feral pigs, especially to hunting preserves, must be controlled.

Proceedings of the seminar, including texts of all presentations are available from Livestock Conservation Institute, 6414 Copps Ave. #204, Madison, WI 53616.

A sub-committee on feral pigs, made up of representatives of the USAHA committees interested in the problem, was appointed by the president of USAHA at the request of this sub-committee. Black presented the report of that sub-committee, headed by Dr. George Beran of Iowa State University, as it involves the swine brucellosis program (appendix B) and moved approval of those recommendations insofar as they refer to the swine brucellosis program. The motion was seconded by W. C. Stewart and carried.
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Dr. Richard Fite of the policy and program development staff of APHIS, reported on a review which is underway of the swine brucellosis eradication program. He heads a seven-member committee, six members from APHIS and Dr. Merle Lang of Iowa representing the industry, which is charged with answering the question: "Should USDA eradicate swine brucellosis and if so how should it be done?" Issues being studied are: should there be a program at all?; feral swine; budgeting and the surveillance program. The committee is charged with reporting prior to the start of the USDA budget process for FY'92. Five members of the committee were in attendance at the meeting.

Dr. Janet Payeur of the National Veterinary Services Laboratory at Ames, IA, reported on studies to test feral swine for serologic evidence of selected bacterial and viral diseases and to examine them by bacterial culture for infection (appendix A).

Dr. Terry Taylor, APHIS-VS AVIC for Virginia, reported a case history of brucellosis infection in a swine herd in that state which may have involved feral swine. The case was disclosed as a result of testing to qualify for sale of feeder pigs in the pseudorabies program. The herd, containing 25 breeding animals, is in the southeast part of the state and the owner is primarily a peanut farmer. Repeated testing in the herd confirmed that it was infected with brucella suis. The herd has been depopulated. The only two swine herds within 1.5 miles of the herd have been tested and are negative. Sources of breeding stock within the last five years were tested and negative, except that two of the source herds had been sold out, so could not be tested. Symptoms in 1987 have lead to the conclusion that the herd had been infected for some time. During the past two years there have been two reports of positives at slaughter plants where this owner sold sows, but the positives could not be traced. It is considered a good possibility that the infection was purchased. The owner said there are no feral pigs in the area. The state game department indicated the only feral pigs in the state are on False Cape off the east coast, but that opinion is disputed by others. This farm is surrounded by hunting clubs, but they say they don't hunt feral swine and haven't brought any in. Monitoring of the area will continue.

Dr. Frank Mulhern of the National Pork Producers Council discussed actions needed to prevent feral swine from exposing domestic swine to brucellosis. He said while there is evidence of infection in feral swine, there is no proof of current transmission. The studies proposed by the sub-committee on feral pigs are critical to success of disease eradication efforts. He moved, seconded by Black, that the swine brucellosis sub-committee urge APHIS-VS to call a meeting to identify the variations in possible transmission of disease from feral pigs to domestic pigs, to develop specific studies of feral swine and to define a monitoring system to find transmission of disease from feral to domestic pigs. Motion carried.

Don Gingerich, president of the National Pork Producers Council, commented that pork producers support completion of the eradication effort.
He called first for action to eliminate the infection in presently known infected herds, then gear up the surveillance program to find new infections.

Dr. M. A. Van Buskirk of Pennsylvania reported on an importation of reindeer which were card positive to swine brucellosis. The reindeer originated in a herd in Alaska which is known to be positive. They were bled in Alaska and then shipped to Pennsylvania before the test results were known. The animals were slaughtered, but no brucella suis was recovered from them and contacts in Pennsylvania are negative.

Mulhern discussed actions needed to begin the three-year eradication effort developed by VS staff. He said there are three stages to an eradication effort: (1) panic, (2) cooperation and (3) apathy. The apathy stage is what makes programs go on forever. Many states with most of the pigs have been free for many years, but the cost of testing continues. Producers in those states are protesting the continuing cost. What’s needed is to get rid of the infection in infected herds, eliminate the infection and then eliminate the program. The three-year plan has received sympathetic response from congressmen. It would require a federal budget of $10 million over three years. Considering the USDA budget cycle, FY ’92 is the first budget in which it could be included. Dr. Mulhern proposed an organized, coordinated industry-wide effort to get rid of the disease by 1995, but noted that the results of the study being conducted by APHIS may have an effect on this plan.

R. R. Ormiston of APHIS was asked about funding for FY ’89. He said the same amount is available as in FY ’88, a total of $674,000 of which $15,000 will be allocated for indemnities, some for staff costs and the rest for surveillance, concentrated in the infected states.

It was noted that previously several million dollars were available from the brucellosis allocation and while overall brucellosis funding has remained fairly constant, even though the number of infected cattle herds has declined dramatically, the amount of the overall federal brucellosis budget allocated for swine brucellosis has been reduced drastically. Chairman Doby volunteered to confer with the chairman of the overall brucellosis committee of USAHA to secure support for an increased share of the brucellosis funds for the swine brucellosis eradication effort.

R. R. Ormiston discussed draft 2 of new proposed Uniform Methods and Rules for the swine brucellosis control/eradication. This draft is a response to a large number of comments and suggestions for changes after distributing of a proposed first draft. If approved by USAHA the revision can be put into effect quickly.

Some discrepancies between the proposed UM&R and the recommendations of the feral swine sub-committee approved by the swine brucellosis sub-committee and some inconsistencies in the proposal were pointed out. On motion by Black, seconded by Lang, the sub-committee approved the proposed UM&R subject to revision of the feral swine requirements based
REPORT OF THE COMMITTEE

on discussions between VS staff and Dr. Beran and also subject to annual review. The motion carried.

There being no further business, the meeting was adjourned at 5:30.

BRUCELLOSIS IN FERAL SWINE FROM FLORIDA

Janet B. Payeur, D.V.M., Ph.D.
Darla R. Ewalt, M.S.
Ronald L. Morgan, D.V.M., M.S.
Dean A. Stevens, Jr.
Patricia L. Geer
National Veterinary Services Laboratories
Science and Technology
Animal and Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa 50010

INTRODUCTION

The European wild pig (Sus scrofa) is the progenitor of the domestic and wild breeds of swine. Wild swine are potentially susceptible to all of the viral, bacterial, and fungal diseases of domestic swine. Diseases may be manifested as clinical cases or as carrier states. Wild swine populations are well established throughout much of the southeastern United States, Texas, California, and Hawaii. Because wild swine are susceptible to and may serve as reservoirs for diseases of domestic swine, disease among these swine is of concern to wildlife and domestic livestock interests.

Clark et al. conducted a survey of California wild swine for antibody to selected zoonotic disease agents between 1981–1983. Antibody to Brucella species was detected in 15% (21/136) of the samples. In a study by Corn et al. in Texas, antibody to Brucella species was found in 3.2% (4/124) of the swine tested. Brucella suis biovar 1 was isolated from four swine in two populations, but only one hog was seropositive. Brucella abortus strain 19 was isolated from one hog which was seronegative.

Becker et al. in a survey in Florida isolated Brucella suis biovar 1 from more than 9% (9/95) of the feral swine. Fifty-three percent (50/95) of the animals were positive to at least one serological test for brucellosis in a battery which included complement fixation (CF), standard tube (STT), rivanol (RIV), and card test. Cervical and inguinal lymph nodes and seminal vesicles were the most common sources of the organism. Wood et al. in a survey in South Carolina found more than 23% of the feral swine positive using the card test for brucellosis. Infected boars commonly shed large number of B. suis in semen, and venereal transmission of brucellosis has been demonstrated experimentally and under natural conditions.

Zygmont et al. determined that 6% (21/352) of the wild swine in seven
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populations in Arkansas, Florida, Georgia, Louisiana, and South Carolina were seropositive for brucellosis in at least one of the following tests: card, STT, plate, buffered plate antigen, CF, or RIV. \textit{Brucella suis} biovar 1 was isolated from three of these seropositive feral swine located in two areas in Florida and one area in Louisiana.

OBJECTIVE

The purpose of this study was to test feral swine for serologic evidence of selected bacterial and viral diseases and to examine them by bacterial culture for infection with brucellae and mycobacteria.

MATERIALS AND METHODS

In September of 1988, three adult feral sows (1–2 years old) were captured in Hendry County, Florida, and shipped to the National Veterinary Services Laboratories (NVSL), Ames, Iowa. One sow (2027) aborted the second day after arrival, and the fetuses (7) and placenta were cultured for \textit{Brucella} species. One of the sows (2026) had four piglets on November 10, 1988.

Sera from three captured adult feral sows and four piglets were screened for several bacterial, viral, parasitic, and rickettsial diseases. Sera were collected every 2 weeks for 32 weeks and tested for antibody to \textit{Brucella} species by the following tests: card, CF, RIV, STT, and particle concentration fluorescence immunoassay (PCFIA). Standard card test results are recorded as either negative or positive. Standard tube test results are interpreted in the following ways: Negative = I 1:25 or lower, Reactor = 1:25 or higher. The RIV test is interpreted in the following ways: Negative = 1:25 or lower, Reactor = +1:25 or higher. The CF test is interpreted in the following ways: Negative = I 1:20 or lower, Reactor = 1:20 or higher. The degree of fixation of complement is as follows: 1+ = 25%, 2+ = 50%, 3+ = 75%, 4+ = 100%. The PCFIA test is interpreted in the following ways: Negative = S/N values greater than 0.70, Suspect = S/N values less than or equal to 0.70 and greater than 0.25, Reactor = S/N values less than or equal to 0.25.

The swine were skin tested intradermally for mycobacteriosis using 0.1 ml Avian and Mammalian Old Tuberculins. After 40 weeks, the animals were necropsied and the following specimens were collected for culture: mandibular, superficial inguinal, hepatic, bronchial, and mesenteric lymph nodes, spleen, lung, kidney, liver, blood, feces, and urine.

The swine were serologically tested for antibody to the following diseases or agents: \textit{Brucella}, \textit{Leptospira interrogans}, \textit{Mycoplasma hypopneumoniae}, \textit{Actinobacillus pleuropneumoniae}, \textit{Toxoplasma gondii}, \textit{Eperythrozoon suis} (EPE), \textit{Q Fever}, encephalomyocarditis (EMC), hemagglutinating encephalomyelitis (HEV), porcine parvovirus (PPV), pseudorabies (PR), swine influenza (SI), transmissible gastroenteritis (TGE), vesicular stomatitis (VS) [New Jersey and Indiana], porcine enterovirus (PEV), Venezuelan
equine encephalomyelitis (VEE), western equine encephalomyelitis (WEE), and eastern equine encephalomyelitis (EEE).

The serum neutralization test (SN) was used to test for EMC (1:32), TGE (1:8), PEV serogroups 1–8 (1:32), VEE (1:10), WEE (1:10), EEE (1:10), and VS (1:8). The hemagglutination inhibition test (HI) was used to test for HEV (1:10), PPV (1:16), SI (1:10), VEE (1:10), WEE (1:10), and EEE (1:10). An enzyme-linked immunosorbent assay (ELISA), a latex agglutination test (LA 1:40), and serum neutralization test (SN 1:4) were used to test for PR. The indirect hemagglutination test (IHA) was used to test for EPE (1:10) and *Toxoplasma gondii* (1:8). The CF test was used for Q fever (1:10), *Mycoplasma hyopneumoniae* (1:8), and *Actinobacillus pleuropneumoniae* (1:8). The indirect fluorescent antibody (IFA) test was used to test for toxoplasmosis. The microagglutination test (MAT) was used to test for *Leptospira interrogans* (1:100). Fecal samples were collected and examined by flotation for parasites and their eggs and cultured for mycobacteria.

Four additional feral swine from Clay County, Florida, were acquired from a research project at the National Animal Disease Center (NADC), and they were tested for the diseases mentioned above. They had been infected with pseudorabies and TGE while at the NADC.

**RESULTS**

Sow 2026 gave birth to four piglets (two males, two females) 38 days after arrival. She was serologically positive for brucellosis on four tests (card, CF, RIV, STT) for 32 weeks and was classified as a “reactor” for 12 weeks and “suspect” for 20 weeks on the PCFIA (Table 1). She was culture negative for *Brucella* species.

Sow 2026 was serologically positive for *Toxoplasma* (IHA 1:64), PPV (HI 1:64), *Leptospira interrogans* serovars autumnalis (MAT 1:400), ballum (MAT 1:100), bratislava (MAT 1:400), and icterohaemorrhagiae (MAT 1:100). The following parasites were found in the fecal samples: *Oesophagostomum* species and *Trichuris suis*. She was culture negative for *Mycobacteria* species but was skin test positive and showed histologic lesions compatible with mycobacteriosis.

Sow 2027 aborted seven fetuses on the second day after arrival. No *Brucella* species were isolated from the aborted material. The cause of the abortion was probably from stress related to the travel. She was classified serologically “negative” by the card test for 42 weeks, by the STT for 14 weeks, and by the RIV test for 8 weeks. She was classified serologically as a “suspect” for 4 weeks and as a “reactor” for 34 weeks on the PCFIA. After serology, she was classified as a “reactor” for 42 weeks by the CF test (Table 2). *Brucella suis* biovar 1 was isolated from the lung.

Sow 2027 was serologically positive for *Toxoplasma* (1:32), PPV (HI 1:128), PEV serogroup 2 (1:64), *Leptospira interrogans* serovar autumnalis (MAT 1:100) and ballum (MAT 1:100). The following parasites were found in the fecal samples: *Oesophagostomum* species and *Trichuris suis*. She was...
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culture negative for *Mycobacteria* species but was skin test positive for mycobacteriosis.

Sow 2028 was serologically positive for *Toxoplasma* (1:32), PPV (1:256), PR (ELISA and LA 1:40), PEV serogroup 3 (1:32), 4 (1:64), 8 (1:32), *Leptospira interrogans* serovars autumnalis (MAT 1:100), ballum (MAT 1:100), and bratislava (MAT 1:100). The following parasites were found in the fecal samples: *Oesophagostomum* species and *Trichuris suis*. She was culture positive for *Mycobacterium avium* serotype 1 (hepatic and superficial inguinal lymph nodes and spleen). She was skin test positive for mycobacteriosis. Lesions compatible with mycobacteriosis were seen grossly and histologically in the liver and in the hepatic and mesenteric lymph nodes.

Piglet 1 after serology was classified as a “reactor” on the card test for the first 4 weeks, on the STT for the first 2 weeks, on the RIV test for the first 2 weeks, on the CF for the first 6 weeks, and as a “suspect” for the first 4 weeks on the PCFIA test. She was “negative” on all of the tests for the remaining period until she died at 38 weeks of age (Table 4). She was culture negative for *Brucella* species.

Piglet 1 was serologically positive for *Toxoplasma* (1:8), HEV (1:10), and PPV (1:128). The following parasites were found in the fecal samples: *Oesophagostomum* species and *Ascaris suum*. She was positive on the skin test for mycobacteriosis but was culture negative. No lesions were seen on histopathology. She was pregnant with three fetuses when she died.

Piglet 2 after serology was classified as a “reactor” on the card test for the first 4 weeks, on the RIV for the first 2 weeks, on the CF for the first 6 weeks, and as a “suspect” for the first 4 weeks on the PCFIA test. She was “negative” on all of the tests for the remaining period until she was euthanized at 35 weeks of age (Table 5). She was culture negative for *Brucella* species.

Piglet 2 was serologically positive for *Toxoplasma* (1:8), HEV (1:10), and PPV (1:1024). The following parasites were found in the fecal samples: *Oesophagostomum* species and *Ascaris suum*. She was positive on the skin test for mycobacteriosis but was culture negative. No lesions were seen on histopathology.

Piglet 3 after serology was classified as a “reactor” on the card test for the first 4 weeks, on the STT for the first 2 weeks, on the RIV test for the first 2 weeks, on the CF test for the first 6 weeks, and as a “suspect” for the first 4 weeks on the PCFIA test. He was “negative” on all of the tests for the remaining period until he died at 38 weeks of age (Table 6). He was culture negative for *Brucella* species.

Piglet 3 died at 38 weeks of age from septicemia, pulmonary congestion with edema, and toxemia. He was serologically positive for HEV (1:10), PPV (1:512), and *Leptospira interrogans* serovar ballum (1:200). The following
parasites were seen in the fecal sample: *Oesophagostomum* species and *Ascaris suum*. He was negative on the skin test and on culture for mycobacteriosis.

Piglet 4 after serology was classified as a "reactor" on the card test for the first 4 weeks, on the STT for the first 2 weeks, on the RIV test for the first 2 weeks, on the CF test for the first 6 weeks, and as a "suspect" for the first 4 weeks on the PCFIA test. He was "negative" on all of the tests for the remaining period until he died at 6 weeks of age from a mild hepatitis and interstitial pneumonia (Table 7). He was negative for *Brucella* species on culture.

Piglet 4 was serologically positive for PPV (1:256). The following bacterial organisms were isolated from tissues taken at necropsy: *Escherichia coli* from the small intestine, mesenteric and pulmonary lymph nodes, stomach contents; *Staphylococcus* sp from the lung, liver, kidney; *Streptococcus* sp. from the liver, reproductive tract, mesenteric lymph nodes; *Pseudomonas aeruginosa* from the pulmonary lymph nodes; *Proteus* sp. from the reproductive tract and small intestine. He was negative on viral isolation for the following: adenovirus, EMC, HEV, PPV, reovirus, enterovirus, and SI.

Pig 32 was negative for *Brucella* species on culture. He was negative on all of the brucellosis serological tests (card, STT, RIV, CF, PCFIA). He was serologically positive for *Toxoplasma* species (1:8), HEV (1:40), PR (1:8), TGE (1:32), PEV serogroup 1 (1:64), and serogroup 4 (1:32). He was negative for mycobacteriosis on culture and by histopathology.

Pig 33 was negative for *Brucella* species on culture. She was negative on all the brucellosis serological tests (card, STT, RIV, CF, PCFIA). She was serologically positive for *Toxoplasma* species (1:16), HEV (1:80), PR (1:4), TGE (1:8), PEV serogroup 1 (1:64), serogroup 3 (1:32), serogroup 4 (1:32), and serogroup 8 (1:128). She was negative for mycobacteriosis on culture and by histopathology.

Pig 34 was negative for *Brucella* species on culture. She was negative on all the brucellosis serological tests (card, STT, RIV, CF, PCFIA). She was serologically positive for *Toxoplasma* species (1:8), HEV (1:80), PR (1:16), TGE (1:8), PEV serogroup 1 (1:256), serogroup 3 (1:64), serogroup 6 (1:64), serogroup 7 (1:32), and *Mycoplasma hyopneumoniae* (1:32).

Pig 35 was negative for *Brucella* species on culture. He was negative on all the brucellosis serological tests (card, STT, RIV, CF, PCFIA). He was serologically positive for *Toxoplasma* species (1:16), HEV (>1:640), PR (1:16), TGE (1:8), PEV serogroup 1 (1:256), serogroup 2 (1:64), serogroup 3 (1:32), serogroup 4 (1:32), serogroup 5 (1:64), serogroup 6 (1:64), serogroup 7 (1:64), WEE (1:10), and *Mycoplasma hyopneumoniae* (1:128).
BRUCELLOSIS

CONCLUSIONS

Feral swine are often hunted or captured and reared for human consumption and can be a source of infection to both man and domestic livestock. The swine in this study were positive for various bacterial and viral diseases which are of importance to the livestock industry.

Previous surveys of feral swine have shown serological and bacteriological evidence of brucellosis in the population. The data from this study confirms these previous findings. The three sows (2026, 2027, 2028) used in this study were selected because they were card test positive and from a farm with a cow infected with *Brucella suis* biovar 1. The other four swine (32, 33, 34, 35) were selected because they were serologically negative for *Brucella* and pseudorabies. Feral swine in the same area with cattle may be the source of cattle infections.

*Brucella suis* has not been shown to cause abortion or clinical signs in cattle, nor is there any evidence of horizontal transmission. It has been recovered from the milk of cows experimentally exposed to infected swine and may localize in the udder of naturally infected cows and be excreted in the milk.

The incidence of *B. abortus* in cattle is decreasing in the United States, but the incidence of *B. suis* may be increasing. There were 11 *B. suis* biovar 1 isolations from cattle submissions to the NVSL in the 5-year period between October 1, 1982, and September 30, 1987 (Table 8). Between October 1, 1987, and September 30, 1989, there were 40 *B. suis* biovar 1 isolations from cattle submissions to the NVSL. Twenty-four isolations during FY 89 were from an on-going research project at the NVSL. The distribution of brucellae by biovar among submissions to the NVSL during FY 83–89 is shown in Figure 1. The NVSL *B. suis* isolations came from Florida (19), South Carolina (3), Texas (4), Alabama (1), and Iowa (24) (Figure 2). There were 16 *B. suis* biovar 1, 2 *B. suis* biovar 3, and 1 *B. abortus* biovar 1 isolations from swine submissions to the NVSL between October 1, 1984, and September 30, 1989 (Table 9).

Brucellosis has been detected in many wild swine populations within the states of Arkansas, California, Florida, Georgia, Hawaii, Louisiana, South Carolina, and Texas. Abortions and infertility due to *B. suis* infection probably occurs in wild swine, but the number of swine is unknown. Swine brucellosis has been an important problem in commercial swine, but eradication of this disease is proceeding rapidly from domestic swine. Wildlife management agencies should identify infected wild swine populations and discourage relocation of potentially infected wild swine.

Diagnosis of brucellosis may be bacteriological or serological. The card test is the principal means of diagnosis in swine, but various other serum tests have been used. The tests are effective in determining the presence of brucellosis in the herd but have limitations in detecting individually infected animals. It is advisable to use a battery of serologic tests (tube,
card, CF, PCFIA) when trying to identify infected swine. The entire herd must be considered in any control program.13,18

Florida has more feral swine than any other state. Excellent habitat and a lack of natural predators have resulted in a feral swine population estimated at 500,000.19 The domestic swine population is small (approximately 400,000 pigs marketed annually) but widely distributed in Florida, with many part-time farmers raising small numbers. Feral swine are present in all but one of Florida’s 67 counties. The close proximity of feral and domestic swine may allow transmission of infectious diseases from feral swine.20 Although feral swine are not a wild game species in Florida, they are hunted in most counties and trapped on private property. Feral swine may be slaughtered by the trapper, sold or given to individuals, sold through commercial markets for slaughter, or sold to game preserves as hunting stock.19 The value from hunting and trapping was estimated to be $8.3 million in 1980. Feral swine do not contribute significantly to agriculture but are important for recreation.19 The damage to crops and pasturage from feral swine probably outweighs any economic returns to farmers. Recreational benefits make feral swine an important resource in most areas of the state. Degner’s survey indicated that meat from feral swine is widely distributed throughout the United States by hunters who usually give it away rather than sell it.20

Brucellosis in humans has flu-like symptoms including intermittent fever, headaches, muscle and joint soreness, and weakness. The fatality rate is very low, but the disease course may be prolonged and debilitating. Meat-packing plant workers represent about 85% of human brucellosis cases. About 80% of these cases are associated with \textit{B. suis} from swine.12,21,22 Hunters and livestock producers are frequently infected with \textit{B. suis} when handling and dressing feral swine.5,8,22 Swine were the source of brucellosis for man in 39% of the cases in Florida during the period from 1963 to 1975. In 28 confirmed human cases during 1974 and 1975, 565 involved swine contact and 22% occurred in hunters.8 About 100,000 feral swine are killed by hunters each year in the 12 southeastern states.8 Hunters should be advised of the presence of swine brucellosis in endemic areas.5

Brucellosis caused by \textit{B. suis} has significant public health implications. This species is much more pathogenic for humans than other brucellae present in the United States. Infected swine usually have more \textit{B. suis} organisms in their tissues than do cattle infected with \textit{B. abortus} and represent a considerable danger to people exposed to them.16

\textit{Brucella suis} biovar 1, \textit{Mycobacterium bovis}, \textit{Mycobacterium avium}, and \textit{Herpesvirus suis} readily infect wild swine. These organisms can infect domestic livestock and complicate disease eradication programs. Newly introduced or relocated swine should be adequately quarantined and have tuberculin and serological tests to prevent the spread of these diseases to domestic swine and cattle populations and to humans.1,8
BRUCELLOSIS

The presence of brucellosis and pseudorabies in wild swine supports the hypothesis that these animals are reservoirs of these swine diseases. Control of wild swine relocation for hunting and movement in and around commercial swine farms may help to minimize exposure of domestic swine. A natural reservoir of infection in the feral swine population is strongly implicated but not yet proven.

Wild hogs are exposed to zoonotic agents in their environment and people entering these habitats and handling these animals are at risk. Feral swine are simultaneously perceived as a potential national disaster in terms of exotic and domestic diseases and as a hunting/recreational asset.

REFERENCES

REPORT OF THE COMMITTEE


BRUCELLOSIS

Table 1. Results of brucellosis serological tests for Sow 2026.

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R = Reactor  S = Suspect  NT = No test  N = Negative

Table 2. Results of brucellosis serological tests for Sow 2027.

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R = Reactor  S = Suspect  NT = No test  N = Negative

Table 3. Results of brucellosis serological tests for Sow 2028.

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R = Reactor  S = Suspect  NT = No test  N = Negative
REPORT OF THE COMMITTEE

Table 4. Results of brucellosis serological tests for piglet 1.

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R = Reactor  S = Suspect  N = Negative

Table 5. Results of brucellosis serological tests for piglet 2.

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<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

R = Reactor  S = Suspect  N = Negative

Table 6. Results of brucellosis serological tests for piglet 3.

<table>
<thead>
<tr>
<th>Test</th>
<th>Weeks</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
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<td>16</td>
<td>18</td>
<td>20</td>
<td>22</td>
<td>24</td>
<td>26</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Card</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
</tr>
<tr>
<td>STT</td>
<td>R</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
</tr>
<tr>
<td>RIV</td>
<td>R</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
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<tr>
<td>CF</td>
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<td>R</td>
<td>R</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
</tr>
<tr>
<td>PCFIA</td>
<td>S</td>
<td>S</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
</tr>
</tbody>
</table>

R = Reactor  S = Suspect  N = Negative
Table 7. Results of brucellosis serological tests for piglet 4.

<table>
<thead>
<tr>
<th>Test</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Card</td>
<td>R</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>STT</td>
<td>R</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>RIV</td>
<td>R</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>CF</td>
<td>R</td>
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<td>N</td>
</tr>
<tr>
<td>PCFIA</td>
<td>S</td>
<td>S</td>
<td>N</td>
</tr>
</tbody>
</table>

R = Reactor  S = Suspect  N = Negative

Table 8. Results of bacteriologic examinations for brucellae from cattle, FY 83–89.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>FY83</th>
<th>FY84</th>
<th>FY85</th>
<th>FY86</th>
<th>FY87</th>
<th>FY88</th>
<th>FY89</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. abortus biovar 1</td>
<td>1732</td>
<td>1180</td>
<td>1153</td>
<td>1014</td>
<td>1203</td>
<td>934</td>
<td>907</td>
</tr>
<tr>
<td>B. abortus biovar 2</td>
<td>71</td>
<td>59</td>
<td>40</td>
<td>36</td>
<td>42</td>
<td>51</td>
<td>47</td>
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<tr>
<td>B. abortus biovar 4</td>
<td>74</td>
<td>45</td>
<td>54</td>
<td>55</td>
<td>34</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>B. abortus strain 19</td>
<td>417</td>
<td>258</td>
<td>215</td>
<td>228</td>
<td>296</td>
<td>434</td>
<td>511</td>
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<tr>
<td>B. suis biovar 1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>11</td>
<td>29</td>
</tr>
</tbody>
</table>

Total isolations 2294 1544 1464 1338 1577 1475 1544
Total submissions 2993 2142 1968 1933 2323 2039 2150
Percent isolation 76.6 72.1 74.4 69.2 67.9 72.3 71.8

Table 9. Results of bacteriologic examinations for brucellae from swine, FY 85–89.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>FY85</th>
<th>FY86</th>
<th>FY87</th>
<th>FY88</th>
<th>FY89</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. suis biovar 1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>B. suis biovar 3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B. abortus biovar 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Total isolations 1 2 3 7 6
Total submissions 17 10 14 31 72
Percent isolation 5.9 20.0 21.4 22.6 8.3
REPORT OF THE COMMITTEE

APPENDIX F

REPORT OF THE SCIENTIFIC ADVISORY COMMITTEE

It is the opinion of the Scientific Advisory Committee that current UM7R calfhood vaccination requirements should not be changed and calfhood vaccination should continue to be recommended for beef heifers in Class B states.

RAPID COMPLETION PLAN — REVISED
(Supplement — April 28, 1989 — from Metcalf to Cargile)

(1) We emphasize the necessity to fully comply with the UM&R requirements to attain goals.

(2) Fully support state technical program review.

(3) Improve education and training of program personnel of low and high incidence states in the most recent diagnostic, vaccination and disease management procedures.

(4) Optimize the expenditure of RCP funds through existing economic and epidemiological simulation models (available at USDA/APHIS & Texas A&M University) of state and national programs.

(5) Restriction of addition of new breeding stock to herds until a negative test and calving to avoid unnecessary exposure.

(6) Mandatory whole-herd vaccination.

(7) In lieu of “as soon as possible” add “vaccinate on the next herd test.”

(8) In lieu of “retested 180 days after whole-herd vaccination” add “retest between 120 and 180 days.”

CONCERNING PROPOSED U M & R CHANGES

(1) We accept the proposed combination of PCFIA and CF for determining MCI reactors; however, we recommend that cattle with PCFIA values of 0.3 to 0.6, and that are not MCI reactors, be evaluated epidemiologically. Furthermore, MCI reactor classification must be equitable between states.

(2) We agree that the card test can be designated as a presumptive test and CITE negative cattle may be technically classified as negative animals; however, we recognize that this classification may interfere with marketing channels.

(3) We accept the proposed reactor criteria for the PCFIA for vaccinates and non-vaccinates and the suspect criteria only for vaccinates.

Due to the known problems associated with latent Brucella abortus infection, the exposed heifers in dairy herds should move from the herd only if “S” branded or spayed. It is our opinion that the optimal age to calfhood
vaccinate is between 4 and 8 months of age, not 3 and 12 months of age. We agree that non-vaccinates may be tested with the CITE test. The Scientific Advisory Committee does not recommend establishing quarantined feedlots in Class Free states; however, spayed cattle can move freely. The risks are too great and is in conflict with the Class Free concept and Rapid Completion Plan.

APPENDIX G

Proposed UM&R Changes
by John Cargile
(See Chapter 2, Part II, Section C, Subsection 3)
(See Chapter 2, Part III, Section C, Subsection 3)
(See Chapter 2, Part IV, Section C, Subsection 3)

Prevalence rate of MCI reactors

MCI reactors are defined as:

A. Cattle positive on the standard card test when the card test is designated as the official test; or,

B. Cattle that are reactors on other officially designated tests.

Calculation of the adjusted MCI reactor prevalence rate is determined by deleting from the gross number of MCI reactors:

1. reactors that originated from known reactor herds,
2. reactors that are traced to out-of-state origins,
and
3. reactors that originated from herds of origin in which the subsequent herd blood test was negative.

(See Chapter 1, Part II, Section 4, Subsection C, 1. c.)

c. Standard Card Test — Standard card test results are recorded as either negative or positive. The card test is to be used as an official test to classify cattle and bison as reactors only under the following circumstances:

1) When conditions and time are such that no other test is available; or
2) When the owner and/or his agent request it because of time or situation constraints; or
3) In stockyards, when the State animal health official specifically designates the card test as the only official test in stockyards. No other test except the BAPA is to be used in stockyards as an official test in those States where the card test has been given the only official test status in stockyards; or
4) In stockyards when the state animal health official designates the card test as the official test, the card test will be the only official diag-
The card test may be used in conjunction with the Standard Plate Test (SPT), Rivanol Test or CITE® test as supplemental tests on vaccinated cattle or bison. Vaccinated cattle or bison which are positive to the card test, must be classified as suspects if all supplemental tests done are negative or suspicious. If any one of the supplemental tests done has a reactor test results, the card test positive animal must be classified as a reactor.

5) To classify cattle and bison as negative on surveillance samples collected at slaughter, on routine samples collected on farms, and on tests of suspicious and affected herd; or

6) To test Market Cattle Identification (MCI) test samples. Cattle and bison that are tested under the MCI program using an approved presumptive test (BAPA or RST) and that are found to be positive on the presumptive test must be retested using the standard card test and the standard plate or tube agglutination test. If there is no further testing, all samples that are positive on the standard card test or standard plate or tube agglutination test will be reported as MCI reactors and traced to the herd of origin. Nonvaccinated cattle that are positive on the standard card test must be reported as MCI reactors for the purpose of State or Area classification.

The card test will not be used to classify cattle and bison as reactors under the following circumstances:

(1) In stockyards when the state animal health official designates the card test as a presumptive test; and,

(2) All Market Cattle Identification (MCI) test samples will be screened with the PCFIA test. Animals will be classified as reactors if they are 0.30 or less on the PCFIA test. Sera with a value of 0.60 or less will be subjected to the Complement Fixation (CF) test. The following animals will be classified as reactors: nonvaccinated animals with CF test results of 2+ 1:20 or higher, (Technicon Test results of 1+ 1:10 or higher); vaccinated animals with CF test results of 1+ 1:40 or higher, (Technicon Test results of + 1:20 or higher).

Results of the standard card test may be used to supplement the results of other tests conducted in the cooperative State-Federal brucellosis laboratory to give the designated epidemiologist additional information when classifying cattle and bison.

(See Chapter 1, Part II, Section 4, Subsection C, 1. h)

h) PCFIA® (Particle Concentration Fluorescence Immunoassay) may be used as presumptive or diagnostic test with the following diagnostic criteria:

1) Negative — S/N values greater than 0.60 [0:70].
2) Suspect — S/N values less than or equal to 0.60 [0:70] and greater than 0.30 [0:25].

3) Reactor — S/N values less than or equal to 0.30 [0:25].

4) S/N values may be interpreted differently if done by a designated epidemiologist.

**APPENDIX H**

**DRAFT**

**Proposed Changes to the Brucellosis Eradication Uniform Methods and Rules**

by Dr. Jan Huber

**Herd blood test**

1. Cattle or bison. The herd blood test must include all cattle or bison six months of age and older, except steers, spayed heifers, official calfhood vaccinates -----------------. *(Change to: The herd blood test must include all cattle or bison six months of age and older, except steers, spayed heifers, bulls less than 18 months of age, official calfhood vaccinates -------.)*

A motion was made and seconded that the proposed change of 1988 for page 20 be left as presently written.

20

3. Testing Requirements

A. Cattle and Bison

1. *Herd blood test* --- Herd blood tests must include all cattle and bison six months of age and older except steers, spayed heifers, official calfhood vaccinates -------. *(Change to: Herd blood tests must include all cattle and bison six months of age and older except steers, spayed heifers, bulls less than 18 months of age (except as specified in Part II, 3,A, 2), official calfhood vaccinates ----------------.--)*

2. *Herd blood test for release of quarantine and postquarantine test* --- The herd blood test that is performed to qualify a herd for release of quarantine or that is performed six to twelve months after a quarantine has been released must include all non-neutered cattle and bison six months of age and older, ------. *(Change to: The herd blood test that is performed to qualify a herd for release of quarantine or that is performed six to twelve months after a quarantine has been released must include all non-neutered cattle and bison (females and males) six months of age and older, ------.)*

235
REPORT OF THE COMMITTEE

A motion was made and seconded that the proposed change of 1988 for page 39 be left as presently written.

39

d. Heifer calves from affected herds — All heifer calves in an affected herd are included under the herd quarantine restrictions. If they are retained in the herd they should be calfhood vaccinated. These heifer calves may only be moved if they comply with the movement requirements outlined in Chapter 2, parts II D5, III D5, IV D5, and V D5 for each Class State.

(Change to: Calves from affected herds — All calves in an affected herd are included under the herd quarantine restrictions. These calves may only be moved if they comply with the movement requirements outlined in Chapter 2, parts II D5, III D5, IV D5, and V D5 for each Class State. If heifer calves are retained in the herd they should be calfhood vaccinated.)

The motion that was made and seconded was incorrect and is withdrawn. The motion amended movement requirements that are to be outlined on pages 110 and 111; not on page 54. The correct motion is to leave the proposed change of 1988 for page 54 presently written.

54

5. Heifer calves from affected herds

(Change to: Calves from affected herds)

All heifer calves in an affected herd, are included under the herd quarantine restrictions. (Change to: All calves ———.) If they are retained in the herd they should be calfhood vaccinated. (Change to: If the heifer calves are ———.) These heifer calves may only be moved if they comply with the requirements of a, b, c or d of this section. (Change to: Calves may be moved only if they comply with the requirements of a and b of this section.)

A motion was made and seconded that the proposed change of 1988 be amended as follows:

1. Option C. will be reinstated.

110

As of October 1, 1988, the movement of calves from affected herds in Class B States will be (Change to: has been) terminated. As of October 1, 1990, no cattle may be moved, from Class B States that are not meeting the standards for progress or that did not meet the standards at any time between October 1, 1988, and October 1, 1990, except steers, spayed heifers, “S” branded cattle, or cattle from certified Free herds. (Change to: As of October 1, 1990, no cattle may be moved, from Class B States that are not meeting the standards for progress or that did not meet
BRUCELLOSIS

The standards at any time between October 1, 1988, and October 1, 1990, except steers, spayed heifers, bulls less than 18 months of age out of any herd not known to be affected, “S” branded cattle, or cattle from certified-free herds.)

a. The heifer calves must be “S” branded or spayed, or (Change to: The calves must be “S” branded or neutered, or)

b. 1) The heifer calves must be quarantined and held separate and apart from the affected adult herd after weaning until they are negative on an official test following the completion of their first calving, or

2) If the heifer calves remain in the affected adult herd, the entire herd shall not be released from quarantine until all the heifer calves have matured and calved, then the entire herd must be tested negative for brucellosis to qualify for quarantine release.

c. (Delete)

d. (Delete)

A motion was made and seconded that Option C be reinstated and amended as follows:

C. In commercial dairy herds, calves, no more than 6 months of age, must:

(1) be part of a herd that is following an approved individual herd plan (IHP), (the IHP will include adult vaccination and a lowered upper-age-limit calfhood vaccination when recommended by the designated epidemiologist), and

(2) the heifers will be calfhood vaccinated (at the ages provided for in the IHP or the UM&R) if they are at least 4 months of age when leaving the herd.

(3) be identified with an official eartag prior to movement, and

(4) be separated from the dam at no more than 7 days of age, and

(5) be quarantined separate and apart from other cattle after movement from the affected herd for at least 30 days.

111.a

a. steers and spayed heifers; (Change to: steers, spayed heifers, and bulls under 18 months of age from herds not known to be affected;)

b. (Delete)

c. (Delete)

d. (Delete)

e. (Becomes item b.)

f. (Becomes item c.) other cattle moving directly from Certified Brucellosis-Free herds. However, a certificate is required for interstate move-
ment of cattle from Certified Brucellosis-Free Herds that are moved for feeding or for breeding. (Change to: other cattle moving directly from certified-free herds. A certificate is required for interstate movement of cattle from certified-free herds that are moved for feeding or for breeding.)

A motion was made and seconded that the proposed change of 1988 for page 119 be amended as follows:

1. Change “herds not known to be affected” to “herds not under quarantine.”

5. Heifer calves from affected herds

(Change to: Calves from affected herds)

All heifer calves in an affected herd, are included under the herd quarantine restrictions. (Change to: All calves in an affected herd, are included under the herd quarantine restrictions.) If they are retained in the herd they should be calfhood vaccinated. (Change to: If heifer calves are retained in the herd they should be calfhood vaccinated.) These heifer calves may only be moved if they comply with the requirements of a, b, c or, d of this section. (Change to: All calves may be moved only if they comply with the requirements of a and b of this section.)

As of October 1, 1988, no cattle may be moved from Class C States except steers, spayed heifers, “S” branded cattle, or cattle from certified Free herds. (Change to: No cattle may be moved from Class C States except steers, spayed heifers, bulls less than 18 months of age from herds not known to be affected, “S” branded cattle, or cattle from certified-free herds.)

a. The heifer calves must be “S” branded or spayed, or  
(Change to: The calves must be “S” branded or neutered, or)

b. (No change)

c. (Delete)

d. (Delete)

A motion was made and seconded that the proposed changes of 1988 for page 123 be amended as follows:

1. Option C. will be reinstated and amended as written for page 111.

2. Change “herds not known to be affected” to “herds not under quarantine.”
A motion was made and seconded to require testing of all test-eligible cattle upon entry into a quarantined feedlot. After discussion, the motion was withdrawn. It was then moved and seconded that parturient cattle in Class A, Class B, and Class C States shall be tested upon entry into a quarantined feedlot. The motion passed.

A motion was made and seconded that 18 months will be the test-eligible age for natural addition heifers and bulls in certified-free herds. During the discussion, it was clarified that this applies to qualifying tests as well as annual recertification tests. The test-eligible age for nonvaccinated purchased additions will continue to be six-months. The motion passed.

It was moved and seconded that MCI reactors will be eligible for reclassification by the designated epidemiologist. The motion passed.

A motion was made and seconded that cattle and bison which test positive to the BAPA test or RST under the MCI program must be retested using the standard card test or other official tests to determine their brucellosis classification. The BAPA test will not be limited to use at recognized slaughtering establishments and specifically approved stockyards. The use of the RST shall not be limited to cooperative State/Federal laboratories. The UM&R and CFR will be made consistent with these changes. The motion passed.

A motion was made and seconded and passed that the UM&R be changed so that a second official test is not required on BAPA negative samples from affected herds.
EPERYTHROZOONOSIS (EPE) IN LLAMAS: A NEW DISEASE?

L. W. Johnson, D.V.M., Ph.D.
Colorado State University

Recently at various veterinary diagnostic facilities around the US, cases of moderate to severe anemia in llamas have been observed where an erythrocyte parasite morphologically suggestive of Eperythrozoon is demonstrated. In nearly all clinical cases observed, some other clinical condition e.g. pneumonia or "ill thrift" has apparently made the affected individual more susceptible to the hemoparasite parasite. The affected individuals are predominantly young (90% < 1 yr) and, at the peak of clinical involvement, demonstrate a hemolytic/nonicteric anemia with a normal regeneration response. An extremely high level of parasitemia is characteristic as well as variable fever and depression. The foregoing clinical and laboratory findings coupled with subsequent convalescent indirect hemagglutination antibody (IHA) titers against an E. suis antigen has allowed us to consider eperythrozoonosis (EPE) as the diagnosis. A recently conducted IHA serologic survey of 222 llamas from seven states and on 22 premises revealed 54 seropositive (>1:40). This prompts speculation that a likely state of premunity exists, which may also account for clinical cases in immunocompromised infected camelids. Treatment response for the clinical cases with anemia and parasitemia using standard doses of tetracycline have been encouraging, however recrudescence following treatment is common unless the underlying primary problem is corrected. The true agent for what appears to be EPE in llamas is unknown. The various Eperythrozoon organism (E. wenyoni and E. teganodes of cattle, E. ovis of sheep and E. suis of pigs) are probably not involved as they are generally regarded as species specific. Vector spread from carrier to susceptible llamas is likely. Prevention for now should be aimed at avoiding contact with known carriers, maintaining closed herds with serological testing (IHA) and quarantining new animals. There must also be some emphasis on vector control.

The question of whether the causative agent could be imported has to be raised. Considering that no condition comparable to it has been observed prior to this past calendar year, it is indeed a new and significant disease. Only extensive serological and epidemiological studies can answer the question. Future work on this condition will hopefully identify the parasite, determine how it can be experimentally transmitted, and provide a llama-specific IHA test. Needed regulatory controls must be addressed.

Acknowledgement for cooperation in preparing this material go to Drs. Norman Evans, Bruce McLaughlin, and A. R. Smith; Mr. Chuck Campbell and the llama community.
DIAGNOSTIC PROCEDURES FOR DETECTING LLAMAS EXPOSED TO MYCOBACTERIA


SUMMARY

Positive tuberculin skin test responses were observed on the lateral wall of the thorax of llamas 18 months after sensitization with Mycobacterium bovis. Histological examination of dermal biopsies of response sites 72 hours after injection of tuberculin revealed predominantly mononuclear cells. Elevated enzyme-linked immunosorbent assay reactions were detected in M. bovis sensitized llamas 42 days after tuberculin skin test.

INTRODUCTION

Tuberculosis has been reported in llamas in the United States and in other countries.¹ ³ Available information indicates tuberculin skin tests conducted in the cervical region or in the perianal region are not useful in detecting llamas exposed to M. bovis.⁴ The objective of this investigation was to obtain information on: 1. the persistence of delayed-type skin hypersensitivity responses to tuberculin, 2. histologic composition of tuberculin responses and 3. ELISA reactions in serum before and 42 days following tuberculin test in M. bovis sensitized llamas.

MATERIALS AND METHODS

Group I included 2 llamas that were tuberculin skin tested once and not sensitized. Group II included 2 llamas tuberculin skin tested four times during the previous 18 months and not sensitized. Group III included 2 M. bovis sensitized llamas that have been tuberculin skin tested four times the past 18 months. At the initiation of the most recent test, (7/31/89), blood serum was collected from all 6 llamas for subsequent indirect ELISA testing using both M. bovis PPD and M. bovis antigens.⁴ ⁵ A second serum sample was collected 42 days later from each of the llamas.

All intradermal test sites were evaluated 72 hours after injection of PPD tuberculin. Uniform dermal biopsies were removed from the injection sites for histopathological evaluation.

RESULTS

Positive tuberculin skin test responses using M. bovis PPD in the axillary area were observed in each of the 2 M. bovis sensitized llamas (group III). However, only slightly suspicious responses were detected in the cervical or caudal fold (Table 1). Dermal hypersensitivity to M. avium PPD tuberculin was detected in Group II llamas. No significant skin test responses were detected in Group I llamas.
Table 1. Summary of Dermal Tuberculin skin testing (7/31/89) in llamas.

<table>
<thead>
<tr>
<th>Group</th>
<th>Axillary¹</th>
<th>Cervical²</th>
<th>Caudal Fold³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. bovis</td>
<td>M. avium</td>
<td>M. bovis</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
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</tr>
<tr>
<td>B₂</td>
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</tr>
<tr>
<td>S</td>
<td>0</td>
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</tr>
<tr>
<td>P</td>
<td>0</td>
<td>3.8</td>
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</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6.1</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>A</td>
<td>8.0</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

¹Increase in skin thickness (mm) after 72 hrs.
²Subjective score 72 hrs after PPD injection; N = normal
³Subjective score 72 hrs after PPD injection; N = normal

A summary of histological findings of tuberculin injection sites is presented in Table 2. Microscopic findings of M. bovis PPD injection sites revealed the presence of perivascular inflammation composed primarily of mononuclear cells in M. bovis-sensitized llamas.

Table 2. Biopsies of tuberculin test in axillary area 72 hours after injection of tuberculin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Histological Description</th>
</tr>
</thead>
</table>
| I     | *M. avium* — no significant changes  
*M. bovis* — no significant changes |
| II    | *M. avium* — llama M: no changes  
llama P: severe perivascular lymphocytic inflammation  
*M. bovis* — llama M: focal necrosis of collagen in upper dermis  
llama P: focal abscessionation, necrosis in subcutis |
DETECTING LLAMAS EXPOSED TO MYCOBACTERIA

III  *M. avium* — llama B: mild perivascular inflammation (mononuclear cells); superficial llama A: moderate perivascular inflammation; upper dermis

*M. bovis* — llama B: moderate perivascular inflammation (mononuclear cells); extending into deep dermis llama A: moderate perivascular inflammation (mononuclear cells) involving upper dermis

Positive ELISA reactions were detected in sera of each of 2 llamas sensitized with *M. bovis* killed cells in oil (Table 3). The ELISA reactions were elevated in llamas 42 days after tuberculin skin tests. Important ELISA reactions were not detected in nonsensitized llamas receiving repeated tuberculin skin tests.

Table 3. Results of serum ELISA.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Group</th>
<th>1st</th>
<th>2nd*</th>
<th>1st</th>
<th>2nd*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>2+</td>
<td>4+</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

*42 days after 1st sample

*Serum dilution 1:160.

DISCUSSION

The findings reported herein indicate that the axillary intradermal site is the most reliable for detecting *M. bovis* sensitized llamas. In addition, the axillary site lends itself to objective rather than subjective evaluation. Repeated tuberculin testing of nonsensitized animals (Group II) has not induced false positives using either the intradermal or serological testing procedures. In addition, it appears that dermal testing of sensitized llamas increases the ELISA reaction at the time interval tested (42 days). This may be useful in the diagnosis of llamas exposed naturally to pathogenic mycobacteria.
Of special note in these studies is the dermal and histological reaction of llama P in Group II to \textit{M. avium} antigen. Because of the large pigeon population at the research site, this animal may be sensitized to or infected with \textit{M. avium}. Subsequent dermal and serological testing of this individual and site peers will be performed.

**REFERENCES**


\footnote{1} Colorado State University, College of Veterinary Medicine, Ft. Collins, CO 80523.

\footnote{2} Iowa State University, College of Veterinary Medicine, Ames, IA 50010.
Chairman: Dr. V. A. Seaton, Ames, IA
Vice Chairman: Dr. L. M. Siegfried, Madison, WI

W. F. Alexander, OK; A. A. Andersen, IA; D. E. Bartlett, WI; R. E. Bohlender, NE; E. A. Carbrey, IA; C. S. Card, AZ; H. M. Chaddock, MI; R. F. Conner, IN; R. A. Crandell, TX; G. L. Crenshaw, CA; C. N. Dobbins, GA; D. P. Ferlicka, MT; A. M. Gallina, WA; L. R. Harrison, GA; L. Johnson, CO; A. T. Kimmell, KS; G. Lambert, IA; A. J. Luedke, CO; C. S. McCain, OK; P. L. McDonough, MA; C. A. Mebus, NY; J. M. Miller, IA; P. A. O’Berry, IA; B. I. Osburn, CA; D. H. Schlafer, NY; J. A. Schmitz, NE; R. D. Schultz, WI; N. Stirling, SD; D. E. Suther, CA; N. R. Swanson, WY; R. M. S. Temple, OH; C. O. Theon, IA; H. A. Waters, VA; R. E. Werdin, MN; C. A. Whetstone, IA.

The meeting was called to order at 1:35 p.m., October 31, 1989 by Chairman, Dr. Vaughn Seaton. There were 21 members and 25 other interested participants in attendance.

Dr. Lynne Siegfried, Wisconsin Animal Health Laboratories, presented a review of bovine spongiform encephalopathy and showed a video-tape of the clinical signs of disease prepared by the Central Veterinary Laboratories, Weybridge, England where the disease was first diagnosed.

Dr. John Post, University of Connecticut, gave an overview of Lyme disease, a tick borne infection caused by the spirochaete, *Borreli* burgdorferi. The signs and lesions of the disease were described for human, dog and horse, then the presentation focused on the disease in cattle. Indirect FA and ELISA titers in cattle are common and sometimes high. Presumed affected animals may show joint swelling, reddened and painful skin on the udder, laminitis, abortions and reproductive failure. A Borrelia has been isolated from the urine resulting in the speculations that the disease may also be transmitted through urine contamination. Work is in progress to clarify some of the questions about the disease in cattle.

Dr. LaRue Johnson, Colorado State University, reported on the results of a study comparing cervical and axillary inoculation sites and the use of *Mycobacterium bovis* and *M. avium* antigens for testing llamas for tuberculosis. ELISA serology and tissue biopsy of the inoculation sites were used to compare the immunologic responses to the inoculation procedures before and after sensitization to the tuberculin antigen. Conclusions reached were that, for the llama, the axillary site was best; sensitized animals were readily detected by tuberculin testing; repeated tests failed to result in false positive reactions; and intradermal tuberculin testing can result in serologic reactions to *M. bovis* by the ELISA test.

Dr. Johnson went on to report on a disease in llamas related to the presence of Eperythrozoon-like structures in the red blood cells. Affected
llamas had hemolytic, regenerative anemia, fever and depression. It was speculated that the disease may be related to the presence of pneumonia, immunodeficiency and stress. A survey of 222 llamas showed that the condition exists in 7 states and that 90% of the affected animals were less than one year old. About 25% of the positive animals were also positive on indirect hemagglutination test using swine erythrozoon antigen. Treatment with tetracycline gives short term relief, with recrudescence occurring in two to three weeks. Prevention and control measures and research to further define the etiology and epidemiology of the disease were proposed.

Dr. Johnson presented a resolution to amend proposed quarantine regulations for Chilean llamas being imported to the United States. It was moved and seconded that the resolution be approved by the committee. After lengthy discussion, the resolution passed by a 9 to 3 vote of the membership present.
LABORATORY PROCEDURES DESIGNED TO MEET THE CHALLENGES ON THE DAIRY

Robert B. Bushnell, D.V.M.
University of California, Davis, CA

Effective dairy microbiology should specifically address the issues of udder infection in the dairy animal, as well as the total aspects of milk quality and sanitation that are associated with milk production.

Bacteriological methods and interpretations must not become an end in themselves but be oriented toward understanding and solving the problems which occur on the dairy.

Established procedures in the laboratory should be adopted and modified as necessary to meet the following objectives.

1. Simple inexpensive systems applied to initial analysis. (Expensive procedures are reserved for special occasions.)

2. Be able to handle large volumes of samples accurately and quickly.

3. Report results rapidly to the practitioner or dairyman. Initial results should be given by phone. Most results are available 24–48 hours after samples are received. No reports should be delayed longer than seven days.

4. Develop a written method of tabulating and reporting results in a meaningful and easily usable format. (E.g., samples in numerical order, cows listed by strings and by type of infection.)

5. Be able to identify the category of each sample received. (E.g., clinical quarters, composite cow, bulk tank.)

6. Subquality samples should not be processed further, but resampling should be requested.

7. Retain all samples in the freezer or refrigerator until you are satisfied with the results. (E.g., Initial bacterial analysis may subsequently indicate a need for Mycoplasma culture.)

8. Be aware that age of sample, method of storage, and presence of other substances may affect the ability to isolate organisms.

9. Send the bill for services with the written report. Routine culturing should run between $2.00–$5.00 per sample. Single bulk tank samples may run higher.

Quality of Sample

We should continually remind ourselves that a laboratory can accurately evaluate a high quality sample which has been carefully taken, stored, and shipped. On the other hand, little can be gained from a carelessly taken or contaminated sample. We should not struggle in the laboratory to analyze an inadequate sample. On the other hand, we should recognize that in some cases a contaminated sample represents the true situation, and we should
be prepared to differentiate between pre-existing growth and post-contamination growth.

Types of Samples Submitted and Microbiological Considerations for Each as Carried Out in Our Laboratory.

Samples from cows showing clinical signs of mastitis should be taken from the foremilk of individual quarters showing abnormalities. Composite samples should not generally be taken from clinical cases.

The degree of abnormality of the sample should be visually noted, and a CMT test is often valuable on normal looking milk. This allows evaluation of the severity of the infection, and indicates samples for additional cultural techniques, and prevents calling highly abnormal secretions "normal from the cultural standpoint."

These samples are cultured fresh as received on washed bovine blood agar media and mycoplasma media. All agar plates are read at 24, 48 and 72 hours. The extended incubation is necessary to identify nocardia, mycobacterin, prototheca, and certain yeasts. Cultures which initially contain bacteria may later include other organisms after extended incubation.

Most clinical cases of mycoplasma will be actively shedding the organisms, shedding of other organisms, however, is extremely variable.

Milk samples which yield no growth in 24 hours on BBA, will be frozen, thawed, and mixed with 50% BHI broth. After 8 hours incubation, they are plated on BBA. This short incubation period will generally give adequate time for the major organism to grow. Extended times encourage multiplication of contaminants.

Highly abnormal secretions that are culturally negative, should be selected for additional microbiological procedures, the sender should be advised to retake fresh samples which may be cultured soon after collection (without refrigeration if possible.)

The more samples submitted, the better the chance of making a correct microbiological determination. It is always more rewarding to request fresh samples, then to keep extending expensive procedures on old samples that yield nothing.

Mycoplasma or secondary contaminants such as nocardia will be present even in cows currently under treatment with antibiotics. Therefore, sampling of existing cases of mastitis in herds with large numbers of incurable quarters is advisable.

Organisms causing clinical disease may not be the same organism responsible for subclinical disease in the same dairy.

Well collected clinical samples should not contain contaminants. 40%-50% of properly collected clinical samples are expected to be microbiologically negative. Isolation of organisms from all clinical samples should make one
LABORATORY PROCEDURES

suspicious of contamination. Many organisms obtained from clinical samples will appear in small numbers. E.g., a pure culture containing 4–5 colonies of E. coli/0.01ml of milk is generally significant in clinically active secretions.

Fresh Cow Samples

A composite sample of the foremilk from all normal quarters is taken 2–3 days after freshening. Abnormal quarters should be sampled separately.

Sampling should include all fresh cows and fresh heifers. Many organisms will be shed at freshening that will only appear intermittently later in lactation. E.g., Mycoplasma species in cows and heifers, and Staph aureus in heifers.

Fresh cow composite samples, unlike clinical samples, can be expected to contain contaminates. Therefore, interpretation of environmental infection must be weighed carefully in these types of secretions.

Generally, environmental infection at or near calving will result in a number of clinical cases of mastitis. Individual clinical cases at freshening, therefore, can better be used to determine these infections.

Sampling of fresh cows allows evaluation of the effectiveness of dry cow therapy (Strept ag), dry cow infection from therapy (Mycoplasma, Nocardia), and heifer infection (Staph aureus, Strept ag, and Mycoplasma), as well as the existence of blind quarters in fresh cows.

This allows the practitioner and dairyman to detect these infections early, before they are spread to other cows in the herd.

Composite Samples of Lactating Cows

Composite samples of lactating cows are used in whole herd cultures to identify cows with contagious mastitis (Strept ag, Staph aureus, Mycoplasma), so that positive animals may be segregated, treated, or culled.

Composite samples are also used on individual cows in a herd with high somatic cells to determine the causative organisms.

They are also used on a sample group of cows, or on a string of cows to determine the average prevalence of infection without the effort of a complete herd culture.

Unlike clinical samples, a level of contamination in composite samples is expected. Those samples which are heavily contaminated, or samples with suspicious organisms in the presence of contamination, are listed either as contaminated or suspects, and resampling of these cows is requested.

We Must be Careful Not to Interpret Contamination as Infection

After initial culture, all samples are evaluated on the CMT test. Cows with high CMT’s (CMT 2–3) that can not be culturally identified, are either submitted for additional laboratory techniques or are reported as suspects.
Cows with blind quarters should be identified on the sample tubes with a code such as an X, to let the laboratory know that the sample does not represent all four quarters. If these cows are culturally negative, they are listed as suspects because of the unknown status of the blind quarter.

**Bulk Tank Samples**

Bulk tank samples yield useful information, but one must be careful not to allow these samples to speak for the udder status of the entire herd. For example, clinically active cows do not contribute to the bulk tank and the amount of milk contributed to the bulk tank by each cow varies. However, the bulk tank sample does accurately reflect changes in a given herd when used routinely, and is extremely valuable at times when the milk is suffering from milk quality problems, particularly of bacterial origin.

A bacteriological method (washed BBA can be used) should be selected that can differentiate mastitis organisms, gross contamination, or incubation problems from improper washing of the milking system.

In the case of bulk tank microbiology, one is interested in the “big picture”, — the impression. One should not dwell on the detailed microbiological procedures on these types of milk samples.

**Microbiological in General**

In general, herds with cell counts higher than 500,000, the offending organisms will be either Staph aureus, Strept ag, or both.

Occasionally one will encounter selected environmental infections, originating from a point source, that will drive cell counts up. Usually these infections are found in the clinically active samples as well, and may initially be overlooked in the composite cow samples.

When these types of organisms are encountered, additional expense and microbiology procedures are warranted.

The API system, for example, is frequently used to identify gram negative bacteria such as Serratia, Klebsiella, or Pseudomonas.

**Environmental Analysis Using Bacteriology**

Use of microbiology to trace organisms to their source and to evaluate contaminations and efficacy of sanitizing procedures is warranted.

Water in the dairy environment, as well as the pipes, cisterns, and hoses that carry water, are often sources of potential pathogens.

Evaluating the ability to destroy these sources of bacteria using sanitizers, can be accomplished using the same microbiological steps.

One should be aware, however, in testing surfaces or solutions in contact with sanitizers, that a quencher to inactivate the sanitizer is necessary at the time samples are collected. Extended contact with the active sanitizer after collection may eliminate the organism and prevent growth on culture media.
LABORATORY PROCEDURES

Sodium thiosulfate and Tween-80 are quenchers which are commonly used.

If a service laboratory is able to communicate directly with the practitioner and dairyman who are submitting samples, more appropriate samples will be taken, procedures in the laboratory will be more efficient, and information developed from the laboratory analysis will be more helpful and timely in problem solving.

REFERENCES


Use of the Blood Agar Plate and Other Special Media for Problem-Solving Through Bulk-Tank Milk Analysis. Robert B. Bushnell.

Evaluation of Laboratory Variables Used in Isolation of Pathogens from Field Collected Milk Sample. Harjot Singh Gill.
REPORT OF THE COMMITTEE ON MASTITIS

Chairman: Dr. T. H. Fuhrmann, Tempe, AZ
Vice Chairman: Mr. N. J. Corlett, Strongsville, OH

J. Adams, VA; B. N. Bhargava, MD; T. F. Conner, IN; M. L. Crandall, VA; F. Dickinson, OH; D. F. Dineen, ME; P. A. Dukas, OH; N. E. East CA; B. J. Edmundson, WA; W. H. Fales, MO; T. W. Freas, IN; D. A. Gable, VA; F. D. Gregerson, CO; D. D. Hancock, WA; M. Huff, CO; F. S. Idtse, WI; D. E. Jasper, CA; W. L. Kadel, KY; E. Keahey, TX; C. A. Kirkbride, SD; J. D. Kopec, MD; J. H. Lang, WI; D. H. Lein, NY; J. C. Lemmermen, FL; E. T. Littledike, NE; T. Matsushita, VA; Gen. T. G. Murnane, TX; M. A. Owen, MA; D. N. Rice, NE; F. J. Schoenfeld, UT; M. Sharar, MD; W. E. Stemler, IL; K. E. Sterner, MI; J. M. Vetterling, CO; D. U. Walker, VT; R. F. Weidner, PA

The meeting was called to order at 1:30 PM on October 30, 1989. Mr. Norm Corlett presided in the absence of the Chairman Dr. T. J. Fuhrmann. A list of attendees is attached.

The minutes of the last meeting were not available so were not read or approved in their absence.

The program consisted of presentations on the subject of laboratory procedures which address mastitis in dairy animals and state, extension, DHIA and industry programs which implement recommendations based on interpretations of laboratory results. Papers presented are attached.

The general theme of the program was to foster development of mastitis diagnostic capability within State Department of Agriculture laboratories by providing information on the need, methods and resources for such services.

Discussion included the need for fast turn around time of results in order to demonstrate to practitioners the usefulness of diagnostic labs.

It was agreed that the committee should focus the attention of government on mastitis as an economic disease. A resolution was passed for submission to the board that USAHA endorse and recognize efforts to secure additional funding for all elements of mastitis control.

A motion was also made and passed to send a representative to the APHIS Policy and Program Development conference to be held in Washington, DC in October 1990 to present the concerns of the USAHA Mastitis Committee to USDA.

Regarding future direction of the Committee a 2nd motion was passed to have the next USAHA Mastitis Committee meeting to be held as a joint committee meeting with AAVLD and to have at least 1/2 of the program devoted to mastitis laboratory diagnostics.

There being no further business, the meeting adjourned at 5:00 PM.
MASTITIS

MILK QUALITY: UNIVERSITY EXTENSION PROGRAM

Kent Hoblet, D.V.M., M.S.
Extension Veterinarian, Dairy and
Associate Professor
The Ohio State University

Extension Veterinary Medicine

The Cooperative Extension Service was created by the Smith-Lever Act 1914 for the purpose of disseminating practical information through the Land Grant Universities. According to a survey conducted in 1985, 39 states have Extension Specialists in veterinary medicine. Of the 119 veterinarians serving as state specialists, 34 had full time appointments, 52 majority time, and 33 less than majority time.1 Currently at The Ohio State University there are 4 veterinarians with individual extension-education responsibilities in either the Dairy, Swine, Poultry or Beef Cattle/Sheep. At Ohio State, extension veterinarians have split academic appointments with tenure in either the Department of Veterinary Medicine (3) or the Department of Poultry Science in the College of Agriculture (1).

Ohio's Dairy Industry

Ohio has approximately 370,000 dairy cows on 5500 farms producing Grade A milk and 2500 farms producing manufacturing grade milk. Dairying is essentially a family enterprise with an average herd size of about 45 cows. The mission of the extension portion (0.65 FTE) of my appointment is to provide health management education to this industry. This is done in cooperation with other university, state and county faculty, allied dairy industry personnel and the state's practicing veterinarians. A major health concern of the dairy industry is the production of quality milk. Over the 15 years that Ohio has had a dairy extension veterinarian the following methods have been used to encourage the production of quality milk:

Laboratory

The Herd Milk Quality Laboratory in the Department of Veterinary Preventive Medicine has been central to educational effort designed to increase awareness of mastitis control. A part-time technician oversees day-to-day activities in the laboratory. Routine diagnostic work has been described.2 It is rather difficult to separate the teaching, research, and service functions of the lab. The laboratory is used for teaching 3rd year professional students, for updating practitioner's skills both informally on a one-to-one basis and formally in a Quality Milk Course held biennially. Research efforts have been as described below. While we do provide fee-for-service milk culturing for the determination of herd prevalence of mastitis pathogens, the primary missions of the laboratory are those of teaching and research.
Teaching

Field Investigation/extension education. The Director of the Ohio Cooperative Extension Service has recognized that the efforts of extension veterinarians are multiplied as they work with the private practitioners who provide services to Ohio’s livestock producers. To this end, we visit, with private veterinarians, herds that are experiencing mastitis and other health-related problems. Because conducting a milking-time evaluation is very time-intensive, efforts are made to use a team approach whenever possible. Often equipment dealers, dairy cooperative fieldmen and others are involved in the investigative process and resultant recommendations. This way, hopefully the educational process is further amplified.

Formal teaching

Veterinarian

Quality Milk Seminar for Veterinarians. We have conducted on a biennial basis a 2-day course with laboratories patterned after the AABP Quality Milk Course.

Producers

Winter Dairy Institutes. During January-February each winter we deliver 12–25 one-day courses with other state extension faculty. Institutes are held throughout Ohio with topics selected in cooperation with an advisory group consisting of county and district faculty.

Producing Quality Milk Seminars for Dairymen. Since 1986, we have held 12 regional Quality Milk Seminars for producers.

Field Days — County-state field days are typically held each summer. Again, topics vary.

Other producer meetings are held in cooperation with county agents, veterinarians and others involved in the dairy industry.

Media. We provide a quarterly newsletter directed primarily toward veterinarians. The circulation is approximately 950. With faculty in dairy science, we contribute to radio spots and extension news releases. We have published leaflets for the Ohio Dairy Guide. The Ohio DHI Cooperative has utilized several of those leaflets which relate to contagious, environmental mastitis and somatic cell counts in their own educational efforts. We have made several videotapes. One, relating to control of environmental mastitis has been AVMA Certified (peer-reviewed) and is available from AVMA for educational purposes.

Research

We have been fortunate in being able to participate in mastitis research that is pertinent to Ohio’s needs and which has ready application in the field. The Nine Herd Environmental Mastitis Field Study led by Dr. K. L.
MASTITIS

Smith of the Ohio Agricultural Research and Development Center, resulted in the acquisition of new knowledge regarding bedding materials, bulk tank bacteriology, and the epidemiology of mastitis in low somatic cell count herds. A completed project, funded by the National Cooperative Dairy Herd Improvement Association, looked at perceptions of producers and veterinarians regarding mastitis control and somatic cell counts. Currently we are analyzing data from a study, funded by The Dairy Farmer's Federation which investigated the economics of the reduction of Staphylococcus aureus mastitis in 3 well-managed herds. These herds were initially identified as a result of an extension-education/investigative visit.

Summary

The mission of extension veterinary medicine is education. Although the above discussion focused on educational methods used in delivering information on milk quality other areas of animal health management must also be addressed. While methodology may vary, our end goal remains to provide useful information and encourage adoption of research-based knowledge in the field.

REFERENCES


COOPERATIVES APPROACH TO RAW MILK QUALITY

Norman J. Corlett, Jr.
Manager of Quality Control
Milk Marketing Inc.
Strongsville, Ohio
October 30, 1989
USAHA Annual Meeting
Las Vegas, NV

Dairy cooperatives have traditionally been concerned with marketing the greatest quantity of milk at the highest price. Quality of milk has always been recognized as an important factor but, it has not been until recently that the word "quality" entered the pricing equation.

Driving by declining consumption of fluid milk and lower prices, dairy producers acted to reverse declining fluid milk sales. Dairy producer financed advertising was initiated to promote fluid milk consumption in the face of the cholesterol scare and quality control programs were instituted to improve flavor and extend shelf-life.
REPORT OF THE COMMITTEE

Advertising has generally resulted in increased consumption of fluid milk. But, the success of quality control programs have become dependent on the “carrot” of over order premiums paid on the basis of quality. Though milk quality pricing programs were pioneered over 10 years ago, it has only been in the last 5 years that somatic cell counts, bacteria counts and other quality parameters have become more common in determining pay prices to dairy producers.

With emphasis on quality, producers now demand more from their professional advisors in university extension and the veterinary community to meet quality pricing program requirements. Demands were also placed on cooperatives and dairy plants to extend field services beyond traditional marketing and regulatory functions to more technically related areas including milking hygiene, equipment function, milking procedures, and general udder health. Identification of mastitis causing bacteria in bulk milk is the most recent addition to the array of services now provided by a few of the largest dairy cooperatives.

Services offered by the cooperative I represent are similar to those provided by others. They are unique, however, in that they are closely coordinated with the veterinary community in the areas we serve.

In the area of mastitis control we offer members bulk milk and individual cow/quarter milk culture services. Bulk milk is screened once a month for the presence of contagious mastitis causing bacteria. The “Bulk Tank Monitoring Report” is mailed to the member, veterinarian, and field representative. When *Staph aureus* or *Strep ag* is isolated, members are encouraged to consult their veterinarian for a diagnosis. Field representatives contact the veterinarian to exchange information on conditions each has noted on the farm and to coordinate recommendations for improvement.

These procedures were initiated in the spring of 1987 as a pilot program in three field territories. Results of the pilot showed the service was a valuable tool in reducing the prevalence of sub-clinical mastitis due to contagious pathogens. The program was therefore expanded and is offered on a voluntary basis at a nominal fee to all members.

At present, approximately 1500 members use the service on a regular basis. Of these approximately 300 participate in the monthly Bulk Tank Monitoring Program. These numbers are expected to double as the remainder of our laboratories become qualified to perform bacteria identification.

We are now summarizing data for a paper to be presented at the National Mastitis Council meeting in February. Field experience shows that bulk milk culture results provide veterinarians a valuable tool for diagnosing hard udder health problems. Further, the close working relationships developed between field representatives and veterinarians provide members ready access to coordinated and prioritized recommendation to improve udder health. Members who implement these recommendations
reduce herd somatic cell counts and the concentration of contagious mastitis causing bacteria in bulk milk. They are also among the growing number of members who receive up to $1.00/cwt premium for their high quality milk. Members who chose the null option continue to have problems and do not qualify for premiums.

Though we are committed to serving all our members, there are areas which are better served by extension and state department of agriculture laboratories. Non members are, of course, dependent on these resources. Judging from the very positive response we have experienced from the members we serve, I suggest there are many dairy producers who can be well served by the increased availability of milk culture services from extension or state department of agriculture laboratories.

MINIMIZING MASTITIS BY COMBINING BACTERIAL CULTURE RESULTS WITH SOMATIC CELL COUNTS USING NEW YORK RECORD SYSTEMS

Philip M. Sears, D.V.M., Ph.D.
Director, Quality Milk Promotion Service/
NYS Mastitis Control Program

The rise in milk somatic cell count (MSCC) in a cow is directly related to the level of a bacterial infection of the gland. Although milk somatic cell count may persist beyond the length of the intramammary infection, the rise in cell count and the persistance of a high cell count, are related to the damage of lactating tissue by the infectious insult. Thus, the utilization of MSCC is an effective method for monitoring infection levels and determining milk loss in a dairy herd. It has been demonstrated in field research that the use of MSCC to select for mastitis treatment is not cost effective and should not be recommended as a general practice. The use of MSCC to monitor progress of a mastitis program and detect early appears to an effective use of the programs.

The use of MSCC alone to manage mastitis without identifying the causative organisms and determining the prevalence of each type of infection, can be misleading and extend the time needed to control the level of infection in the herd. Identifying the causes of clinical as well as subclinical infections is important. The control measures necessary for one may differ from the other. A low MSCC may be indicative of a good control program for the source of the subclinical infections, while missing the management requirements necessary to control clinical infections. However, in herds with high MSCC, clinical infections are generally the same as the subclinical and require the same control. Thus, when MSCCs are used alone without accurate evaluation of the bacteriological prevalence in the herd, planning effective and appropriate measures becomes a hit and miss program based on generalities.
REPORT OF THE COMMITTEE

LINEAR SCORE versus RAW SOMATIC CELL COUNT

The use of linear score (LS) to report MSCC over the raw somatic cell count has the advantage of a direct relationship in calculating milk loss. For each change in LS above, 2, 1.5 pounds of milk is loss per cow/day. The relationship between raw scores range and production losses shown in Table 1. The average of a cow's lactation or the herd average is more accurately calculated when using over the raw average. Similarly, the LS allows an accurate selection of problem cows in the herd and the detection of early problems. Cattle with LS 5 or greater has a probability of 90% or greater of harboring an intramammary infection. While animals with LS 4 or less have less than a 10% probability of of an intramammary infection.

As presented in other papers included in these proceedings, the LS converts raw somatic cell counts from tens of thousands cells per ml to a linear score value of 0.0 through 9.9. The LS is determined by a doubling of raw counts for each whole number from 25,000 (LS 1.0). Table 1 adopted from Shook ADSA, 1982, shows the conversion and projected milk loss.

Table 1: Linear Score Conversion Table

<table>
<thead>
<tr>
<th>Linear Score</th>
<th>Somatic Cell Count</th>
<th>Milk Losses 1st 2nd</th>
<th>Herd Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12,500</td>
<td></td>
<td>Low Mastitis:</td>
</tr>
<tr>
<td>1</td>
<td>25,000</td>
<td>(Pound of Milk)</td>
<td>Culture clinicals</td>
</tr>
<tr>
<td>2</td>
<td>50,000</td>
<td></td>
<td>Monitor equipment</td>
</tr>
<tr>
<td>3*</td>
<td>100,000</td>
<td>200</td>
<td>and environment</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5**</td>
<td>400,000</td>
<td>600</td>
<td>High Mastitis</td>
</tr>
<tr>
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<td>800,000</td>
<td>800</td>
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<td>1200</td>
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The decision to culture all cows in a herd versus selective culturing is not well studied. Knowing the predominate infectious organism in the herd and the level of infection are both important factors when making this decision. Dairies with a high infection prevalence (LS >5) should consider a full herd survey, including complete herd culturing, milking systems and management evaluation. Environment management is as important in these herds as they are in herds with low MSCC. In well managed herds with LS 4 or less selective culturing may be of value. In addition to the herd survey, identifying clinical intramammary infections become an important part of the mastitis control program. With the low level of infections, monitoring individual cows becomes increasingly important to detect problems before the individual infections become a herd problem. Monitoring the milking
system and environment is essential in these herds to avoid clinical mastitis when subclinical infections are low.

**COMPUTERIZED RECORDS FOR INDIVIDUAL DECISIONS**

Table 2 was built using a computerized record system, accessing codes through the Remote Management System (RMS) or the Advance Information Manager (AIM) to build a personalized herd report. To make the most appropriate decision for individual cows in the herd, a list was developed by listing each cow by Lactation Number, Days in Milk, Days Pregnant, Current Milk Production, 305-milk Production, Current Somatic Cell Count-Linear Score, Average Linear Score, and Last Lactation Somatic Cell Count. In addition, culture results was added to the DHIC program for management decision making. The table was created by ordering first on the lactation number, since first calf heifers are good indicators of the current mastitis control program status; and ordered secondly on the current somatic cell count in descending order since the higher somatic cell count cows are likely to be infected, problem cows which should be cultured.

Although the list is made from individual cow records and decisions must be made on individuals, listing cows in order of lactation and including stage of lactation allow the opportunity for observing herd trends and identifying herd problems early as they begin to develop. If the mastitis control program is effective, first calf heifers should not be infected and have a LS >4. In table 2, several heifers were above LS 5 and positive on milk culture. Also noted in the tables these animals have become infected with in the last month and are early into lactation. This appears to be a similar pattern in the second lactation group as well. Thus, the mastitis problem appears to be a recent development, affecting cows after calving. Three areas in this herd should be considered, calf raising, calving area and management practices (including equipment) during early lactation.

It is possible to make some management decisions and determine areas of failure in the mastitis control program by listing groups of cows by MSCC, lactation and stage of lactation, but making the best management decisions can be enhanced when the type of infection is identified using milk culture. Similarly, making management decision on individual cows can be more accurate if the type of infection has been determined prior to the decision. This can be seen from our example in table 2, in which heifers and second lactation cows are enter the milking herd with *Streptococcus agalactiae* and *Staphylococcus aureus*. This herd has a problem with contagious organism, which can be controlled by lactation and dry cow therapy for *Strep ag*, and teat dipping and dry cow therapy for *S aureus*. In addition there may be deficiencies in the milking management of the early lactation cows which have increase the spread of these infections. Both the milking procedure and milking system should be evaluated for deficiencies. The actual management decision of treatment, drying off, segregating or
culling of problem cows must be made on individual cows based on Days in Milk, Production Status, Production and Type of Mastitis. It is easier to make these decisions when all of these categories are placed together for each cow.

Table 2: Dairy herd with predominately Contagious Mastitis

<table>
<thead>
<tr>
<th>BARN NO.</th>
<th>LAC NO.</th>
<th>LTD-M DAYS</th>
<th>DAYS CAR-CLF</th>
<th>C-SD-M POUNDS</th>
<th>RJ-2x305 ACT-MILK</th>
<th>CSC LS</th>
<th>SC-LS LS-LS LS-AV</th>
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<td>8.0 STREP AG, S AUREUS</td>
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Using MSCC alone from DHIC record can be misleading, especially in a well managed herd which has already achieved a very low somatic cell count. It is important to maintain good records on clinical mastitis and culture milk from these cows to identify the source of clinical problems which may be different from those identified in subclinical infections for the herd. Often, the management procedure to control these clinical infections are different from procedures necessary to control the subclinical mastitis. Thus, identifying the organism in clinical mastitis can be important in determining the mastitis management for the clinical mastitis.

Computer programs do not make decisions, but the use of these programs to group cows and allow direct comparison with their herd mates make the management decision of cow records easier. Having complete information on production, reproduction, and mastitis status is essential for making the best decisions in a good mastitis control program.
**Table 3: Dairy herd with predominately Environmental Mastitis**

<table>
<thead>
<tr>
<th>BARN NAME</th>
<th>LAC NO.</th>
<th>LTD-M DAYS</th>
<th>DAYS CAR-CLF</th>
<th>C-SD-M POUNDS</th>
<th>PJ-2X305 ACT-MILK</th>
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WHY FSIS TESTS FOR RESIDUES

William T. Hubbert, D.V.M.
Director, Residue Evaluation & Planning Division
Food Safety & Inspection Service, USDA
Washington, DC


In this presentation, I will first outline the significance of tests in inspection, particularly in relation to residues. Second, I will describe the role of residue testing in accomplishing the FSIS mission.

FSIS Mission

The mission of FSIS is to assure the safety and wholesomeness of the Nation's meat and poultry supply. Clearly, the public perceives the underlying reason for residue testing to be consumer protection.

Inspection

Inspection, antemortem and postmortem, has been the principal means by which we accomplish this mission. Animals found to be unfit for human food are condemned.

Tests-Visual

Tests are intended to detect the presence of something. Antemortem and postmortem inspection really are a series of activities equivalent to tests that are intended to determine the safety and wholesomeness of meat and poultry. Traditionally, we have been able to make inspection decisions on the basis of these observations, or tests, on individual animals as they are slaughtered. Over the years, the state of health of our national herd has improved and, therefore, the percentage of inspected animals passed for food has increased.

Tests-Chemical

As a rule, residues of drugs and other chemicals cannot be detected by visual tests, or observations. Therefore, we have resorted to chemical or biological assays to detect their presence. Although these tests may be able to detect minute concentrations of various chemicals present in tissues as residue, their use cannot be justified simply as an adjunct to traditional visual inspection. Unquestionably, meat or poultry tested and found to have violative concentrations of residues cannot be passed for food. On the other hand, information gained from a single test should never be used solely to determine the fate of a single carcass. Each test result, when combined with the results of other tests for the same chemical in similar animals, adds to the weight of evidence needed to make decisions regarding a particular residue problem.
WHY FSIS TESTS FOR RESIDUES

Specificity-Sensitivity

Given adequate specificity, a test can clearly detect, in other words identify, a single compound or a metabolite of it. The sensitivity of the test, on the other hand, will determine the concentrations that can be detected, beginning with those that are violative and decreasing to smaller positive yet non-violative concentrations. These positive non-violative results represent small fractions of the violative concentrations that potentially pose real human health hazards. Understanding the significance of these results is crucial in deciding what to do about a specific animal. Is the residue concentration found in muscle or other edible tissue greater than the tolerance level established by EPA or FDA?

Problem Solving

Even if we have a rapid, inexpensive, and sensitive test to detect a specific residue, using it only on individual carcasses at slaughter will not solve an associated residue problem. Violative residues result from inappropriate foodborne or other exposure to drugs, pesticides, and other chemicals at some time during the life of the animal. It is far more efficient and cost-effective to prevent that inappropriate exposure among herds and flocks than to separate affected individuals after the fact. That inappropriate exposure may involve such events as failure to observe an adequate withdrawal period for an animal drug or feeding pesticide-treated seed.

Individual vs. Population

The results of residue tests only become truly useful tools in the solution of residue problems when they are analyzed to determine characteristics of populations at risk or changes associated with various control efforts. The relationship of testing to characterization of populations often is overlooked. This is particularly true when residue programs are named for tests, such as SOS, STOP, and CAST. This implies the primary purpose of the program is to detect adulterated individuals without any stated population-related impact. Although the real purpose of the program involving populations at risk may have been stated, it tends to be quickly forgotten or misplaced in favor of detection and condemnation of adulterated individuals as the omnipresent other purpose. In fact, detection of the adulterated individual with subsequent condemnation is far from the universal purpose of concern in the design of residue programs.

Why FSIS Tests for Residues

The two main reasons why FSIS tests for residues are:

1. To be able to determine the level of residue-related risk from meat and poultry, and;
2. To apply this tool in programs of residue risk reduction.

To determine the level of risk, we need accurate estimates of the extent of specified residues. This is particularly important when communicating
the level of residue-related risk to the public. To be an effective tool in risk reduction, testing must focus on populations in which the problem is of significant intensity.

Estimating Risk

Monitoring and surveillance are two widely used terms with essentially identical meaning. We will henceforth use surveillance to indicate programs designed to gather data needed to assess the residue status of defined populations in relation to specific chemical compounds. The most widely recognized FSIS program, previously referred to as monitoring, provides residue data regarding the national herd on an annual basis. This information provides a statistically sound base on which to make assessments. Because the purpose in this instance is to provide data representative of the national herd, it is not an effective method of detecting individual adulterated animals in high risk populations. It will, however, detect adulterated individuals indicative of their distribution in the national herd. Surveillance programs may be designed to assess residue problems in relation to any defined population, time period, geographic area, drug, pesticide or other chemical, or any other measurable characteristic. Surveillance programs may be designed to assess new or potential problems as well.

Reducing Risk

Risk has been defined as the product of hazard times exposure. A chemical in food may be a hazard to the consumer if it presents some carcinogenic or other health-related danger. Residue testing reduces risk by reducing exposure. The obvious direct impact occurs when a carcass found to have a violative concentration of a residue is prevented from entering the food supply. Testing to find violative individuals is only cost-effective when applied to high risk populations. Such high risk populations include all animals with overt evidence of recent drug injection or those in the same or other herd or flock known to have received feed from the same supplier of contaminated feed. It is a highly appropriate tool in any risk reduction program involving a high prevalence of animals presented for slaughter with violative residues. When the prevalence of violations is low, a high rate of testing becomes very difficult to justify. The finding of a violative residue at slaughter is only the beginning of the risk reduction process. FSIS works with investigators from FDA and state agencies who follow up the incident in the field to determine the source and to prevent further violations.

Preventing Exposure

Consumer concerns regarding residues are real. To be effective in allaying those concerns, we need a credible means of demonstrating exposure prevention. The ultimate value of residue testing will be found in programs of quality assurance in which antemortem inspection has gone to the farm
WHY FSIS TESTS FOR RESIDUES

to assure that animals have been raised so that violative concentrations of drug, pesticide, or other chemical residues are not present at slaughter. Primary responsibility for residue prevention rests with the livestock and poultry producer. This preventive approach has been the underlying theme of the Residue Avoidance Program and its successors operative since the mid-1970s. FSIS will continue to work with the industries concerned to develop the most effective means of solving the residue problem, PREVENTION.

Summary

It is evident that residue testing is an important tool and the results provide important information. The purpose of the testing may be SURVEILLANCE, i.e. data gathering to assess a problem; it may be RISK REDUCTION, i.e. detection of adulterated animals in high prevalence populations; or it may be RISK PREVENTION, i.e. quality assurance in populations for which residue avoidance/prevention programs are in effect.
REPORT OF THE COMMITTEE ON ENVIRONMENTAL RESIDUES

Chairman: Dr. C. H. Graham, Kansas City, MO
Vice Chairman: Dr. W. T. Hubbert, W. Bowie, MD

J. Adams, VA; W. B. Buck, IL; H. H. Casper, ND; D. R. Cassidy, IA; W. O. Cook, IL; R. A. Gessert, MI; H. S. Gosser, MO; R. E. John, IL; W. E. Ketter, MD; L. F. Kubena, TX; R. B. Landy, GA; M. M. Mamminga, IA; L. McGovern, VA; G. W. Meyerholz, DC; G. D. Osweiler, IA; J. F. Robens, MD; P. F. Ross, IA; L. G. Sullivan, MI; M. A. Thomas, TX; J. Webb, DC.

The Environmental Residue Committee met at the Riviera Hotel, Las Vegas, Nevada, on the afternoon of October 31, 1989. Eleven members and four visitors attended.

The Committee deliberations were primarily on water quality and mycotoxin contamination of feedstuffs.

Water Quality

Dr. Bill Owen, Iowa State University, presented an overview of the ISU Livestock Health Surveillance Program. This well-funded study included 88 swine, 46 cow calf, 25 feedlot, and 15 sheep operations randomly selected, on a statistically sound basis, throughout Iowa. A wide variety of parameters were evaluated, including water. The data did indicate livestock health problems are multi-factorial, drugs and vaccines will not replace poor management, and that water quality, as a single entity, was not a cause of health problems.

The Chairman shared a verbal report on Missouri water quality supplied by Mr. John Howland, Chief Planner, Water Pollution Monitoring, Jefferson City, MO. Mr. Howland reports ground water does not appear to be a cause of livestock health problems in Missouri, and that surface water quality has improved over the past 20 years because of better municipal waste treatment plants.

Mycotoxin Contamination of Feedstuffs

Mycotoxin contamination of the 1989 feedgrains is minimal and only in a few areas. This is because of much more favorable weather during growing season and harvest. Reported problem areas:

South Texas — Dr. John Reagor, Texas A&M, reported aflatoxin contaminated corn in the 500-600 ppb range; not as high as during 1988 season. Elevators are utilizing screening tests to identify contaminated corn, and removing fines to reduce levels to <200ppb. There have been recent wild geese deaths in the area.

Death losses due to leucoencephalomalacia have occurred in 7 groups of horses in this area. The losses are associated with Fusarium moniliforme contaminated corn screenings used by 2 local mills to make a commercial horse feed.

Southeastern Iowa — Dr. Gary Osweiler, ISU, reported swine losses
ENVIRONMENTAL RESIDUES

due to acute pulmonary edema, associated with feeding of corn screenings. A toxin from *F. moniliforme* is suspected.  

*Western Illinois* — Dr. Bill Buck, UOI, Ill., reported 26% of 121 corn samples submitted were above 20ppb aflatoxin. Nine (9) were over 100ppb with the highest at 400ppb.

Dr. Jane Robens, ARS, Beltsville, shared a report "Recent and Current Research — *Fusarium moniliforme*", by Dr. Ben Voss of Richard Russell Center, Atlanta, GA., with the committee. A toxic factor from *F. moniliforme* (FM) contaminated corn and associated with equine leucoencephalomalacia, culture material of toxic FM strain MCR 826 from S. Africa, and culture material of FM RRC408 are being studied. This toxic factor is currently identified as fumonisins $B_1$ and $B_2$. There is a correlation between total fumonisin $B_1$ and $B_2$ levels in test diets fed rats and hepatotoxicity. Preliminary studies indicate fusaric acid produced by FM strain MCR 826 is phytotoxic to corn seedlings and fusaric acid C is immunosuppressive to mice. Larger quantities of fumonisins $B_1$ and $B_2$ are needed for further investigation.

Dr. Roger Harvey, Texas A&M, presented information on aflatoxin binding of hydrated aluminosilicate. These studies were in lambs and turkeys. At the 2% use level, hydrated aluminosilicate protected lambs from clinical effects of aflatoxin, but there were reduced liver weights and less than normal weight gain. Turkeys are extremely sensitive to aflatoxin. Hydrated aluminosilicate at 2% in diet, provided some protection. Dr. Harvey is looking at other compounds that bind aflatoxin. He emphasized that aluminosilicates differ in their aflatoxin binding capacity. His work has been with a hydrated aluminosilicate — Nova Sil. The value of this product or other aluminosilicates in binding other mycotoxins has not been demonstrated.

Dr. Frank Ross, NVSL, Ames, reported on the Federal Grain Inspection Service evaluation of 6 commercially available aflatoxin screening test kits. All behaved statistically identical. They must be properly cared for, used as directed and only on approved feedstuffs. A lengthy discussion followed on use of screening tests, their limitations and impact in the marketplace. These are screening tests only.

A sound judgment on value of, use of, or disposal of commodities can only be made after confirmatory testing with proven dependable methods. The Environmental Residue Committee of USAHA believes that potential problems associated with widespread use, or screening tests by non-technically trained persons, are serious, and recommends that a joint USAHA-AAVD session be devoted to screening test technology and use at the 1990 annual meeting.

The Environmental Residue Committee voted to support a resolution submitted by the Mycotoxin Committee of AAVLD to that organization on the need for a broadly supported study of mycotoxin contaminated forages.
AVAILABILITY OF VACCINES FOR FOREIGN ANIMAL DISEASES
C. A. Mebus, D.V.M., Ph.D.¹
J. A. House, D.V.M., Ph.D.¹
M. A. Mixson, D.V.M.²

If a foreign animal disease were to enter the United States (U.S.), the objective of the Animal and Plant Health Inspection Service (APHIS) would be to eradicate the disease. Eradication procedures used would depend on the disease and the extent of the outbreak. Diseases which spread by contact and/or have a limited distribution could be quickly controlled by slaughter and disposal. The other extreme is those diseases which are spread by flying arthropod vectors or via aerosol; these could very quickly become widely disseminated. In the latter type of disease outbreak, vaccines would have a place in the eradication plan. Let us use a specific disease as an example. Rift Valley fever (RVF) is a mosquito-borne viral disease that infects many species of animals and man. If a person in the viremic stage of the disease returned to the U.S. and was bitten by a mosquito, this insect could then infect ruminants which are the primary amplifier of RVF virus and the source of infection for other mosquitoes. The result could be an outbreak of RVF in the U.S. In a disease of this type, vaccination would play an important role in the control and eradication of the disease. If ruminants could be quickly immunized, this would stop the amplification of the virus, infection of mosquitoes, and thus, stop or decrease the rate of spread of the disease. Other arthropod-borne diseases of concern are African horse sickness, lumpy skin disease, and heartwater. For highly infectious airborne diseases such as foot-and-mouth disease and hog cholera, vaccines would also be useful in eradication if the disease became so widespread that immediate slaughter could no longer be justified.

There are good vaccines for many diseases that are considered foreign to the U.S.; however, the problem is that many of these vaccines are not available for use in the U.S. Vaccines for animal diseases foreign to the U.S. are not produced in the U.S. because a special high containment facility and, in some cases special legal authorization are required. In addition, previous discussions with commercial vaccine producers have indicated a reluctance to produce these vaccines, for they do not want to be associated with exotic agents because of adverse publicity which such vaccine production might cast on their other products. Vaccines for many of these diseases are produced in foreign countries; however, before a vaccine that is to be used in the U.S. is produced by such a laboratory, the laboratory and all aspects associated with vaccine production would have to be approved by the United States Department of Agriculture (USDA). A proposed solution to this problem is to develop genetically engineered vaccines. The whole or viable virus is not needed to produce these vaccines; thus, they could be produced anywhere within the U.S. Unfortunately, the development of...
these vaccines may take many years or may not be achievable with current technology; thus, it would be prudent to develop interim sources of vaccine. One option would be to develop an international reagent production laboratory operated by an international staff in a country in which a variety of agents can be propagated and which is politically acceptable to many countries. The intent of having this type of laboratory would be to produce vaccines which would meet standards acceptable to USDA. An alternative is to identify the diseases for which the U.S. wants vaccines available and then to work with selected laboratories to develop facilities and protocols that are acceptable to the USDA. To begin this process, USDA will evaluate available vaccines for safety and value in the control and eradication of disease. For vaccines determined to be important, the necessary regulatory work will be accomplished to determine if the vaccine could be used in the United States.

REFERENCES

1. United States Department of Agriculture, Animal and Plant Health Inspection Service, Science and Technology, National Veterinary Services Laboratories, Foreign Animal Disease Diagnostic Laboratory, P.O. Box 848, Greenport, NY 11944.

2. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Emergency Programs, Federal Center Building, Hyattsville, MD 20782.
If you are concerned that the 500 pregnant Hereford heifers you traveled with from Dallas to southern Mexico 3 weeks ago are feverish and anemic, they probably have piroplasmosis. After all, now that the heifers are in the tropics, they have 3 to 1 chance of being infected with a tick-borne pathogen. On the other hand, if the cattle you graze near the ostrich you just brought onto your ranch are also feverish and anemic, you should be aware that they too may have contracted a tick-borne disease.

Tick-borne disease is one of the major problems facing the world livestock industry. In the U.S., while better diagnostic methods have diminished the threats due to viral and bacterial disease agents, the threat from tick-borne diseases seems to have increased. The purpose of my talk is to explore the reasons why this may be so.

Texas cattle fever, or babesiosis, was prevalent in 14 southern states, including Texas, in the early part of this century. Eradication of the vector tick was the best method of disease control. A compulsory dipping program, initiated in 1905 and completed in 1942, succeeded largely because livestock owners cooperated closely with state and federal regulatory personnel, because there was a substantial research investment to support the program, and because the climate associated with the geography in the southern U.S. favored the eradication effort.

Eradication eliminated the tick vector except along the Texas-Mexico border. Since 1942, vigilance has become a key element in maintaining freedom from fever ticks. A quarantine zone monitored by USDA/APHIS was established along the border, and was matched with a program of the Texas Animal Health Commission that deals with tick infestations outside the quarantine zone. Due to the efforts of these 2 agencies, the U.S. has had no problems with tick-borne diseases for nearly 50 years. Once again, research investment into tick biology and disease epidemiology has added to the success, as has the weather associated with the regional geography.

Predictably, almost 50 years later we also find complacency. The livestock industry accepts only a minimum level of regulatory activity. Many regulatory officials and ranchers alike are concerned that the regulations themselves are out-dated and based on what we now know is incomplete information, with no accommodation for new research findings on tick biology and ecology.

These concerns are well illustrated by a review of the basic assumptions of the current state and federal tick programs, namely that all cattle are dipped, the dip always works, and the tick infestation is recent and active. Certainly many cattle are missed; they may have been smuggled, or may
TICK-BORNE DISEASES

have hidden in thickets. Even more important are the deer, nilgai and perhaps other exotics that are not dipped. The dip may not work when acaricide resistant ticks from Mexico spread to the U.S. Lastly, completely compelling research results show that under certain weather conditions, immature ticks can remain on the ground longer than the 9 month period that the current program requires cattle to be dipped or pasture to be vacated after an infestation is detected.

While it may be problematic that the U.S. remain free of exotic ticks and tick-borne diseases, it is very difficult to assess the risk and to sort out the dimensions of the threat to the livestock industry. The tick-borne diseases include those caused by the intraerythrocytic protozoa or piroplasms, and those caused by the rickettsiae. The diseases and the domestic livestock most commonly affected are listed below:

Piroplasms
- Babesia—Cattle, Horses, Sheep, Goats and Pigs
- Theileria—Cattle, Sheep and Goats

Rickettsiae
- Anaplasma—Cattle, Sheep and Goats
- Ehrlichia—Cattle, Horses, Sheep and Goats
- Cowdria—Cattle, Sheep and Goats

Several of the above diseases are problems in the U.S. and always have been. Several more are occasional problems in one or another animal species. The immediate concern is cowdriosis, or heartwater.

Many tick genera can transmit the piroplasms and rickettsiae listed above, including those favoring temperate climates—Ixodes, Haemaphysalis, and Dermacentor, and those that do better in the tropics—Rhipicephalus, Boophilus, Hyalomma, and Amblyomma. Various species of most of these genera are present in the U.S. However, of most concern are the tropical species of Amblyomma, the bont tick, the primary vector of heartwater in southern Africa.

The priorities for the future are based on what we’ve learned from the past: Continued vigilance, research investment and better use of our geography.

Vigilance is more than waiting. Better vigilance includes a regulation that all animal species from countries with ticks and tick-borne diseases be subject to inspection and/or treatment with an acaricide. Building on our cooperative relationships with laboratories in other countries will improve our understanding of epidemiology, particularly the accidental hosts that could introduce exotic ticks and tick-borne diseases.

The research investment into all areas of animal health, not just the diseases we are talking about today, must not erode further. Better evaluation of the biology of exotic ticks and tick-borne diseases, particularly in wild animals, is critical. Heartwater can be introduced either by
infected animals or infected ticks; an unapparent infection in the wild could well become a problem in captivity. We must, for instance, consider the consequences of a heartwater introduction into a white-tailed deer population. With exotic ticks, the need is to figure out the interconnections between a host in a foreign country, with another important host species in the U.S. Finally, while expert systems and computer projections provide enticing results about disease spread, they serve us best by promoting a better assessment of the effects of the changing ecology on the risk to the livestock industry.

Geography can be very important. The U.S. must share an interest with Mexico, Canada and the Caribbean. Activities with mutual benefit would include surveillance, control of diseases and the control of the disease vectors.

Additional needs, particularly at the USDA level, include a policy on the use of effective therapeutic drugs that are available in other countries but have not been licensed in the U.S. We also need a policy on the use of vaccines that might be useful, even on an experimental basis, in the event of a disease outbreak. Lastly, we need a practical policy that will allow continuing surveillance for ticks and tick-borne diseases, before a worst case scenario can occur. It is highly questionable whether a disease like heartwater would be diagnosed before it became established in the wildlife population. Keep in mind that the discovery of ticks on black rhino and ostrich were fortuitous, what about the several hundred ostrich that were imported last year? The industry should demand that people who know what they are about be allowed to look for ticks and diseases where they think they might be occurring.
REPORT OF THE COMMITTEE ON EPIZOOTIC ATTACK

Chairman: Mr. J. B. Finley, Encinal, TX
Vice Chairman: Dr. H. A. McDaniel, Hyattsville, MD

J. B. Anderson, TN; J. L. Blair, VA; W. W. Buisch, NY; R. G. Burdett, OR; J. J. Callis, NY; T. M. Cook, DC; A. H. Dardiri, NY; G. E. Dittberner, CAN; A. K. Eugster, TX; W. D. Felkner, IA; M. J. Gilsdorf, MD; W. C. H. Glaze, TX; P. R. Henry, CO; B. R. Heron, CA; M. C. Howard, CA; J. L. Hyde, NY; L. P. Jones, TX; D. W. Luchsinger, VA; E. T. Mallinson, MD; R. H. McCapes, CA; C. A. Mebus, NY; N. Meyer, VA; M. A. Mixson, MD; C. J. Nelson, DC; J. E. Novy, MD; R. E. Omohundro, CO; J. S. Orsborn, Jr., CA; B. I. Osburn, CA; E. C. Sharman, GA; W. G. Sterritt, CAN; D. L. Thompson, CA; O. H. Timm, CA; M. A. Van Buskirk, Jr., PA; S. A. Vezey, GA; S. T. Wilson, Jr., MD.

This committee meeting was called to order at 1:33 p.m. There were 57 members and guests present.

Dr. Gale Wagner presented a stimulating and thought provoking discussion on the Vulnerability of the United States to Tick-Borne Diseases. The biggest problem is complacency and a false sense of security. Deer and other alternate host may be the most likely host to bring these exotic pests into this country because they are not always thoroughly inspected or treated. Efforts to keep exotic ticks and parasites out of U.S. must be strengthened and better plans are needed to deal with them when they are found in this country.

The availability of vaccines for foreign animal diseases was presented by Dr. C. A. Mebus. A plan for evaluating vaccines for use in the U.S. in the event of an outbreak is being developed. Risk analysis studies are also planned to provide additional data on use of these vaccines.

He also summarized other developments at the Foreign Animal Disease Diagnostic Laboratory, Plum Island, NY during the past year.

Dr. Jaun Guy was applauded and sincerely complimented by the committee for his presentation on the hemorrhagic disease of rabbits campaign conducted in Mexico. The management and technical proficiency demonstrated during this successful campaign proved Mexican officials are equally or more prepared to mount an emergency response than U.S. officials. His explanation of the policies and procedures used also vividly demonstrated that much creativity and ingenuity were required. Thanks to these dedicated individuals the Western Hemisphere is again free, or almost free, of this plague that cost Mexico about 50 million dollars.

Dr. John Mason presented another success story about the Mexican-U.S. Commission for exotic disease monitoring. It is truly an outstanding program. However, Dr. Mason also suggested several issues that need to be reconsidered. Hog cholera in Mexico is a serious threat to the United States, but because commission rules prohibit them from working on diseases not
exotic to both the U.S. and Mexico, they have to consider that each suspected outbreak might be African Swine Fever to conduct the necessary investigations. The existing policies are equally outdated for bovine tuberculosis, screwworms, equine encephalitis, Newcastle disease, ticks and blue-eye disease of pigs.

Drs. Roger Drummond and Vic Nettles updated the committee on the Caribbean bont tick situation. Nymph and larva stages of this tick have been found on mongoose and cattle egrets. The fact that these cattle egrets fly from one island to another may seriously complicate eradication plans.

This committee endorsed four resolutions from other committees and approved five new resolutions presented.
PESTICIDES AND FOOD SAFETY: 
PERCEPTION VS. REALITY

James H. Steele, D.V.M., M.P.H.

INTRODUCTION

Is food grown with pesticides safe? How can we be sure? Do farming operations that depend on chemical control of insects, weeds and plant diseases present health hazards for the consumer? What is government doing to protect us?

These and other questions about food safety arise with increasing frequency. Unfortunately, there are not always answers which satisfy everyone. Or, perhaps, the answers come in the form of overly technical jargon that fails to tell the consumer whether he or she can buy fresh fruits and vegetables without health concerns.

Today American consumers get mixed messages about the safety of their food supply. Almost daily we hear about dangerous "chemicals" in our food, frequently in the form of pesticide residues. Indeed, in 1983 concerns about residues of one particular pesticide, Ethylene Dibromide (EDB), caused a near panic, resulting in the Environmental Protection Agency (EPA) banning this chemical because it was found in trace levels in some foods — and was considered dangerous because it caused cancer in high doses in laboratory animals.

On the other hand, scientists have been assuring us for years that our food supply is very safe and getting even safer. For example, the National Academy of Sciences produced a landmark report in 1987 confirming that the American public faces no threat from pesticide residues at the dinner table. Many other reassurances from respected experts make the same point — there is no scientific basis for apprehension about the safety of our food supply from pesticide residues when pesticides are properly used.

POSITION STATEMENT

The American food supply is very safe and getting even safer. That's the consensus of the experts. For example, the National Academy of Sciences (NAS), an eminent scientific body, studied the issue intensely for two years. The Chairman of the NAS study panel unveiled its 274-page report on May 20, 1987 with an unqualified reassurance; the American food supply is very safe and getting even safer. Later the same day, under questioning by a U.S. Senate Committee, the EPA Assistant Administrator of Pesticides, declared "the food supply is wholesome. It is safe. The challenge to the EPA is to take some of the recommendations in this (NAS) report and improve on it."

Even in the face of such seemingly irrefutable evidence, public concern about food safety persists.
The facts do not appear to support such concerns. Scientists specializing in the fields of nutrition, toxicology and related areas find little or no factual basis for public concern over food safety related to properly-used pesticides.

The Commissioner of the Food and Drug Administration (FDA), stated it clearly in testimony before the U.S. Congress when he said that microbiologically contamination of food is a critical public health issue: "As a microbiologist, I believe that such microbial contamination poses a much greater risk to consumer health than pesticide residues."

We suspect that much, although perhaps not all, of the public's concern about food safety is a clash between public perception and scientific fact. To be sure, there are instances where pesticides have exceeded safe standards and have otherwise been misused and resulted in illnesses.

The California watermelon scare in 1985 is an example. In that case, a grower blatantly disregarded the law and applied a pesticide on a crop for which it was not registered. The result was transient illness for several hundred people who consumed the tainted melons although, fortunately, no one was permanently harmed by this accident. There are occasional findings by the FDA of residues in excess of tolerances — but rarely so large that they exceed the built-in safety factors of 100-fold or more.

It appears that as with many other issues, the public receives most of its information — and concern — from media reports. Media commentators and writers are rarely scientists. When they report that some chemical has been detected someplace it shouldn't be, too often they conclude their reports with, in effect; "We think there's danger but we don't know how serious it is." Every time that happens, the viewer or reader is left with the uneasy feeling that the presence of any detectable amount must somehow represent a serious health danger or hazard.

Dosage is what makes chemical presence dangerous. "Pure" mountain water, if analyzed by modern detection technology, shows trace amounts of many chemicals — including arsenic and other naturally occurring chemical substances. That doesn't mean the water isn't good because, again, the dose makes the poison.

Because we can now detect trace quantities of chemicals that are barely there — expressed as parts per billion or even trillion — we are now finding elements and compounds we never knew were present. (A part per trillion is equivalent to 1 gram of salt in 10,000 tons of potato chips or one second in 320 centuries.) There are far more chemicals and "poisons" occurring naturally than mankind has ever created in laboratories. Many of the naturally-occurring chemicals are far more toxic than the man-made substances. e.g. Toxins produced by bacteria, protozoa, plants, snakes, insects, fish and amphibia.

It gets down to being able to distinguish smoke from fire. When an alarm is sounded about pesticide residues on food, it frequently turns out to be trace amounts well below the stringent safety standards set by the regula-
PESTICIDES AND FOOD SAFETY

tory agencies. It's often like someone shouting "fire!" in a crowded arena just because someone lights a match. Such unwarranted alarms send everyone running for the exits unnecessarily.

A leading contemporary commentator states:

How extraordinary! The richest, longest lived, best protected, most resourceful civilization, with the highest degree of insight into its own technology, is on its way to becoming the most frightened.

Is it our environment or ourselves that have changed? Would people like us have had this sort of concern in the past? ... Today, there are risks from numerous small dams far exceeding those from nuclear reactors. Why is the one feared and not the other? Is it just that we are used to the old or are some of us looking differently at essentially the same sorts of experience?

Production of a singly new pesticide is a long, enormously expensive, complicated scientific procedure designed to produce pesticides that are safe when used properly, i.e., according to a prescribed set of instructions and directions. The eight-to-ten year procedure includes exhaustive testing and scientific review. Both the manufacturer and the government regulatory agencies are deeply involved, with the same goal of ensuring that the farmer and the general public have an abundant yet wholesome supply of food which is safe for human consumption.

Thus The American Council on Science and Health concludes that the American food supply is very safe and getting safer as technology continues to advance. That is because the American system of regulating food safety is the best that regulatory agencies anywhere in the world have devised and is constantly being improved.

PESTICIDES, HISTORICALLY SPEAKING

Chopping weeds with a hoe and manually removing the worst of the insects was how most early Americans spent spring and summer on the farm.

Schools started late and closed early to make children available for these manual tasks and other farm chores. Often, the most prosperous farmers were those with the largest families — to do the work that otherwise could not be done. Modern cultivation machinery was still far in the future.

So too were modern pesticides. Sulfur was used as early as 1000 B.C. for fumigation. Farmers continued to apply it into the early 1800s to control insects and plant diseases. By the late 1800s, kerosene emulsions, lead arsenate and copper compounds had come into widespread use to kill certain pests. But they were not very reliable.

Not until the World War II period did modern man-made pesticides make their introduction. DDT was the most celebrated of the new discoveries. Its
remarkable ability to control many insects, including malaria-bearing mosquitoes, was hailed worldwide as a scientific miracle. The innovation was of such significance that the inventor was awarded the Nobel Prize. Within the next few years, dozens of other new man-made pesticides would be discovered and marketed.

**Actual Annual Incidence of Lethal Poisoning in the U.S.**

<table>
<thead>
<tr>
<th>Year</th>
<th>From Agricultural Chemicals</th>
<th>From All Other Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>152</td>
<td>N.A.</td>
</tr>
<tr>
<td>1970</td>
<td>4</td>
<td>5299</td>
</tr>
<tr>
<td>1971</td>
<td>43</td>
<td>5313</td>
</tr>
<tr>
<td>1972</td>
<td>38</td>
<td>5380</td>
</tr>
<tr>
<td>1973</td>
<td>32</td>
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<td>4608</td>
</tr>
<tr>
<td>1980*</td>
<td>24</td>
<td>4331</td>
</tr>
</tbody>
</table>

*Last year for which data have been released. In 1980, a total of 15 of 4331 accidental poisoning deaths were attributed to cleaning and polishing agents, disinfectants, paints and varnishes.


Simultaneously, horse-drawn implements were rapidly yielding to the revolution favoring tractor-drawn tillage and harvesting machinery. All of this was timely for agriculture, as steadily increasing numbers of farmers decided they preferred to work in the cities instead of hoeing corn and plucking worms from cotton and other crops.

The public’s strong initial acceptance of the new miraculous agricultural chemicals began to fade with the 1962 publication of Rachel Carson’s Silent Spring. Her warnings about the potential environmental consequences of some pesticides made consumers realize that in addition to their benefits, pesticides also posed risks for man and the environment.
PESTICIDES AND FOOD SAFETY

Major FDA Events from 1969-1987
(as selected by the FDA)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Number of Major Events*</th>
<th>Number of Pesticide-Related Major Events</th>
</tr>
</thead>
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</tr>
<tr>
<td>1970</td>
<td>3</td>
<td>0</td>
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<td>1971</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>1972</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>1973</td>
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<td>1974</td>
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<td>1976</td>
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<td>1978</td>
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<td>1</td>
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<td>1980</td>
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<td>0</td>
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<td>1981</td>
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<td>1982</td>
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<td>1</td>
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<tr>
<td>1986</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>1987</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>TOTALS</td>
<td>182</td>
<td>6</td>
</tr>
</tbody>
</table>


*Major FDA Events include such matters as foodborne illnesses aboard trains; nitrite and nitrate in meat and poultry: vinyl chloride polymers in contact with food; chlorofluorocarbon propellants in food, drug, and cosmetic containers; tolerances for PCBs in milk, dairy products, poultry, eggs and fish; full ingredient labeling for all frozen dessert products; fish and shellfish poisonings and contamination; moratorium on saccharin; reduction of lead in cans used for food; N-nitrosamine in baby bottle nipples; radiation for foods; aspartame; sodium labeling; salmonellosis; pasteurization of soft cheeses; cholesterol labeling of food products, and others.
Congress took notice. In 1972, it shifted federal authority for the control of pesticides from the U.S. Department of Agriculture (USDA), where it had resided for decades, to a newly established federal agency called the Environmental Protection Agency (EPA).

Today, the EPA retains the front-line responsibility for weighing the risks of pesticides against their benefits. DDT and most of its close chemical relatives of that era have long since been removed from the market or their uses highly restricted to specific applications by certified pest control operators.

Rapidly replacing DDT and related compounds was a new generation of agrichemicals which were more environmentally acceptable. So innovative are some of these new agricultural chemicals that mere ounces per acre will control what once required pounds and with far less persistence.

Today, the EPA bases registration decisions for new pesticides on test data provided by manufacturers and other applicants following strict EPA protocols or instructions.

Required studies include testing to show whether a pesticide has the potential to cause adverse effects in humans, fish, wildlife, and endangered species. Potential human risks include acute reactions such as toxic poisoning and skin and eye irritation, as well as possible long-term effects like cancer, birth defects, or reproductive system disorders. Data on "environmental fate," or how a pesticide behaves in the environment, are also required so that EPA can determine, among other things, whether a pesticide poses a threat to ground or surface water.  

**SOCIETAL BENEFITS OF PESTICIDES**

In 1910, the typical American farmer produced enough to feed seven people. Today, the typical farmer feeds about 79 people, including 26 in foreign countries.

How can the farmer of the 1980s produce over 80 percent more per acre than the preceding generation? The answer is modern, scientific farming, using "tools" like pesticides, new seed varieties and automated equipment. Pesticides alone account for one quarter of the yield increase per acre.

Agricultural chemicals, along with fertilizers and mechanization, have made the American farmer the world's most efficient food producer. That means quality as well as quantity. Even with these efficiencies and continuing improvements, American agriculture is hard pressed to compete with foreign producers.

As with the other modern tools of agriculture, pesticides meet very specific needs. To produce crops successfully, farmers must compete with...
PESTICIDES AND FOOD SAFETY

10,000 species of insects
1,500 plant diseases
1,800 kinds of weeds
1,500 types of nematodes (worm-like organisms)

These pests can reduce yields or damage crops so severely that the food or fiber is not marketable. Unchecked, they can totally wipe out a crop.

In 1986, U.S. farmers spent $4.0 billion on pesticides. That seemingly huge sum was only about three percent of their total spending for production goods and services.

Chemical control of insects alone helps farmers' yields significantly:

<table>
<thead>
<tr>
<th>Crop</th>
<th>Percent Increase</th>
<th>Total 1987 Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>100 Percent</td>
<td>321,346 metric tons</td>
</tr>
<tr>
<td>Corn</td>
<td>25 Percent</td>
<td>19,280,760 metric tons</td>
</tr>
<tr>
<td>Onions</td>
<td>140 Percent</td>
<td>202,273 metric tons</td>
</tr>
<tr>
<td>Potatoes</td>
<td>35 Percent</td>
<td>1,753,500 metric tons</td>
</tr>
</tbody>
</table>

(Source: USDA Crop Production 1987 Summary, January 1988)

Studies in Iowa and Illinois show that herbicide weed control saved farmers up to 9.2 percent over the cost of mechanical tillage of corn and up to 11.7 percent over mechanical tillage of soybeans. At the same time, herbicide weed control boosted corn yields 9.4 bushels an acre and soybeans 7.9 bushels an acre over yields with mechanical weed control. Savings and yield improvements like these make the difference between profit and loss.

In Minnesota tests, the Council for Agricultural Science and Technology reported in 1984 that chemical control of weeds in corn resulted in an energy gain 35 percent higher than that from mechanical cultivation. Chemical control required 32 percent less energy and production was 11 percent higher.

In the fall of 1987, consumers across the country felt the direct impact of crop loss when retail prices for iceberg lettuce nearly quadrupled. It approached $2.00 a head in some areas! The cause was a September 1987 invasion of sweet potato whiteflies in California's Imperial Valley and in the vicinity of Yuma, Arizona.

The whiteflies spread a virus called “the infectious yellows.” The disease did not appear until two to three weeks before harvest, in late October and early November. One Agricultural Extension Service advisor said:
The plant would be all nice and green on Friday, but you’d go back and look on Monday and you couldn’t believe it’s the same thing. The leaves are all yellow and browning. In a week, it’s garbage.\textsuperscript{7}

The damage to the lettuce crop sharply drove up prices for substitute crops, too. Prices of romaine and other lettuces almost doubled.

The lettuce crop loss is an extreme example. In Wisconsin tests, insect damage prevented green peppers from producing any fruit, reduced the average weight of cabbage heads from 3.5 pounds to one pound and damaged 86 percent of the sweet corn.\textsuperscript{8} Although it is unlikely to happen to that extent, such losses on a national basis would skyrocket the consumer price of produce several-fold.

These examples dramatize the economic importance of proper and timely pesticide use. Without it, the tremendous quantities of food needed to ensure reasonable consumer prices would not be possible.

These studies do not readily demonstrate one key benefit of pesticides: their impact not merely on the external appearance of products but on their quality.

We don’t eat worms when we bite into an apple or an ear of corn. We don’t find aphids or insect parts on spinach, lettuce or leafy vegetables. We detect no mites on strawberries, soft fruit or citrus. There are no fungi or harmful bacteria accompanying insect damage. What would consumer acceptance be if we saw insect excrement on produce, not to mention the health effects if we consumed it in our food? That’s the difference the proper use of agricultural chemicals makes.

**RELATIVE RISKS**

Year after year, FDA Total Diet Studies (Market Basket Studies) show that pesticide residues in food are well below safety limits and pose no health risks for consumers as noted by the FDA Commissioner in testimony before Congress.\textsuperscript{9}

Year after year, public opinion surveys find that consumers are concerned about the potential health hazards of pesticide residues on food. For example, the Food Marketing Institute’s (FMI) 1988 update on “Consumer Attitudes & the Supermarket” determined that 75 percent of the respondents considered pesticide residues to be a “serious hazard.” The percentage of concern has remained at about that level since FMI started asking the question in 1984.


Base: Split Sample of 508 and 511 Members of the Shopping Public

Q: How concerned are you about residues, such as pesticides and herbicides? Would you say that these residues are a serious health hazard, somewhat of a hazard or not a hazard at all?
PESTICIDES AND FOOD SAFETY

<table>
<thead>
<tr>
<th>Year</th>
<th>Serious Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>77%</td>
</tr>
<tr>
<td>1985</td>
<td>73%</td>
</tr>
<tr>
<td>1986</td>
<td>75%</td>
</tr>
<tr>
<td>1987</td>
<td>76%</td>
</tr>
<tr>
<td>1988</td>
<td>75%</td>
</tr>
</tbody>
</table>

(Source: Trends, 1988, Food Marketing Institute, Washington, D.C.)

Even when the facts demonstrate conclusively that a product or substance presents virtually no risk, with some people there is the very strong perception of risk. The chart below from Scientific American illustrates how markedly the perception of risk can differ from the actual risk. The chart also shows how differently various occupational age groups regard identical risks.

One commentator observed:

The dominant perception for most Americans (and one that contrasts sharply with the views of professional risk assessors) is that they face more risk today than in the past and that future risks will be even greater than today's. Similar views appear to be held by citizens of many other industrialized nations. These perceptions and the opposition to technology that accompanies them have puzzled and frustrated industrialists and regulators and have led numerous observers to argue that the American public's apparent pursuit of a "zero-risk society" threatens the nation's political and economic stability.\(^1\)

The central issue of concern for many consumers is the alleged relationship between cancer and exposure to pesticide residues in food, especially fresh fruits and vegetables.

Researchers have found that natural pesticides are ingested in our diet at least 10,000 times more by weight than are man-made pesticide residues. They are by far the main source of "toxic chemicals" ingested by humans.\(^2\)

Assuming that residues of man-made pesticides on the food we eat remain within regulatory limits (tolerances), how great is the risk — even if some of these substances have been shown to cause cancer in laboratory animals? That, it seems to us, is the fundamental question which most concerns consumers.
<table>
<thead>
<tr>
<th>ACTUAL LEVEL OF RISK</th>
<th>HOW THEY RANKED IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>1</td>
</tr>
<tr>
<td>Alcoholic Beverages</td>
<td></td>
</tr>
<tr>
<td>Motor Vehicles</td>
<td></td>
</tr>
<tr>
<td>Handguns</td>
<td>--Pesticides as Ranked by College Students</td>
</tr>
<tr>
<td>Electric Power</td>
<td></td>
</tr>
<tr>
<td>Motorcycles</td>
<td></td>
</tr>
<tr>
<td>Swimming</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>--Pesticides as Ranked by Women Voters</td>
</tr>
<tr>
<td>X-Rays</td>
<td></td>
</tr>
<tr>
<td>Railroads</td>
<td>10</td>
</tr>
<tr>
<td>General Aviation</td>
<td></td>
</tr>
<tr>
<td>Large Construction</td>
<td></td>
</tr>
<tr>
<td>Bicycles</td>
<td></td>
</tr>
<tr>
<td>Hunting</td>
<td></td>
</tr>
<tr>
<td>Home Appliances</td>
<td>--Pesticides as Ranked by Business People</td>
</tr>
<tr>
<td>Fire Fighting</td>
<td></td>
</tr>
<tr>
<td>Police Work</td>
<td></td>
</tr>
<tr>
<td>Contraceptives</td>
<td></td>
</tr>
<tr>
<td>Commercial Aviation</td>
<td></td>
</tr>
<tr>
<td>Nuclear Power</td>
<td>20</td>
</tr>
<tr>
<td>Mountain Climbing</td>
<td></td>
</tr>
<tr>
<td>Power Mowers</td>
<td></td>
</tr>
<tr>
<td>Scholastic Football</td>
<td></td>
</tr>
<tr>
<td>Skiing</td>
<td></td>
</tr>
<tr>
<td>Vaccinations</td>
<td></td>
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<tr>
<td>Food Coloring</td>
<td></td>
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<tr>
<td>Food Preservatives</td>
<td></td>
</tr>
<tr>
<td>PESTICIDES</td>
<td></td>
</tr>
<tr>
<td>Prescription Antibiotics</td>
<td></td>
</tr>
</tbody>
</table>

ACTUAL LEVEL BASED ON SCIENTIFIC DATA

Spray Cans -- 30

Perceptions of risk may differ markedly from actual risk. On the left, 30 various causes of accidental death are ranked in order from government
PESTICIDES AND FOOD SAFETY

statistics. The risk from spray cans was the lowest among the causes of accidental death; risk from pesticides was in the lowest order. On the right is how three population groups ranked pesticides.\textsuperscript{11}

Unfortunately, there is not a simple "yes" or "no" answer. Like everything else where conclusions depend on science, it becomes a judgement call based on the best data available. Let's look at some of those facts.

PESTICIDE TOLERANCES

As stated throughout this report, the most basic principle of toxicology is that the dose makes the poison. Almost invariably, when chemicals are found to be carcinogenic in animal tests, it is because the animals were responding to the effect of massive doses of the chemical over relatively long periods of time. Saccharin is a good example where these doses were far higher than any low-level dose to which humans would be exposed.

From such tests and other supplemental studies on a particular pesticide used on food crops, a tolerance is set by the EPA and enforced by the FDA. A tolerance is designed to assure the consumer that any pesticide residues remaining on foods they buy are well below the dose which might injure human health. It is not arbitrary. Rather, it is a very specific level determined after careful research.

Using the results of toxicity studies and field trials, regulatory agencies set tolerances for the "most extreme" conditions of proposed use. That means the maximum application rate, the maximum number of applications and the shortest interval after chemical application prior to harvest and the maximum market share.

Such a combination of conditions would almost never occur simultaneously in real life. Tolerances are deliberately established by this method so that there is virtual certainty that the actual residue will not exceed the tolerance even under "worst case" conditions.

The EPA Administrator said recently: "Pesticide residues rarely are present at tolerance levels in ready-to-eat food commodities and in many cases may be undetectable by the time the food products reach the consumer."\textsuperscript{13}

The safety of the tolerance is confirmed through long-term animal feeding studies using several different species of test animals. These toxicity studies are then used to determine what is called the No Observable Adverse Effect Level or NOAEL. This is the level at which a chemical under study has no harmful effect on the most sensitive test animal.

Then, a safety factor of at least 100 is applied. Based on the result, an Acceptable Daily Intake (ADI) is set. The ADI represents how much residue can be ingested by a typical person every day over a 70-year lifespan without ill effect. What this means for the consumer is that any residues
that might remain on food are at a level far, far below those which have no effect on the most sensitive animal.

It is also worth noting that the maximum residue is the amount permitted on a crop at the time of harvest. By the time most fruits and vegetables reach a consumer’s kitchen, the pesticide will have degraded to minuscule or trace amounts far below the allowable tolerance if they exist at all. Washing, peeling or cooking will reduce any trace amounts even further.

Consumers should not assume that because it is permitted, the tolerance amount (upper limit) will be found in foodstuffs they purchase. The fact is that the FDA’s monitoring program finds that less than 3 percent of the market basket food samples tested container residues above acceptable tolerances (but rarely above the 100-fold safety factor). In fact, the California Department of Food and Agriculture reported that it found no residues whatsoever in 80 percent of the food samples it tested.

The FDA samples a greater proportion of imported foods, mainly fresh produce, for pesticide residues than it does domestically grown food. The incidence of illegal pesticide residues is about the same as for domestic foods and do not constitute a hazard to consumer health.

Residues begin to degrade shortly after the pesticide is applied. Sunlight, air, bacterial action, and rain will usually break down the compound. Also, irrigation helps wash the chemical off the crop.

Where necessary, EPA requires a pre-harvest interval, a period between the last application of the pesticide and the time the crop is harvested. During this time, natural processes listed above reduce the pesticide residue to levels well within limits at harvest.

The degradation of residues between the field and the table is confirmed by FDA’s Total Diet Study (TDS) or “Market Basket Survey.” The TDS determines the dietary intake of pesticides, minerals, other industrial chemicals, heavy metals and other contaminants by measuring their concentration in food as eaten. The TDS allow identification of trends and checks the effectiveness of food safety regulations.

TDSs have been conducted since 1964. Originally, the study was based on food consumption of a 16-to-19-year-old male. In 1974, it was expanded to include typical diets for infants (6 months old) and toddlers (2 years old). In 1982, the diets were once again revised based on two nationwide surveys covering about 50,000 people.

**HOW TO MINIMIZE RISKS**

Even though EPA limits the amount of pesticide residues that may legally remain on food, the consumer can and should take certain additional precautions.
PESTICIDES AND FOOD SAFETY

Here are some suggestions:

- Thoroughly wash and/or peel fresh fruits and vegetables.
- Cook or bake foods to held reduce residues.
- Trim and discard fat from meat and poultry.
- Use a varied diet and avoid eating the same foods over and over.

For further information, you may wish to obtain a copy of the ASCH booklet titled "Pesticides in Your Home and Garden." Please send $2 and a stamped (75 cent) self-addressed envelope to:

American Council on Science and Health
1995 Broadway
New York, NY 10023

ALTERNATIVES TO CHEMICAL CONTROLS

Some people concerned about pesticide safety advocate eliminating any potential risk by totally banning all agricultural chemicals. They contend that other equally effective means of controlling pests are already available. Let's look briefly at the state of the art of several alternatives to agrichemicals.

Many alternative ways of controlling insects, weed and plant diseases are being studied. Pest control science is not a static endeavor. Just as there are extensive programs to improve man-made pesticides, there are also parallel efforts examining other approaches. Substantial sums are being invested researching alternative methods. Some of these alternative approaches have received much media attention.

- Biotechnology is perhaps the one that has achieved the most widespread publicity recently. It is the newest approach. Some scientists view it as very feasible for the long term. Accordingly, many organizations have committed millions of dollars to new research and plan to spend even more.

Using diseases to kill pests is a variation of the biological control technique. One well-known application is the use of milky spore disease to control Japanese beetle grubs. In effect these spores serve as a chemical or biological insecticide.

The development and utilization of new diseases is time-consuming and costly. The major difficulty is that the biological effect is slow because of the requirement for specific environmental or climatological conditions that favor the proliferation of the disease. In the meantime, the crop can be destroyed by the pest. Farmers are unlikely to take this risk when chemical products will provide them with almost immediate control and protection of their crops.

- Genetic control is a method that has produced excellent results from time to time. It centers on evolving crop varieties with resistances to
specific diseases. For example, work on breeding various kinds of resistances into wheat has contributed to significant increases in productivity.

- Still another technique is Integrated Pest Management (IPM). Farmers adopting this approach integrate cultivational practices, genetic resistance in plants, modified cropping sequences and appropriate tillage to minimize pest populations. Farmers and other trained people scout the fields to identify pest populations and to apply the appropriate pesticide. The chemical is used minimally and only if necessary in accordance with a damage or pest population threshold to prevent economic loss of the crop.

IPM without agricultural chemicals can be effective in some instances. An example is the use of parasites or predators to control pests. A well-planned IPM program can decrease the use of farm chemicals. Increasingly, farmers have learned to manage their crops with greater precision using lower rates of chemical application, relying on scouting and expanding their IPM program for a more balanced approach to pest control.

- Good Field Practices is an approach related to IPM. Essentially it means the farmers use the best management practices in growing their crops. Availing themselves of state or university-sponsored applicator certification programs is what many farmers have done to enhance safety and efficiency.

In addition, equipment calibration clinics and seminars are widely available. Aerial fly-ins and workshops for computer-assisted calibration and swath analysis are still other ways to enhance farm operations as are industry-sponsored workshops for farmers, consultants, and professional applicators.

Discussions about alternatives to pesticide use tend to imply that agricultural chemicals are used on all food crops all of the time. That is not the case at all. For example, a 1976 survey of six regions found that only 27.7 percent of corn acreage was treated for insects. That figure ranged from a low of 9 percent to a high of 45 percent. The point to be noted here is that most of the acreage was not treated for insects. The circumstances clearly did not require it.

Four years later in 1980, the circumstances changed. Some 52.4 percent of the corn acreage required treatment. The range in acres treated in those six regions was from 17.2 percent to 91.5 percent. The message is that judicious use is the rationale the American farmer follows, applying no more than is necessary.

A clear indicator of the absence of viable alternative methods is the fact that for many farmers, pesticides are the approach of choice. Few have selected other means, such as organic farming, because they are not as reliable, efficient or cost-effective. If another means of controlling pests and increasing yield within the same safety parameters existed, farmers very likely would quickly adopt it.
CONCLUSIONS

Much of the public's concern about food safety is basically a clash between public perception and scientific fact. It's easy for that to happen, especially when the U.S. scientific literacy rate continues to average only 3 to 4 percent.

Most instances of public information and concern originate from news media reports. They almost always pertain to the presence of a pesticide residue found on a food. The message the reader or viewer comes away with is that presence equals serious danger. In effect, the perception created is that all pesticides are "dangerous."

The other half of those stories is rarely mentioned. When it is, it generally doesn't register. That other half, however, makes the difference between scientific fact and public perception.

The essential matter is how much of the chemical was found and, even more importantly, how that level compares with the stringent safety levels mandated by the regulatory agencies.

Dosage is what makes presence of a chemical dangerous. The "pure" mountain streams we all treasure often contain traces — presence — of things like arsenic. It is not significant because the level of presence — the dose — is far below the danger level.

The largest potential sources of harm in foods are by far, first, microbiological contamination and, next, nutritional imbalance. The risk from environmental contaminants is 1,000 times less, and the risk from pesticide residues is 100 times smaller again.

What is most appealing about biotechnology is that it offers the prospect of melding science and engineering principles to the processing of materials by biological agents.

If it lives up to its promise, however, biotechnology will not come into being on a broad scale for many years. An enormous amount of research and development work needs to be done to demonstrate effectiveness, efficiency and safety. That is typical of major new scientific undertakings; they do not reach fruition overnight. Sometimes they do not make it at all. That is an investment risk and a judgment each research organization must make.

• Another widely touted approach is organic farming. Sometimes called "sustainable agriculture," its basic appeal is that it does things the "natural" way, avoiding completely the use of man-made chemicals. In addition to uncertainties about food quantity, organic farming has difficulty in producing the quality level consumers expect and prefer. Blemished fruit and wormy vegetables are typical results of organic farming.

• Biological control is another method employed with varying results. Sometimes successful and sometimes not, the biological approach uses natural enemies, such as parasites or one sex of pest species to control another. The use of sterilized male flies, mosquitoes and bollweevils to mate.
with females and produce either no offspring or infertile eggs, for example, has been tried with good results. These techniques work best in small isolated areas but they have been known to be effective where specific geological or cultural conditions exist.

Biological control is one area where biotechnology may make breakthroughs. But many years of research will be required before the farmer can think about relying on it.

Food is very safe when pesticides have been applied properly, in accordance with label instructions. Farmers and applicators cannot legally use any agricultural chemical on any crop and cannot use dosages higher than those prescribed in label instructions.

Food reaching the table will rarely approach the tolerance level set by the government. That tolerance level itself has a safety factor of at least 100 built into it.

Government's assurances of safety could be further strengthened if Congress would appropriate more money to FDA for residue monitoring. There is public support for such an endeavor. An April 1988 Wirthlin Consumer Survey reported that Californians see a need for food safety monitoring in the market and are willing to pay for it.

There has been progress on the federal government level. The FDA has recently decided it will share its sampling results publicly on a more timely basis than in the past.

There are many data that show that the nation's food supply is very safe and becoming even safer. Continuing public concerns will doubtless move regulatory agencies to take additional steps. In the end, the result will be additional verification which will help bring public perception more into conformance with scientific reality. Meanwhile, ACSH sees no reason why consumers need to be concerned about health hazards from properly applied pesticides.

GLOSSARY OF TERMS

Active Ingredient — the substance in a pesticide designed to control the target pest.

Biotechnology — the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.

Carcinogen — a substance that can produce cancer in experimental animals or is known to do so in humans.

Delaney Clause — a provision of the Federal Food, Drug, and Cosmetic Act which states that any substance that causes cancer in man or animals cannot be added intentionally to foods.
PESTICIDES AND FOOD SAFETY

EPA — U.S. Environmental Protection Agency, the principal federal government regulatory body with responsibility for safety of most foods.

FIFRA — The Federal Insecticide, Fungicide, and Rodenticide Act, the principal federal law governing pesticide regulation.

Fumigation — the use of chemicals in the form of gas or vapor to destroy noxious insects, nematodes, and unwanted plants.

Fungicide — a chemical used to protect plants and seeds from fungus infection or to cure fungus infection.

Herbicide — a chemical used to control weeds and other undesired vegetation.

Inert Ingredient — a substance in a pesticide not intended to kill a pest; examples include emulsifiers, solvents, wetting agents, carriers, dilutants, and conditioning agents.

Insecticide — chemical used to control insect pests.

Mutagen — a substance or agent that produces genetic changes in living cells.

NAS — National Academy of Sciences

Nematodes — microscopic worm-like organisms.

Nematocide — a chemical used to control nematodes.

Organic Farming — production of crops entirely without using man-made chemicals and fertilizers during growing, harvesting, shipping and storage stages.

Persistence — the duration of a pesticide’s effectiveness after application in amounts sufficient to kill pests.

Pest — any organisms injurious to plants, man, animals, or other articles or substances.

Pesticide — any substance used to control or destroy insects, weeds, rodents, disease-causing organisms and other types of pests which attack living organisms or spread disease among plants and animals.

Registered Produce — a product that has successfully undergone EPA’s registration approval process.

Registration — EPA’s original approval of a label for a completely new pesticide on a specific agricultural product.

Reregistration — satisfactory EPA completion of the re-examination of data supporting a previously registered pesticide.

Residue Tolerance — the amount of pesticide residue permissible in or on a raw agricultural commodity.

Rodenticide — a pesticide used to kill rodents.

Tolerance — (see residue tolerance)

Total Diet Study (“Market Basket Study”) — an FDA program which
measures pesticide residues in foods eaten by consumers to determine actual dietary intakes of pesticides. Samples of individual food items are prepared and cooked as they would be in the home before they are tested for residues.

Toxic — poisonous, relating to toxin, caused by the toxin.

USDA — U.S. Department of Agriculture, responsible for the safety of meat and poultry products.

APPENDIX

1988 PESTICIDE MONITORING RESULTS IMPLY FOOD IS SAFE

The Food and Drug Administration said in its second annual pesticide residue monitoring program that its 1988 survey results show the continuing safety of the U.S. food supply relative to pesticide residues. The report said that in 1988 a total of 18,114 samples of foods were analyzed (7639 domestic and 10,475 import), an increase of 25% over the total number of samples analyzed in 1987. The foods were from all 50 states and 89 countries. FDA said no residues were found in over 61% of the samples, compared with 57% in 1987, and less than 1% had violative residues.

REFERENCES/

6. Calculated from USDA Fact Book.
PESTICIDES AND FOOD SAFETY


15. 1987 Pesticide Residue Annual Reports, California Department of Food and Agriculture.


ABSTRACT

Much of the public’s concern about food safety is basically a clash between public perception and scientific fact. It’s easy for that to happen, especially when the U.S. scientific literacy rate continues to average only 3 to 4 percent.

Most instances of public information and concern originate from news media reports. They almost always pertain to the presence of a pesticide residue found on a food. The message the reader or viewer comes away with is that presence equals serious danger. In effect, the perception created is that all pesticides are “dangerous.”

The largest potential sources of harm in foods are by far, first, microbiological contamination and, next, nutritional imbalance. The risk from environmental contaminants is 1,000 times less, and the risk from pesticide residues is 100 times smaller again.

Food is very safe when pesticides have been applied properly, in accordance with label instruction. Farmers and applicators cannot legally use an agricultural chemical on any crop and cannot use dosages higher than those prescribed in label instructions.

There are many data that show that the nation’s food supply is very safe and becoming even safer. Continuing public concerns will doubtless move regulatory agencies to take additional steps. In the end, the result will be additional verification which will help bring public perception more into conformance with scientific reality. Meanwhile, American Council on Science and Health sees no reason why consumers need to be concerned about health hazards from properly applied pesticides.

Public health has continued to improve throughout the 20th century. There are less infectious diseases, poisoning, and accidents except for man made health problems: caused by smoking, alcohol and guns in the U.S.

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EMERGING CHALLENGES AND CONCERNS
AN ENIGMA OF ADVANCING TECHNOLOGY

Edward L. Menning, D.V.M., M.P.H., Dipl. ACVPM

Microbiological foodborne disease has steadily been increasing for decades in the United States and other affluent countries. Conservative estimates are well over 5,000,000 cases per year in the U.S. with thousands of deaths at a cost of up to $23 billion. What has allowed for this increased microbial disease incidence?

Most of the infectious diseases of humans discovered in the past 50 years have been zoonoses. Almost all of the recently emerging infectious foodborne diseases are zoonoses. In general these organisms occur in or on healthy or subclinically diseased or carrier food animals which increasingly commingle on farm, feed lot, holding pens, slaughter rooms, and for which vaccines are ineffective.

Prior to 1945
    botulism
    staphylococcal food poisoning
    salmonellosis
    were the only three authentic foodborne diseases known

1945–1975 the following were shown
    C. perfringens–1946
    Vibrio parahaemoliticus–1960's
    Bacillus cereus–1955–1960
    We also learned food could be a vehicle for hepatitis virus, and for Shigella spp.

Since 1975
    Emerging pathogens proven
        Campylobacter
        Yesinia enterocolitica
        Vibrio vulnificus
        Listeria Monocytogenes
        —E. coli 0157-H7 and 3 others
        Aeromonas hydrophila
        Plesiomonas shigelloides
        Norwalk Virus
        Toxoplasma gondii
    Opportunistic pathogens—roles in p.H. is not certain
        10 genera of enterobacteriaceae among which
            Klebsiella pneumoniae
            Serratia marcescens
            Pseudomonas cocovenenans
            often fatal “Bonkrek Poisoning” in Indonesia
EMERGING CHALLENGES AND CONCERNS

Flavobacterium farinofermentans
severe poisoning in China in fermented cornmeal

With the proliferation of ethnic foods in this country don't be surprised when these and/or others show up.

Add to the above some
Corynebacteria spp.
Streptococci spp.
Cryptosporidium spp.
Sarcocystis spp.
e tc.

Enigmatic Happenings

We used to feel totally secure with pasteurization of milk and indeed it is a wonderful public health process that has helped to eliminate many human epidemics of typhoid, septic sore throat, diphtheria, T.B., etc. But something has gone wrong. Between 1982 and 1985 three large epidemics occurred in the U.S. from good old safe pasteurized milk—1985–20,000 people in Chicago with Salmonellosis.

We always knew you shouldn't eat cracked, checked or low grade raw eggs but we also knew grade A intact raw eggs were safe. Wrong again. In the last three years over 5,000 cases of S. enteritidis have been attributed to grade A eggs.

We always knew that C. botulinum produced the world's most potent toxin under many conditions but never in an environment of pH less than 4.6. Wrong again. It does produce toxin in some conditions below 4.6.

We always knew that safe refrigeration, under 45°F, protected from all significant growth of pathogens. Wrong again. C. botulinum, type E grows and produces toxin slightly less than 40°F. Listeria monocytogenes, Yersinia enterocolitica, Aeromonas hydrophila, various strains of E. coli and numerous other of the emerging or opportunistic pathogens grow quite well, thank you at 39°F.

Cheese aged over 60 days was known to be safe from pathogens if not from preformed toxins. Wrong again—Salmonella spp. and Listeria spp. can survive much longer than 200 days.

We always knew that Y. pestis caused deadly bubonic plague and Y. pseudotuberculosis caused little or mild disease. Wrong again. Researchers in Europe have just shown that Y. pestis by flipping one gene (triggered by unknown but natural occurrences) becomes Y. pseudotuberculosis and vice versa. Bacillus cereus is well known to cause food poisoning. It's close cousin B. thuringiensis or "B.T." which we're spraying by the tons all over this country to kill certain insect larvae does not produce any disease in humans—yet. What if they can flip genes like Yersinia?

Consumer Attitude and Industrial Happenings

The absolute trend is toward food products with
more convenience
greater variety
more natural
lower cost
This has led us to have products with
less artificial ingredients
less preservatives
less salt
less sugar
less nitrates
longer refrigeration
vacuum packaging
less cooking
longer shelf lives
cheaper ingredients
faster slaughter
more processing and mixing
more microwaving
avoidance of “highly processed”
drying
canning
freezing

This has now led to an explosive interest in refrigerated distribution of minimally cooked, low acid, no preservative foods through an unreliable refrigeration system. This is frightening enough, but the marketers also want extended shelf lives of up to 60 days via inpackage pasteurization and/or controlled package atmospheres.

These are the ideal situations for cold tolerant and anaerobic pathogens to develop.

Always on the side of bacteria are multiple handling, time and temperature abuse.

Microwaves cook food by vibrating water molecules. Much of the moisture near the outside of the food evaporates, leaving the surface cooler than the inside. The air around the food remains cool too—unlike the searing hot air in a conventional oven—so that organisms present on the surface have a greater chance of surviving. Thus while the center of a hamburger may be steaming hot, the edge may remain cool—and contaminated. A study at Georgia Southern College compared Salmonella inoculated chickens that were either cooked conventionally or microwaved. Those cooked conventionally contained no live bacteria, but eight out of nine microwaved chickens did. And several other studies have shown that hot dogs, hamburgers and tomato soup, all contaminated with bacteria, had higher levels of surviving organisms after microwaving than after conventional cooking.
EMERGING CHALLENGES AND CONCERNS

There has been a revolution in the potential for disease from farm or sea to your table. On the Ohio farm where I was raised we would go to the henhouse, catch a chicken (which had eaten only feed from our farm), kill it, plunge it into boiling water, remove the feathers, carefully remove the viscera, wash it, and immediately "cook the hell" out of it. If the bird were diseased and anything escaped cooking, only my family would have become ill. Since then, there has been a massive technological change in every stage of handling fresh meat from farm to table, except in control of pathogens for which there has been a concomitant standstill or even worsening. Animals are now fed contaminated feeds from all over the country or world, are raised in monstrous close-quartered numbers, are commingled in feed lots from around the country, are further commingled at slaughter plant holding pens, and are further crosscontaminated in the slaughter plants (beef, pork, chicken, etc.). Production lines go so fast that the equipment furthers the accidental fecal contamination. Water temperature in the scald tanks is often so low that it essentially kills nothing. All this is then further crosscontaminated by the cutting up, grinding up and mixing that is now expected. Finally these mixed-up products are refrigerated, shipped, and received by the retail outlets sometimes under variable temperature condition. They then are held and sold "fresh" for many days. In the U.S. these foods move through 25,000 food manufacturers, 35,000 wholesalers, 250,000 food stores, 275,000 restaurants and all households. This problem begins with animal feed and the environment on the farm. It proceeds to be compounded and exacerbated with each step leading to the ultimate consumer who indeed can make it worse.

To muddle the situation more is meat from wild, exotic or game animals. Ranching of exotic or game animals is becoming big business. Much of the meat has little or no inspection. Furthermore, legislatures in their zeal to save money or deregulate are removing some safeguards such as Michigan did this year when it passed an amendment that allows all nonprofit groups to serve all wild game (specifically introduced for muskrat) for public dinners regardless of origin. The only requirement is a sign at the dining room entrance "Consumer Beware. The wild game served at this facility has not been subject to state or federal inspection." Most of the common foodborne pathogens are prevalent in such animals in addition to more exotic pathogens and possible toxic contaminants.

Making the above more of a threat is the actual decreased education of the American consumer as to what dangers are now known to lurk in the shadows of their meat cases. To this day most people cook pork well because they have been educated about trichinosis. Now there is a new ELISA test to test pork for trichinae so that pork might be sold labeled to show that trichinae are probably not present thus intimating that it's safe to eat the pork rare or raw. This totally ignores the much more serious threat of toxoplasmosis especially to pregnant women, from the over 30% of pork positive for it. Most people, from my own observations and questions, have
no idea that meats, poultry and seafood should be considered already contaminated with one or more serious pathogens which will rub off on their hands, sinks, counters, cutting boards, knives, etc. and then contaminate the next item touched. In commercial food establishments there have been drastic changes in the retail work force with extremely high turnovers. A less stable force means less training and knowledge about how to safely handle foods creating a greater public health threat in restaurants, etc. Home economics and other homemaking procedures are now "out" for most females and males. Even grandmother no longer exists to teach proper habits.

There have even been changes in the population at greatest risk, i.e. those who would most easily contract these diseases and then be most severely affected. The extremely young are still in this group but many more children are surviving with other debilitating diseases and hence even more susceptible to foodborne disease. The elderly are still in this group but their overall numbers are rapidly increasing and they too are surviving with many more other weakening conditions. The already debilitated who are not young or elderly also still exist and their numbers also are rapidly increasing due to immunocompromising factors such as antirejection drugs used in transplant surgeries, chemotherapies for cancer patients and of course AIDS. All of the above categories are much more likely to get all of the diseases we're discussing, as well as to be more severely infected with a higher mortality. Plus—the much more frequent travel of most people makes it very difficult to relate illnesses of groups back to specific food incidents.

Some Facts About Some Agents

**Campylobacter**
- low infective dose 500
- swine 38–61%
- chicken 54–87%
- turkey 34–92%
- at over 1 million/gm
- Estimated U.S. morbidity/mortality per year 2,100,000/2,000

**Salmonella**
- some have low infective dose 100
- dairies in California 15–20% positive of which 25% of salmonellae were resistant to 5–12 antibiotics
- swine 3–18%
- chicken 4–61%
- beef 3%
- Estimated U.S. morbidity/mortality per year 1,900,000/2,000

**C. perfringens**
- heat labile and stable strains
EMERGING CHALLENGES AND CONCERNS

poultry—up to 60%
veal—up to 80%
generation time at 106–113°F @ 7–8 minutes is one of the fastest known
Estimated U.S. morbidity/mortality per year 10,000/?
Yersinia enterocolitica
grow well 40°F
especially in swine >9%
Estimated U.S. morbidity/mortality 3,200/?
Escherichia coli
EPEC, EIEC, ETEC, EHEC
EHEC 0157:H7 = Hemolytic uremic syndrome
EHEC only one from animals and statistically from beef
Estimated U.S. morbidity/mortality per year 50,000/?
Toxoplasma gondii
oocysts in cat feces
bradyzoites in meat
pigs 30%
sheep 20%
1 tissue cyst = 1,000 bradyzoites
158°F kills
Estimated U.S. morbidity/mortality per year 3,000 newborns with severe pathology. Of these 1,500 from pork
Listeria monocytogenes
grows well at 39°F
beef 15–20%
broilers 15–80%
Estimated U.S. morbidity/mortality, 1,700 cases in 1986 with 450 deaths and 100 still births
Aeromonas hydrophila
grows well 33 to 39°F
can compete with background bacteria
poultry 98%
other meats 70 to 100%
Vibrio vulnificus
fulminating septicemia—ingestion
progressive cellulitis—wound
U.S. morbidity/mortality in 1988 were 45 cases reported with 17 deaths (25 & 11 from raw oysters)
Chronic Implications of Enteric Bacterial Pathogens
A wide variety of degenerative diseases have been linked recently to foodborne pathogens. These include endocarditis, colitis, food allergy, arthritis, rheumatoid conditions, autoimmune disorders, neoplasia and various chronic enteric inflammatory/hyperplastic diseases.
There is considerable evidence that ankylosing spondylitis is caused by
permanent genetic alteration of human tissue due to bacterial plasmid transfer by these organisms. Belgium research considers Yersinia to be their leading cause of acute and chronic joint disease. Of 18,000 salmonellosis patients from Chicago in 1985, over 3-1/2% have now developed rheumatoid problems.

Implicated in these chronic sequelae are Yersinia spp., Salmonella spp., Campylobacter jejuni, Shigella dysenteriae/flexneri, Escherichia coli, and Klebsiella pneumoniae.

Other chronic plus acute implications for the future include the fact that there will be millions of immunocompromised people due to AIDS, transplants, cancer therapy, etc. all of whom will be susceptible to diseases that heretofore have caused disease only in food animals.

Diarrheal episodes and diarrheal disease are often considered to be acute events of limited duration; a review of current literature indicates that this is not true. According to Dr. Douglas Archer,

Diarrheal episodes caused by many bacteria, viruses, protozoans and other parasites cause alteration of intestinal structure and function. Consequences of such diarrhea-associated gut alterations include loss of normal defense mechanisms against secondary opportunistic pathogens. Additionally, loss of endogenous nutrients and malabsorption of essential nutrients result from diarrheal episodes; the consequences of such losses, even of a single essential nutrient, is compromised immune function, which predisposes to further infection. The net result of such events in some persons is long-term debilitating diseases(s) such as allergy, autoimmune disorders and neoplasia.

Economic Loss Due to Enteric Disease

According to Dr. Sanford Miller, former Director FDA Center for Food Safety and Applied Nutrition,

More careful attention must be paid to microbiological concerns, describing problems of assuring microbiological safety as the most perplexing and hazardous problems of food safety. In presenting his case, Miller noted that more than 81 million cases of diarrhea of foodborne origin probably occur in the U.S. every year, and the estimated average costs for such food-associated illness are over $23 billion per year. The indirect costs to the economy from such illness, he added, may equal—or even double—these direct patient-related costs.

We're not talking here as has been said in the past, that, “foodborne illnesses are a little cathartic that is periodically good for you.”

REFERENCES

EMERGING CHALLENGES AND CONCERNS


Disseminated Mycobacterium bovis infection from BCG vaccination of a patient with AIDS. MMWR 1985: 34: 227–228.


REPORT OF THE COMMITTEE ON FOOD ANIMAL HYGIENE

Chairman: Dr. A. W. Bailey, Oklahoma City, OK

Vice Chairman: Dr. J. L. Blair, Annandale, VA

Dr. D. M. Bedell, GA; Dr. L. G. Billingsley, CA; Dr. C. W. Carraway, NC; Dr. W. J. Charminski, WV; Dr. G. E. Dittberner, CAN; R. W. H. Dubbert, VA; Dr. R. Ford, DC; Dr. C. S. Johnson, TX; Dr. J. B. Kaneene, MI; Dr. C. C. King, SC; Dr. J. C. Leighty, MD; Mr. M. M. Mamminga, IA; Dr. B. Mathis, AZ; Dr. C. S. McCain, OK; Dr. F. U. McCasland, TX; Dr. C. O. McCullough, VA; Dr. F. C. Okino, IL; Dr. J. C. Paige, MD; Dr. J. R. Priester, AL; Mr. L. D. Woodson, KS.

The Food Animal Hygiene Committee was called to order by Chairman, Dr. Alfred W. Bailey at 1:30 PM, October 30, 1989. Thirty-one (31) persons including thirteen (13) committee members were in attendance.

Dr. Janice Webb, Staff Officer, Residue Evaluation and Planning Division, FSIS, USDA updated the committee on several current residue issues. Two FSIS studies were done on roaster pigs comparing residues with those found in market hogs. Roaster pigs are defined as being 2 to 4 months of age, and 40 to 125 pounds in weight and are generally feeder pigs purchased for immediate slaughter. Roaster pigs appeared to have less residues of antibiotics, comparable levels of sulfonamides, and increased levels of carbadox. Roaster pig sampling is continuing in the FY 1990 residue sampling plan. An aflatoxin sampling survey was conducted on market swine. There were no aflatoxin residues found in 160 muscle samples; 8 samples were positive for aflatoxin but only one was above the 0.5 ppb enforcement level established by FDA. The Calf Antibiotic and Sulfadiazine Residue Test (CAST) program for bob veal was aided in reducing the level of residues from approximately 7% in 1978 to less than 3% in 1989. Improvements in the CAST program are currently under review by FSIS. Expanded laboratory testing has been done by FSIS on in-plant positive STOP tests (Sulfa Test On Premises). The findings indicate the need for development of a newer, more sensitive in-plant test for the detection of sulfonamides and antibiotics. Memos of Understanding (MOUs) represent voluntary programs between FSIS and industry with the primary objective of prevention of residues at any point at which they may occur in the production chain. Currently such programs cover 40% of the turkeys; 20% of the broilers; 3% of the fed cattle; and 0.1% of swine slaughtered in the U.S.

Dr. Ed Menning, Executive Vice President of the National Association of Federal Veterinarians gave a presentation entitled, "Microbiological Food-Borne Diseases . . . Emerging Challenges and Perspectives." There was widespread interest in the presentation and at the request of the Committee, the entire paper is being published in the USAHA proceedings.

Dr. Bill James, Chief, Project Management Branch, Slaughter Inspec-
tion Standards and Procedures Division, FSIS, USDA described a pilot program being tested in a poultry slaughtering plant. The study utilizes HACCP (Hazard Analysis Critical Control Point) principles and focuses on human pathogens and the microbiological safety of the finished product. Preliminary findings demonstrate that bacterial hazard control in the processing plant are measurably, but not completely effective. The scope of the study has now been expanded to include the farm environment in an effort to reduce the level of live bird contamination. A study of the effect of organic acids in feed is part of this expanded project.

Dr. Joe Blair chaired a discussion of various fish and seafood inspection bills and proposals currently being considered by Congress. There was a great deal of interest by state program officials in participating in a cooperative seafood inspection program, provided there were provisions for interstate shipment of such products.

The Food Hygiene Committee went on record in support of the Salmonella Committee resolution entitled: “Foodborne Diseases and Biosecurity/Disease Preventions Programs.”
LUMPY SKIN DISEASE
James A. House

Introduction. Lumpy skin disease (LSD) of cattle is caused by a capripoxvirus; and is closely related to the viruses which cause sheep and goat pox (SGP). The Office International des Epizooties considers LSD one of the “List A” diseases, which are “communicable diseases considered to have the potential for very serious and rapid spread, and which have the ability to result in serious economic loss.” The persistent spread of LSD, its entrenchment in endemic areas, and newly documented occurrence in Egypt dictate a renewed interest in this disease.

History. Lumpy skin disease was first described as “pseudo-urticaria” in Northern Rhodesia (Zambia) in 1929. Over a decade later, it was reported in Bechuanaland (Botswana) in 1943. The disease did not spread rapidly until 1944-45 when it was reported in Southern Rhodesia (Zimbabwe), South Africa, and in 1946 in Swaziland and Portuguese East Africa. It was estimated that 8 million cattle (75% of the population) in South Africa were affected with LSD by the end of 1946.

The first epidemic in Kenya in 1957 was associated with a high prevalence of mosquitoes. In 1970, cases of LSD were identified in the Sudan. Between 1971 and 1984 the disease appeared in Chad, Ethiopia, Niger, Nigeria, and the Ivory Coast. Kuwait, in 1986, was the first country outside of Africa to report LSD. In 1988 LSD was reported in Egypt. Figure 1 depicts the historic spread of LSD.

The infectious nature of LSD was described in 1945, and the justification for classification as a member of the poxvirus group was presented.

Characteristics of the Virus. The Neethling strain of LSD virus (LSDV) from South Africa is the prototype strain. The poxviruses are very large (300 to 450 x 170 to 260 micrometers) double-stranded DNA viruses. The molecular weight of their DNA is between 130 and 240 x 10^6 daltons. Poxviruses possess up to 30 structural proteins and several non-structural proteins. There are several major proteins, one of which cross-reacts with most vertebrate poxviruses. External filaments and membranes containing structural proteins can be observed by electron microscopy.

The capripoxviruses are ether-sensitive and do not produce hemagglutinins. Cross-protection studies between strains of LSDV, and between LSDV and strains of SGP virus (SGPV) could not distinguish these viruses from each other. Isolates of LSDV from a wide range of coun-

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*United States Department of Agriculture, Animal and Plant Health Inspection Service, Science and Technology, National Veterinary Services Laboratories, Foreign Animal Disease Diagnostic Laboratory, P.O. Box 848, Greenport, NY 11944
tries in Africa were serologically identical.\textsuperscript{14,28} Using agar gel immunodiffusion (AGID) with purified viruses, a major capripoxvirus immunoprecipitation line was noted from sera 15 days post infection (DPI); a second line usually appeared by 37 DPI and a third line was noted about 50 DPI with sera from severely affected animals.\textsuperscript{22} Animals severely affected with \textit{Parapox ovis} (contagious pustular dermatitis, also called ORF and contagious ecthyma) developed a line of precipitation to the first antigen recognized by convalescent capripoxvirus sera in the AGID test. Although the reactions were enhanced using S\textsuperscript{35} labelled antigens, the test could not distinguish between SGPV and LSDV.

Other workers using crude antigens noted 1 or 2,\textsuperscript{3,5} 2 or 5,\textsuperscript{29} and 6 or 8\textsuperscript{16} lines, the latter two reports claiming that the differences distinguished between strains of SGPV. The controversial and varied results of the AGID test make it of questionable diagnostic value. Restriction endonuclease (RE) studies on strains of SGPV and LSDV showed the close relationship between the capripoxviruses.\textsuperscript{6,18} Direct and indirect fluorescent antibody (FA) studies do not distinguish between the capripoxviruses.\textsuperscript{14}

\textbf{Cultural Characteristics.} LSDV replicates in numerous cell cultures.\textsuperscript{33} The cells most frequently used for isolation are lamb testicle (LT), lamb kidney (LK), calf kidney and calf testicle. The virus consistently grows ten-fold better in testicle than in kidney cells; maximum titers are achieved in 3 to 4 days.\textsuperscript{30} Fetal bovine lung (FBL) and ovine choroid plexus cells show comparable susceptibility to LT cells; the Madrin Darby bovine kidney (MDBK) cell line also supports the growth of LSDV.

Cytoplasmic inclusion bodies usually surrounded by a halo are seen with hematoxylin and eosin (H&E) stain in infected cells about 1 DPI. With Giemsa stain, the cytoplasmic inclusion bodies appear purple. Chromatin migrates toward the nuclear membrane leaving a vacuole in the center of the nucleus. Viral inclusions are observable in infected cell cultures with phase microscopy and by FA. Approximately 95\% of the virus in cultures is cell associated.\textsuperscript{30}

LSDV replicates in embryonating chicken eggs (ECE) without producing remarkable lesions.\textsuperscript{19,39} Optimal titers were obtained by inoculating 5 to 7 day ECE, incubating at 33.5 to 35°C, and harvesting after 4 to 6 days of incubation. Viral titers were highest in the chorio-allantoic membrane where small, white, non-hemorrhagic plaques were noted in 7 to 9 day old eggs.\textsuperscript{39}

\textbf{Stability of the Virus.} Poxviruses are notoriously stable; LSDV is stable over a pH range of 2 to 10 for 1 hour at 37°C and 14 days at 2 to 4°C.\textsuperscript{31} It is stable at 37°C for 5 days, 3 months at 25°C, and at least 6 months at 4°C at pH of 6.6 to 6.8.\textsuperscript{40}

\textbf{Epidemiology and Transmission.} Epidemics of LSD have been associated with the rainy season and locations (river basins) conducive to insect multiplication.\textsuperscript{12,15,19,24,40} The most common means of transmission in cattle is by insect bite. The LSDV was experimentally transmitted by \textit{Stomoxys}
LUMPY SKIN DISEASE

calcitrans but not by biting lice (Mallophaga), suckling lice (Damalinia spp), or Culicoides nubeculosus. Mosquitoes, predominantly Culex mirificus but also Aedes natronius, in heavy concentrations were observed during the first epizootic of LSD in Kenya and associated with transmission.

Virus shed in oculonasal discharges may account for a minor amount of transmission. Sheep and goats in contact with cattle affected with LSDV do not develop disease or antibody to LSDV or SGPV under field conditions. Likewise, cattle maintained in close contact with sheep and goats infected with SGPV do not develop disease or antibody to LSDV or SGPV.

*Incubation.* Depending on the dose of virus experimentally inoculated intradermally, a local reaction occurs in 6 to 20 days. One to 12 days after development of the local reaction, a fever may occur. If a generalized disease occurs, it usually begins about the second day of fever. The ability of the virus to generalize varied from 3 of 59 animals infected to 40 to 50%. This variation may reflect differences in animal susceptibility, viral strains, passage history, dose, or route of inoculation.

Under natural conditions the incubation period is from 2 to 5 weeks after exposure. Pathogenesis of LSD. Following intradermal introduction, the virus replicates locally in a wide variety of cells, predominately macrophages but also endothelial, epithelial, muscle, and nerve cells. Thus, LSDV is able to replicate in all of the 3 main embryonic cell types. The virus causes hydropic degeneration and swelling of cells, in early lesions, eosinophilic intracytoplasmic inclusion bodies (ICIB) are evident. Vasculitis, thrombosis, and viral induced necrosis result in central necrosis of the nodular lesion called a sitfast.

The stages in development of the LSD nodule are: 1) macule–typified by erythema; 2) papule–erythema plus edema; 3) vesicle—a stage not grossly evident in LSD; hydropic degeneration and coalescence of vacuoles are noted in epithelium histologically; and 4) scab or sitfast—the final stage of the LSD lesion where necrosis secondary infection with bacteria and scab formation is common. Tissues peripheral and deep to the sitfast organize into a connective tissue mass leaving a permanent scar.

During the replication of virus locally, the virus is cleared to local lymph nodes by macrophages. Viral replication results in lymph node enlargement (up to 10 times greater than normal size) caused by macrophage accumulation, lymphoid proliferation, edema, congestion, hemorrhage, and necrosis.

A viremia (mainly associated with macrophages) occurs, and the virus spreads to numerous secondary sites in the integument, respiratory, and urogenital systems.

*Host Range.* Lumpy skin disease by definition is a capripoxvirus induced disease of cattle. Sheep and goats can be experimentally infected with LSDV; a nodule indicating local viral replication may occur at the site of
intradermal inoculation, but generalized disease does not occur. Antibodies are induced which are cross-protective for SGP.\textsuperscript{6}

A survey of wildlife species from Africa found no antibody in 38 of 44 species and 6 with low prevalence.\textsuperscript{20}

Antibodies to LSDV but not to cowpox virus were found in the African buffalo (\textit{Syncerus caffer}) in forest areas adjacent to where LSD is endemic.\textsuperscript{12} In another survey, 1,413 African buffalo sera from sub-Saharan Africa were negative for antibody to LSDV.\textsuperscript{20} African buffalos have not been observed with clinical LSD. Two 3 week-old seronegative calves did not develop signs of disease when infected with LSDV.\textsuperscript{44}

Experimental infection with the Neethling strain of LSDV in a 4-month old seronegative giraffe (\textit{Giraffe camelopardalis}) and an impala (\textit{Aepyceros melampus}) a few weeks old caused the death of both with lesions typical of LSD. The impala had a significant rise in antibody prior to death.\textsuperscript{44}

Similarly, upon inoculation, a seronegative adult male black wildebeest (\textit{Connochaetes gnou}) did not develop clinical signs or antibody to LSDV.\textsuperscript{44}

\textit{Morbidity and Mortality}. The morbidity rate in cattle varies from 3 to 85\%; mortality is usually 1 to 3\%.\textsuperscript{1,7,19,24,40} There are reports of high mortality up to 75\% (Diesel 1949), but it was not clear what complicating factors may have been involved. Immunosuppression could allow this versatile, pantropic pathogen to cause severe disease. Contamination of an anaplasmosis vaccine of bovine blood origin with LSDV caused disease in 493 of 1,260 vaccinated cattle, and 111 died.\textsuperscript{15}

\textit{Clinical Signs}. The clinical signs of LSD have been described by numerous authors.\textsuperscript{7,6,19,24,40} The first clinical sign is a swelling in the skin (nodule) up to 3 to 4 cm in diameter, followed by a fever (104 to 106°F) which may persist for up to 3 weeks. Increased salivation, ocular nasal discharge, depression, and anorexia occur prior to and concurrent with the development of the skin lesions. The name LSD comes from the most distinctive clinical sign produced by LSDV—the skin nodule. Early skin lesions are raised, and the hair may stand erect. These deep intradermal nodules, which may be from 1 to 6 cm in diameter, occur essentially anywhere on the body but especially in the skin of the muzzle, nares, eyelids, lower ear, perineum, tail, and the oral and nasal mucosa. The nodules may resolve or become necrotic to form a sitfast.

Enlargement of the superficial lymph nodes such as the prescapular or prefemoral is evident especially if skin is affected in their field of lymph drainage.

In severe cases, lameness is common as a result of lesions producing edema and inflammation of the tendon sheaths (tendosynovitis) and the joints (synovitis). The virus may also produce pox lesions on the coronary band and in the laminar tissue of the feet.
LUMPY SKIN DISEASE

Involvement of the testicles as well as a persistent fever may produce temporary or permanent sterility. Abortion may occur, likely the result of prolonged fever. Decreased milk production and dramatic weight loss are common. Complications by secondary bacterial infections may cause abscess formation and discharge from lesions.

Necropsy Lesions. The gross pathology of LSD has been reviewed and described. At necropsy skin nodules are congested, edematous, hemorrhagic, and necrotic, and involve all layers of the epidermis and dermis; adjacent muscular involvement is common. Peripheral lymph nodes are often greatly enlarged up to 10 times greater than normal size.

The mucous membranes of the nares and the turbinates may have proliferative pox lesions up to 3 cm in diameter which may coalesce. Pox lesions are common in the mucosa of the oral cavity, pharynx, and larynx. Mucosa of the epiglottis and trachea commonly show pox lesions. Retropharyngeal and submandibular lymph nodes may be greatly enlarged.

The lungs are variably affected in animals with generalized disease. Randomly distributed focal pox lesions, which are congested and edematous nodules, may be palpated. Lobular atelectasis may be due to occlusion of bronchioles by proliferative lesions. Pleuritis with vasculitis may occur. When lung involvement is present, the mediastinal lymph nodes are enlarged.

Tendosynovitis may be accompanied by edema and congestion of adjacent tissues and excess synovial fluid, and fibrin may form in joints. Pox lesions may occur in the kidneys and in the mucosa of the urinary bladder.

FIELD DIAGNOSIS

LSD has 3 salient features which aid in its diagnosis: 1) random distribution of skin lesions; 2) skin nodules which are deep and involve all layers of the epidermis; and 3) the development of necrotic sit fasts. Diseases to consider for differential diagnosis include bovine herpes mammillitis, urticaria, insect bites, *Hypoderma bovis* infection, photosensitization, detmatomycoses, and streptothricosis.

Sample Collection for Virus Isolation. Samples should be aseptically collected, sealed in sterile leak-proof containers, and sent immediately to the laboratory on wet ice. If greater than 48 hours is required to reach the laboratory, freeze and ship under dry ice being certain to tape the screw caps to prevent CO₂ from entering the containers. Collect the following: 1) biopsies from the periphery of at least 2 early skin lesions from at least two acute cases; this may be done surgically, preferably with a 6 or 8 mm diameter biopsy punch; 2) biopsy from an enlarged lymph node; obtain by aspiration with a 16 gauge needle; and 3) heparanized blood taken early in the disease from a febrile animal.

Sample collection for pathology. Collect samples and fix in neutral buffered 10% formalin: 1) biopsies—collect as in #1 above; 2) from necropsy—a
HOUSE

full set of organs with special emphasis on collection of those organs having
lesions, e.g., skin, lymph nodes, turbinates, tracheal mucosa, lung, etc.; and
3) for transmission electron microscopy (TEM), a skin biopsy at the edge of
an acute lesion can be sliced 1 mm thick and fixed in 10% buffered formalin
but preferably in 2% glutaraldehyde in phosphate buffered saline. Sections
may also be selected from areas of paraffin blocks which have inclusions
and early lesions when viewed histopathologically. These may be sectioned
for TEM and usually yield evidence of capripoxviruses.21

A sample preserved on wet ice may be processed for negative staining
following lysis of cells with distilled water to release LSDV. The virus is
adsorbed onto grids, stained with phosphotungstic acid and examined.17
Demonstration of a pox virus by EM from a bovine skin nodule along with
the appropriate clinicopathological history is diagnostic of LSD.

Sample collection of serology. Collect serum from animals in the acute
phase (fever with early nodules), chronically affected (sitfasts present), and
21 to 28 day convalescent sera from respective acute cases for virus neu-
tralization (VN), neutralization index (NI), or indirect fluorescent antibody
(IFA) serological studies.

LABORATORY DIAGNOSIS

Virus Isolation. Stranding of infected cells (see cultural characteristics)
is typical of LSDV. The virus may be identified by neutralization with a
reference serum. Using constant-serum and varying-virus (neutralization
index), a reduction of 1.5 logs or greater by specific immune serum iden-
tifies the virus as a capripoxvirus. Association with a history compatible
with LSD supports the identity as LSDV. In endemic areas where LSD and
SGP occur, laboratories must take precautions to avoid cross-contamination
of cultures since routine tests cannot differentiate these viruses. Definitive
identification of LSDV is a previously free area where SGP occurs should
be done by specific virus neutralization followed by inoculation of sero-
negative cattle and production of generalized LSD to differentiate LSDV
from SGPV.

Microscopic Pathology. The histopathological lesions of LSD have the
following key features: 1) ICIB in early lesions 2) a significant vasculitis
with thrombosis in the dermis, and 3) necrosis of dermal tissue. Electron
microscopy reveals pox viruses approximately 300-400 x 170-260 nm upon
negative staining or within the cytoplasm of infected cells by TEM.

Serology. Convalescent serum having a NI of 1.5 logs are considered
positive for antibody to capripoxvirus. Virus neutralization titers of 1:4 or
greater against 100 TCID50 of LSDV indicate infection with capripox-
viruses.13 Specific fluorescence with IFA using sera diluted 1:5 or greater
indicates infection with capripoxvirus.13 Because of the equivalent sero-
logical reaction of antibodies between LSDV and SGPV using VN, NI, and
IFA, these reactions must be associated with a history of clinical LSD to
have significant meaning. Care should be taken to note vaccination history
since LSD vaccines and SGP vaccines, which are used to control LSD, elicit antibodies to LSDV and SGPV.

PREVENTION AND CONTROL.

Control of insects may aid in diminishing exposure to LSDV. Use approved insecticides and repellants as recommended by the manufacturer. Restriction and control of animal movements and importation is important since cattle with lesions and scabs serve as the source of virus for insects or environmental contamination.

An attenuated LSD vaccine has been developed by workers in South Africa by passage of the virus on the chorio-allantoic membrane of embryonating chicken eggs 20 passages and later in lamb testicle cell cultures (60 passages). Antibodies which last at least 3 years are induced with 5,000 to 10,000 TCID$_{50}$ of virus. Following vaccination, swelling may occur at the site of vaccination, but generalized disease has not been reported.$^1$

Vaccines for SGPV have been used successfully to control LSD in Kenya,$^9$ taking advantage of the close antigenic relationship of the capripoxviruses. The SGPV strain was not attenuated for sheep and goats but apparently did not spread from cattle to contact sheep or goats. Countries free of SGP should consider this particular vaccine as a potential source of infection for sheep and goats with this agent.$^{11}$ However, attenuated SGP vaccines may not present the noted risk.

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LUMPY SKIN DISEASE


Figure 1. The geographical distribution of lumpy skin disease; from the “OIE Disease Information,” Vol. 1, No. 1, 1988), updated and modified (printed with the permission of O.I.E.).
NECROTIC HEPATITIS OF RABBITS:
A FATAL NEW PARVOVIRAL DISEASE OF RABBITS

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SUMMARY
A new disease of rabbits, first called rabbit hemorrhagic disease syndrome, has been reported in the People's Republic of China since 1984. Since 1986 it has been reported in most countries of Europe by such names as necrotic hepatitis of rabbits (NHR) and X disease. In 1989, the disease spread rapidly through 13 states in Mexico. Domestic rabbits die in 2 to 3 days with few clinical signs except terminal epistaxis and sometimes convulsions. Mortality may reach 80–90%. Primary sites of replication are in the small intestine crypt and villous epithelium, hepatocytes, and splenic lymphocytes. Necrosis with fibrin thrombi as a result of a terminal coagulopathy is found in many organs. The similarities of the disease described in Mexico to Chinese and European outbreaks and the evidence for a parvovirus etiology are summarized.

INTRODUCTION
A disease of domestic rabbits causing high morbidity and mortality was first reported in the Nanking region of the People's Republic of China in 1984. The disease spread rapidly, killing 470,000 Angora rabbits by July of that year. Since 1984, the disease spread to northern China and as far west as Tibet. The disease was reported in Korea in 1985 and spread throughout the country by 1987. Until 1988, the disease was not reported outside of China and Korea. However, in 1988, an identical disease syndrome was reported in many countries of Europe, including Czechoslovakia, East and West Germany, France, Italy, and Spain. A similar disease was reported from Sweden called European Brown Hare Syndrome,4 which was first suspected to be caused by a severe hepatotoxin. Personal communications with Spanish investigators indicate that the European hares are dying of this disease in the wild and that raptors and other wild predators are starving due to the loss of prey.

There are nearly as many names for this disease as outbreaks. They include: rabbit viral haemorrhagic disease,10,20 rabbit plague,3 rabbit viral haemorrhagic pneumonia,16 rabbit viral septicemia,18 rabbit viral sudden death,9 picornavirus hemorrhagic fever,1 X disease of rabbits,2 infectious necrotizing hepatitis,11 and necrotic hepatitis.5

The loss of rabbits in various outbreaks has been staggering. In Korea, 150,000 rabbits were reported lost between 1985 and 1987.9 In Mexico, where an eradication campaign has been underway since February 21, 1989, over 65,000 rabbits have died and nearly 100,000 have been killed to
control the disease. Similar large numbers of rabbits have been lost in Italy and other European countries. One of the most devastating effects of this disease has been the loss of entire research colonies of rabbits in China.

The spread of the disease from country to country has been suspected to be due to trade in rabbits, rabbit meat, and furs. The Korean outbreak is suspected to have come from China through trade in rabbit fur. The source of the Mexican outbreak was traced to the importation of 18 metric tons of rabbit meat from China through the United States to a supermarket chain outside Mexico City. The outbreak in Germany may have been due to trade in Angora rabbits with China. It is even possible that the disease may have been in Germany, perhaps in wild hares, before 1984. Since there is virtually no regulation of live rabbits or rabbit meat and fur commerce between most countries of the world, including the United States, continued spread of this disease seems likely.

The signs of disease in all these outbreaks are similar. Predominantly young adult and adult rabbits die suddenly after 6–24 hours of fever with few clinical signs. Most rabbits appear depressed in the final hours and may show a variety of neurologic signs including excitement, incoordination, opisthotonus and paddling, and sometimes emitting a terminal squeal. A few rabbits may have terminal epistaxis. Gross pathologic lesions include congestion and hemorrhages in the lungs, a fine lobular pattern of necrosis in the liver resulting in a pale liver, and splenomegaly. The latter two lesions, though most consistent, are subtle. Hemorrhages in many other organs have also been reported leading to a confusing list of lesions attributed to this disease.

**ETIOLOGY**

A virus has been purified from infected rabbit tissue from most major outbreaks. The liver, spleen, and blood have yielded sufficient virus for negative stained preparations for electron microscopy. Most workers report finding a non-enveloped, roughly spherical viral particle with small surface projections. There are differences in the size of the virus reported by different workers. These have ranged from 25–40 nm, with most reports closer to 30 nm. Our measurement with 1% phosphotungstic acid staining at pH 8.5 using a Zeiss EM10 electron microscope have been between 25 and 30 nm. The smaller particles appear to be empty capsid structures which account for about half the population. The liver has yielded the highest concentration of virus, and most workers have used extract of this organ for virus purification. In our experience, this has been a difficult procedure with the end product often contaminated with large amounts of protein, ribosomal RNA, and nuclear DNA. Results of virus and nucleic acid extraction from different laboratories vary widely. Whether the virus contains single stranded RNA or DNA is still debated. Likewise, the classification of the virus varies. Korean workers and early reports from China have called it a picornavirus, while German, Italian, and Spanish workers believe it to be a calicivirus. Our work and more recent reports...
NECROTIC HEPATITIS OF RABBITS

from China indicate that it is caused by a parvovirus.

Difficulties in the identification of this virus stem mainly from the fact that no method has been found to cultivate this virus in vitro. Many different primary cells and cell lines have been used without success. Only rabbits have been found to be susceptible to infection. Therefore our investigations to identify the virus have been done using infected blood, and liver and spleen tissue sections, and the hemagglutination inhibition (HAI) test.

Our investigation of the agent causing the Mexican outbreak has concentrated on light and electron microscopy and immunostaining of infected tissue sections to complement HAI Serologic tests using monoclonal antibodies to many paroviruses. These results are reported elsewhere and will be briefly summarized here.

Transmission electron microscopy of liver, spleen, and intestine demonstrated intranuclear inclusion bodies in hepatocytes, splenic lymphocytes, and small intestinal crypt epithelial cells. Adjacent to these dense globular inclusions were linear and paracrystalline arrays of 25 nm empty capsid structures. Virions were found free in the cytoplasm of these cells and in cytoplasmic vacuoles. This represents the typical morphogenesis of a parvovirus.

Immunostaining of cryosections of infected rabbit spleen using the avidin-biotin complex-alkaline phosphatase (ABC-AP) indirect method was used to compliment HAI tests for serological identification of the parovirus. These results indicate that the virus is most closely related to porcine parovirus and minute virus of mice, a murine parovirus. It is not closely related to the endemic lapine parovirus or the bovine or carnivore paroviruses.

PATHOLOGY

An extensive description of the lesions and pathogenesis is in preparation and are summarized here. The hepatic lesion is most consistently found even in rabbits killed early in the infection. There is severe diffuse portal hepatic necrosis with cytoplasmic and nuclear swelling with margination of the chromatin. Small multiple intranuclear inclusion bodies can be found in the swollen nuclei. Only the centrolobular hepatocytes are spared resulting in the characteristic gross pattern of pale necrosis outlining each lobule.

Another common lesion which has not been previously reported is necrosis of the small intestinal crypts and villi. This may vary in severity and is often a segmental lesion involving only one area of the small intestine. The spleen is also variably affected with necrosis in the red pulp and marginal zone. The liver, small intestine, and spleen seem to be the primary target tissues of this virus, and viral replication in the nucleus of affected cells has been confirmed by electron microscopy and immunostaining.
The complicating pathologic change which seems to be the cause of the sudden death in NHR is a severe disseminated intravascular coagulopathy (DIC). It is rapidly progressive, resulting in acute thrombosis of major vessels as well as capillaries. Any organ may be affected, and the severity of vascular infarction varies. Pulmonary vein thrombosis is the basis of the pulmonary congestion and hemorrhage seen in many cases. Similarly, the terminal neurologic signs can be attributed to microthrombi in the brain. Frequently, there is massive infarction of the kidneys with both renal vein thrombi and diffuse glomerular thrombosis. In most fatal cases, the DIC results in fibrin deposition in the entire red pulp of the spleen, sometimes causing massive splenic necrosis due to infarction. The pulmonary and renal vascular thrombosis and fibrin deposition in the spleen are most remarkable and, though rarely seen in other diseases, are the result of DIC and are not directly attributable to viral replication. It is the hepatic necrosis which is so characteristic and consistent in this disease and, therefore, makes us feel that necrotic hepatitis of rabbits is the most descriptive name.

**DIAGNOSIS AND CONTROL**

Diagnosis of the disease is based on hemagglutination (HA) of human type O RBCs with extracts of infected liver, spleen, or blood. Immunostaining of the liver and spleen can be used to confirm the diagnosis. As many as 20–40% of rabbits in an outbreak may recover and can be diagnosed by serology using the hemagglutination inhibition (HAI) test.

We have shown that the virus is shed in the feces of infected rabbits. Fecal contamination and any blood or infected tissues seems to be the most likely source of infectious virus. Aerosol transmission is not likely to be an important means of disseminating this virus unless feces or blood are aerosolized during cleaning operations. The virus is very stable in the environment as is typical of other paroviruses. Therefore, thorough cleaning and disinfection of all equipment, cages, houses, and vehicles with which infected rabbits have contact is essential for the control of an outbreak. Disinfectants which have been used to control NHR include One-Stroke Environ,™ Vanadine,™ formalin, and household bleach. Extensive inactivation studies have not yet been done due to the lack of an *in vitro* system to assay the virus.

Control of the disease in countries where it is now endemic has been based on strict sanitation and maintenance of closed rabbit colonies. Vaccination has been used to control outbreaks in China and some countries of Europe using a formalin inactivated extract of infected liver. The vaccine offers protection for about 6 months. Field observations and our preliminary laboratory results suggest that recovered domestic rabbits may carry and shed the virus in the feces for up to a month. Therefore, recovered rabbits may serve as a source of new outbreaks and should not be introduced into unaffected colonies.
NECROTIC HEPATITIS OF RABBITS

The effect of NHR on wild populations of rabbits is still in question. It is likely that this disease infects European brown hares (*Lepus europaeus*) and the varying hare (*Lepus timidus*). Recent personal communications indicate that wild rabbits are dying in Spain. Mortality rates in the wild rabbits are unknown, but the losses have been significant and raptors are starving as a result. In our preliminary infectivity studies, cottontail rabbits (*Sylvilagus* sp.) did not show clinical signs of disease, but a domestic rabbit put in the same room with them a week later did die. This suggests that cottontail rabbits may shed the virus, at least for a short time. A similar study done with Volcano rabbits (*RomeroLagus diazzi*) did not result in clinical disease. Further studies need to be done on hares (*Lepus* sps.) in the United States, including several species of jackrabbits and snowshoe hares which are in the same genus as European brown hares.

CONCLUSIONS

Necrotic hepatitis of rabbits is a new and devastating viral disease which has recently emerged from the family of Parvoviruses much as canine parvovirus appeared in the 1960s. The canine virus was believed to have mutated from feline panleukopenia virus. In this instance, NHR virus probably mutated from the procine and rodent subgroup of parvoviruses. In any case, NHR is rapidly sweeping the globe causing major epizootics in the naive domestic rabbit populations. As in the 1960s with the canine epizootic, the disease is spreading virtually unchecked in a species which can move across international borders almost without restriction. Since the United States has practically no restriction on live rabbit importation and little control over rabbit meat or fur commerce, we can expect this disease to enter the United States soon. It is likely to occur first among pet rabbit breeding stock, due to the high mobility of these rabbits and their risk of exposure on the show circuit. It will be a major challenge to our veterinary animal disease specialists to try to control its spread. Though the USDA has little authority to regulate rabbit diseases, there will be pressure to take steps to control the death losses, especially when NHR threatens the highly valuable medical research rabbit colonies throughout the United States.

REFERENCES


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PATHOGENESIS OF FOOT-AND-MOUTH DISEASE IN GUINEA PIGS USING IN SITU HYBRIDIZATION

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Foot-and-mouth disease (FMD), caused by an aphthovirus (Family Picornaviridae), is a highly contagious disease of cloven-hoofed animals which causes severe economic losses when introduced into non-endemic areas. The pathogenesis of FMD has been the subject of numerous investigations using a variety of techniques including histopathology, virus isolation, and fluorescent antibody (Burrows et al., 1981; Sutmoller and McVicar, 1976; McVicar and Eisner, 1983; Yilma, 1980). Results have been inconclusive and sometimes contradictory with respect to delineating primary sites of viral replication. The recent development of in situ hybridization (ISH) as a tool to identify FMD virus (FMDV) in cell culture prompted the initiation of this pathogenesis study to try to determine preferred locations for viral replication.

Five adult guinea pigs were inoculated intraepithelially in the right hind foot pad with $10^6$ TCID$_{50}$ of FMDV Asia I. Animals were euthanized with carbon dioxide at 0, 4, 10, 24, 48, and 72 hours postinfection (hpi). Selected tissues (both hind foot pads, left front pad, tongue, spleen, heart, lungs, and pancreas) were collected in 4% paraformaldehyde for histopathology and in OCT over liquid nitrogen for in situ hybridization. A portion of left hind foot pad was collected from every animal for virus isolation.

By 24 hpi, guinea pigs were depressed and inappetent, with vesicles forming at the site of inoculation. By 72 hpi, vesicles were forming on the contralateral (noninoculated) hind foot pad.

Histologically, the most dramatic lesions were in the inoculated foot pads which by 24 hpi had multifocal vesicle formation. At 72 hpi, extensive mature vesicles were present on the contralateral hind foot pad.

Virus isolation from the noninoculated foot pad confirmed the presence of virus only at 24, 48, and 72 hpi. All other samples were negative for the presence of virus.

The probe for in situ hybridization was 500 base pairs of biotinylated RNA, from the section of the genome encoding for the enzyme polymerase. Results of in situ hybridization are presented in Table 1.

Viral nucleic acid was detected with certainty in the noninoculated fore and hind foot pads as early as 10 hpi, well before any histologic changes associated with FMDV infection. The pattern of hybridization was segmental, with a group of several cells in a given area of the lower stratum spinosum staining positively whereas adjacent similar areas were free of
positive reaction. These tissues remained consistently positive for the presence of viral nucleic acid up to the end of the experiment at 72 hpi. At this time, in the fore pad, even though virus had first been detected with certainty in that tissue 62 hours previously, there was still no microscopic evidence of FMDV-induced damage in the histologic section. Similarly, tongue tissue was positive by ISH at 4, 48, and 72 hpi and yet there was never any microscopic evidence of degeneration or vesicle formation. Research in cattle has demonstrated that the virus is widely disseminated to many areas of epidermis, with lesions developing only in areas subjected to mechanical trauma or unusual physiologic conditions (Gailiunas and Cottral, 1966; Gailiunas, 1968). This would explain why lesions are most likely to develop on tongue, interdigital cleft and teat. It may be that the guinea pigs, once they became depressed and inappetent, were reluctant to move about the cage or eat, so that although virus was present in the fore foot pad and tongue, there was insufficient physical activity to result in the development of vesicles.

A surprising result was the presence of positive hybridization reactions in the cytoplasm of alveolar macrophages and selected epithelial cells of lung. This reaction was weak at 4 hpi and progressed to strong positive staining by 24 hpi. It appears that in the guinea pig the lung is a site for early viral replication. In livestock the role of the lung in early replication of FMDV has been the subject of numerous investigations. Some workers believe that alveolar macrophages may serve to transport inhaled, replicating virus into the circulation (Sutmoller and McVicar, 1976) whereas other studies have shown that the lung is not important in early replication and dissemination of virus (Burrows et al, 1981). In either case, it should be noted that whereas it is well-established that livestock are readily infected with FMDV by aerosol exposure, guinea pigs cannot be infected in this manner. Consequently, the replicating virus detected in the lungs of these animals was derived from the systemic circulation.

In summary, ISH demonstrated the presence of FMDV in a number of epidermal and visceral tissues very early in infection, well before the onset of clinical disease or pathologic lesions. It appears that, in the guinea pig, the virus is widely disseminated to foot pads and tongue, with epidermal lesions resulting only in areas subjected to physical stress. In addition, ISH indicated that the lung is a target organ for early viral replication in the guinea pig. This technique could prove useful in further exploring the pathogenesis of FMD in the natural hosts.
Table 1. Results of *in situ* hybridization on guinea pigs inoculated with FMDV

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± equivocal staining; slight increase over background  
+ weak positive staining  
++ positive staining  
+++ extensive positive staining  
NA tissue not available for ISH

REFERENCES


UNCONTROLLED MOVEMENT OF GERM PLASMA IN
LATIN AMERICA, THE DANGERS, PROBLEMS, AND
SUGGESTED SOLUTIONS

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In the next twenty minutes I will address a subject which has been swept under the rug for at least twenty years. I am referring here to the smuggling of germ plasma, in particular of bovine semen in Latin America.

The import of cattle, semen or embryos from areas where exotic animal diseases exist may cause the introduction of such diseases into the livestock population of the importing country.

There are two main approaches to avoid the risk of such disease transmission. The first one is the “zero risk” policy, which relies on the total import prohibition of animals or animal products from countries which have one or more foreign animal diseases. To be effective, this policy requires a strong, well informed customs service and animal health force at all ports of entry, efficient baggage inspection facilities, and incinerators at airport and harbors. Its success depends on the collaboration of the livestock industry and an informed general public.

The “zero risk” policy appears to avoid all risks of disease introduction; however, if the demand for the foreign germ plasma exists, illegal importation of semen or embryos will occur. This situation creates two serious problems:

1. In the absence of sanitary measures and official control there is a grave risk of animal disease introduction.

2. The absence of official certification creates serious doubts as to the origin of the genetic material. The registration of the resulting offspring is either left blank or made under false parentage, jeopardizing the livestock improvement programs in the importing country.

The second approach to prevent the introduction of a foreign animal disease through germ plasma importation is based on a series of sanitary measures which have an accumulative effect with regard to decreasing the risk of disease introduction. The risk which is deemed acceptable must be weighed against the expected benefits of such importation of breeding stock, semen, or embryos.

The “International Animal Health Code” of the Office International des Epizooties (OIE) contains detailed animal health norms for the international movement of animals and animal products. The objective is to decrease the risk of animal disease introduction through imports to an acceptable minimum.

The International Embryo Transfer Society (IETS) has published manu-
als for the import/export of bovine embryos. These manuals describe procedures and techniques to make this form of international exchange of genetics the safest form with regard to disease transmission.

The South American countries maintain a zero risk policy with regard to the importation of Zebu genetics from the Indian subcontinent. The countries north of the Colombian-Panama border have done the same for genetics which originated from FMD countries south of that border and other countries that they consider FMD infected. However, sectors of the livestock industry in most tropical and subtropical areas of the Americas—justifiable or not—perceive a need for the importation of genetics which has resulted in a significant uncontrolled movement of germ plasma: (1) germ plasma from the Indian Subcontinent to South America and (2) germ plasma from South America to the Panama, Central America and Mexico.

1. GERM PLASMA FROM THE INDIAN SUBCONTINENT TO SOUTH AMERICA.

Because of the existence of exotic types of FMD, rinderpest, and other agents not occurring in South America, all importations of cattle and bovine semen and embryos are prohibited. During the sixties, because of strong pressure from the Brazilian livestock industry, a limited number of Zebu cattle were imported under official government control from India. However, in spite of the official import prohibition important amounts of contraband semen were imported. Anyone who has visited a cattle show in Brazil will recall Zebu cattle decorated with Indian necklaces receiving enthusiastic applause! Some of the smuggled genetics also found their way to other South American countries.

The South American Commission for the Control of Foot-and-Mouth Disease (COSALFA) recognized the animal health risk of the uncontrolled semen importations from the Indian subcontinent and requested the Pan American FMD Center to prepare a guide for the purpose of formulating regulations and procedures for facilitating the importation of genetic material, thereby eliminating the risk in such operations. This guide should contain norms and procedures as well as references to the need for skilled personnel and physical installations (laboratories, quarantine areas, etc.).

In response to this request, the Center prepared a guide based on the OIE norms and the norms of the IETS with procedures and protocols for the consideration of the all South American countries.

At the XVI Annual Meeting of COSALFA in Cartagena, Colombia 30–31 March 1989, the draft of the guidelines was approved and the Panamerican FMD Center was requested to prepare a final guide for the importation of breeding stock, semen and embryos. This document will be available early next year and can be obtained from the COSALFA Secretariat, Centro Panamericano de Febre Aftosa. Caixa Postal 589, 20001 Rio de Janeiro, Brazil.
2. GERM PLASMA FROM SOUTH AMERICA TO PANAMA, CENTRAL AMERICA AND MEXICO.

The smuggling of bovine semen and embryos from Brazil to countries north of the Colombian-Panama border are of particular interest to the members of USAHA. I will discuss this flow of uncontrolled movement in some more detail.

In April of this year, the question of contraband of bovine semen and embryos and the resulting animal health hazards were discussed during a symposium on biotechnology in San Jose, Costa Rica, organized by the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), in collaboration with the University of Wisconsin. The discussion centered on recent advances in technology which would make it possible to import—with acceptable risk margin—bovine semen and embryos from FMD countries.

As a result the Technical Committee of OIRSA recommended that OIRSA with the assistance of the Panamerican FMD Center/PAHO and APHIS/USDA prepare a draft of standards for the international germ plasma trade for the Region. The Panamerican FMD Center was requested to prepare a project and budget for presentation to international funding agencies. The Center prepared the project and submitted it to PAHO and OIRSA.

Because of an urgent request from the Executive Director of OIRSA and the importance of the issue for the animal health situation for the Region, the Director of PAHO authorized the needed funds for the study. During July, August and September, Dr. Edwin Perez from Costa Rica and I served as consultants of the Panamerican FMD Center/PAHO on the project. Our terms of reference were as follows:

"Develop a draft protocol for the safe importation into the countries of OIRSA of germ plasma from South American countries affected by FMD. This protocol must take into account:

—The needs of the region for the importation of Bos indicus breeds within the framework of the official livestock improvement programs;

—recent advances in technology

—the need to guarantee the continuous freedom of FMD of the region.

The protocol must be simple, effective and practical in order to prevent illegal international movement of germ plasma, which can have disastrous consequences for the livestock industry in the region, in case a foreign animal disease is introduced."

We visited all Central American countries, as well as Panama and Mexico. Our terms of reference are stated in the copy of the project. We met with the veterinary officials and representatives of OIRSA and APHIS, and, whenever possible, with representatives of the livestock industry and importers of semen.
In each of the countries we reviewed the FMD prevention program and existing animal health regulations. We concluded that with the exception of Mexico, the countries did not have a well structured animal health system which would enable them to confront an emergency produced by the introduction of a foreign animal disease.

We discussed the needs for the eventual modifications of the regulations. We also tried to determine the perceived and real needs for the importation of the Brazilian genetics and the extent of the uncontrolled movement of semen and embryos. In each of the countries we asked the question: “Does the cattle industry need the Brazilian genetics?”

In Costa Rica and Guatemala the answer was definitely affirmative. The opinion of the official Mexican authorities was negative. They declared that Mexico has obtained sufficient genetic material of Brazilian origin from several sources and expressed the hope that Mexico soon will be in a position to export zebu genetics. We have reason to believe that this view is not fully reflective of the livestock industry’s opinion.

The livestock owners in Nicaragua are interested to obtain zebu genetics from Brazil, but the present economic crisis prevent the importation. The Director of the National Center for Genetic Improvement stated that his institute has the required embryo transfer technology and wishes to obtain Brazilian embryos for the production of sires for the AI station. The institute has the financial means to do this.

In Honduras there is not much interest for zebu genetics, due to the tendency to change from meat production to milk production. There was interest in the importation of genetics from EEC countries. Similar opinions were obtained in El Salvador and Panama.

During the last decade germ plasma from Brazilian origin has been imported into the tropical zones north of Colombia. Part of these imports have been through legal channel, such as the import of zebu cattle, which were moved through the Harry S Truman Import Center at Fleming Key, Florida. Although this was a USA importation, quite a few of these animals went to Mexico and some of them apparently to Guatemala. Semen was also imported by ABS from the Artificial Insemination Center PECPLAN/BRADESCO in Uberaba, Sao Paolo, Brazil. Some of it has found its way south of the USA border.

Illegal semen imports are apparently frequent as evidenced by the type of cattle displayed at cattle shows. Characteristically, at present, several of the judges at the shows are Brazilians. Among the Central American farmers there is a joke: “If one would play a samba at the cattle shows, most cattle would dance!”

Not many embryos are being moved clandestinely, the limiting factor being the present lack of expertise at the receiving end. However, quite soon, the required expertise will become available on a much larger scale.
Finally, we tried to develop protocols, which might be acceptable to the veterinary authorities of the Region and which offer an acceptable risk level compared to the large risks created by the smuggling of semen and embryos.

Because bovine semen and embryos differ considerably with regard to disease transmission risks, two separate protocols for semen and embryos were drafted. As a basis for the semen protocol we used the protocol of APHIS/Brazil for the importation of Brazilian semen into the USA. For the embryo protocol we used the IETS manual.

The safeguards in the protocol with regard to SEMEN are based on:
- Existence of favorable animal health conditions in the country and areas where the artificial insemination (AI) unit is located;
- Isolation of the bulls in AI unit, disease history and observation of the animals of the unit and particularly of the donor bulls;
- Serological testing and microbiological examinations to determine the absence of pathogenic agents of relevant diseases;
- Prevention of accidental contamination of the semen;
- Official certification that there were no disease outbreaks, including further laboratory tests, of the semen donors and the other animals of the AI unit, while the semen is in quarantine storage;
- Official observation of the inseminated cows and epidemiological surveillance of the area in the importing country.

The proposed draft protocol for semen differs from the protocol USDA/Brazil principally on three points:
1. The use of the so called VIA antibody test.

In this protocol the VIA test is not used to exclude individual bulls as semen donors, but as an epidemiological tool to monitor the possible unapparent spread of FMD in the herd of the AI Center as a whole. This change reflects the opinion of the Panamerican FMD Center for the correct use of the results of this test.

2. The Panamerican FMD Center is the Reference Laboratory for the diagnostic tests.

3. The semen is not fully liberated once all tests are satisfactorily completed, but its use remains under official supervision. An animal disease surveillance must be established in the area where the semen is used.

The safeguards of the protocol with regard to bovine EMBRYOS are based on:
- Existence of favorable animal health conditions in the country and area where the herd of the donor dams is located;
- The use of officially approved semen;
GERM PLASMA IN LATIN AMERICA

—Collection and handling of embryos by special accredited and registered embryo collection teams, with highly trained technicians;
—Treatment of embryos in accordance with the procedures and methods described in the Manual of the International Embryo Transfer Society;
—Testing of embryo collection and washing fluids by sensitive microbiological techniques, to demonstrate the absence of FMD;
—Prevention of accidental contamination of the embryos;
—Official certification that there were no disease outbreaks, (including further diagnostic tests of the embryo donor dams and, if necessary, of other animals of the farm) while the embryos were in quarantine storage;
—Official observation of the recipient cows and epidemiological surveillance of the area in the importing country.

With regard to the proposed embryo protocol the following observations can be made:

1. In addition to commonly used animal health considerations, the proposed draft protocol is based on the categorization of diseases by the IETS Import/Export Committee, 1989 which reads:

   “Based on a 1988 review by the Research Subcommittee of the International Embryo Transfer Society (IETS) Import/Export of available research and field information on infectious diseases that have been studied apropos the risk of their transmission through embryo transfer, the IETS has categorized those diseases as follows”

   “Category 1

   Diseases or disease agents for which sufficient evidence has accrued to show that the risk of transmission is negligible provided that the embryos are properly handled (Manual of the IETS) between collection and transfer:
   —Enzootic bovine leukemia
   —Foot and mouth disease
   —Brucella abortus (cattle)”

   This negligible risk refers to “worst scenario conditions.” The animal health requirements required by the proposed protocol will exclude such extreme conditions. However, I must mention that much of the bio-safety which is provided by embryo transfer depends on the expertise and seriousness of the Embryo Transfer Team.

2. The Panamerican FMD Center is the Reference Laboratory for the diagnostic tests.

3. The embryos are not fully liberated once all tests are satisfactorily completed. Their use remains under official supervision. An animal disease surveillance must be established in the area where the embryos are being used.

   At this stage the drafts of the protocols are being considered by the national authorities, OIRSA and APHIS. They will be presented and
discussed during the technical Committee meeting of OIRSA this November. The protocols will then be discussed and hopefully approved by the Region's Ministers of Agriculture. We do not think that acceptance of the proposed procedures will completely eliminate the clandestine movement of semen and embryos. However, we definitely hope that—in the near future—it will eliminate an important animal health risk.

In view of present conditions and the need to keep the OIRSA Region free of exotic animal diseases, we recommend that the veterinary officials of the Region modify the import regulations, taking into account the advances of technology. This relates particularly to the norms for the importation of genetics from FMD affected countries. However, the OIRSA member countries must maintain uniformity in their animal health regulations.

We recommend the strengthening of the animal health services of the OIRSA member countries with regard to their capability of response to the introduction of a foreign animal health emergency systems, similar to that of Mexico. A prerequisite for success in dealing effectively with the control and eradication of a foreign animal disease is the creation of an emergency fund to finance such operations.

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REPORT OF THE COMMITTEE ON
FOREIGN ANIMAL DISEASES

Chairman: Dr. J. L. Hyde, Ithaca, NY
Vice Chairman: Dr. W. W. Buishch, Scotia, NY

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The meeting of the Foreign Animal Diseases Committee opened with a presentation by Dr. Douglas Gregg of a paper developed by Dr. Corrie Brown on "The Use of In-Situ Hybridization for the Detection of Foot-and-Mouth Disease Virus in Cell Culture." (The full text of this paper is published elsewhere in the proceedings). It was reported that infection of tissue culture monolayers could be detected as early as two hours after infection. In contralateral non-innoculated foot pad epithelium, FMD-RNA could be detected as early as six hours post inoculation (about two days before vesicle formation). This RNA probe could be used to aid in the rapid diagnosis for FMD and for pathogenesis studies.

Dr. Donald O. Morgan discussed the application of Monoclonal Antibodies to the diagnosis of Foot-and-Mouth Disease (FMD). A monoclonal antibody (MAB) specific for a highly conserved "internal antigen" of the 12 s protein subunit (12 sps) of FMDV provides the basis of an inhibition ELISA (IHELISA) for the diagnosis of FMD. The antibody reacts with 12 sps for six of the seven FMDV serotypes (except SAT,) but did not react with any of the other animal viruses tested. This IHELISA does not involve infectious FMDV and can thus be used without the spread of contagion.

Dr. Werner Heuschele presented an update on research on malignant catarrhal fever (MCF) at the San Diego Zoo's Center for Reproduction of Endangered Species (CRES). Results were reported on molecular biologic studies conducted by Dr. Bruce Seal and Rob Klieforth. Preliminary findings of collaborative studies at the NVSL, Foreign Animal Disease Diagnostic Laboratory, Plum Island and at the San Diego/CRES facility
have revealed that at least one alcelaphine herpesvirus-1 (AHV-1) cloned DNA segment is able to detect, by blot hybridization, AHV-1 viral genome in infected cell cultures and in DNA extracted from peripheral blood lymphocytes of MCF antibody seropositive wildebeest, and discriminates this from other bovid herpesvirus DNAs.

Dr. James House reported a joint project has been completed between Tufts University and USDA (APHIS and ARS) on rinderpest vaccine. The attenuated Kabete O rinderpest vaccine (“Plowright Strain”), was adapted to the vero cell line. Studies on purity, immunogenicity, and safety were conducted in accordance with USDA requirements for licensing and all tests were satisfactory.

Studies on various stabilizers and lyophilization cycles resulted in a product stable up to 200 days at 37°C. The vaccine seed and cells are available for emergency use in the USA and for international distribution. The Pan African Rinderpest Campaign has adopted the production system and will utilize it in rinderpest control and eradication procedures in Africa. (The full text of this paper appears elsewhere in the proceedings).

Dr. Charles Mebus presented an extremely interesting paper on the current developments in attenuated Rift Valley Fever Vaccine Production. (The full text of this paper appears elsewhere in the proceedings).

This was followed by a presentation by Mr. Bert Hawkins on the concerns of the International Llama Association for the proposed quarantine procedures for the importation of llamas. Dr. Antonio Ramirez then reviewed the Foot-and-Mouth Disease situation in domestic camelids (Alpaca & Llama) in Peru. He noted that 80% of Peru’s camelids are in Southern Peru and are generally uncontrolled in their movement between Peru and Bolivia.

Dr. Roger Drummond presented a detailed paper on the problems associated with the implementation of the pilot tropical bont tick project. The project was initiated in September, 1987, with the signing of a PASA between USDA and USAID. Since then the information and evaluation component under the control of USDA-OICD has made progress in research on Antigua on wildlife hosts of the tropical bont tick by the Southeastern Cooperative Wildlife Disease Study; on economics of the tick and dermatophilosis by the University of Florida; and on the biology and control of the ticks on Guadeloupe by IEMVT (French Research Organization, SECWDS, and USDA-ARS). Unfortunately, little progress has been made on the pilot tick eradication program by USDA-APHIS on Antigua. Originally, the program was to use Amitraz and Atroban sprays, but since then Bayticol pour-on has been developed and is a highly effective tick control treatment. Unfortunately, it is not registered by EPA for use in the U.S. and is not permitted to be used by USAID. This year an environmental assessment team reviewed data used to register Bayticol pour-on in West Germany to see if it was sufficient to register it in the U.S. The team found that there were critical data gaps in oncogenicity, metabolism in soil, toxicity and
FOREIGN ANIMAL DISEASES

other areas. Thus, there has been no change in the status of the pilot eradication program on Antigua. The committee was seriously concerned that more progress had not been made in protecting our industries from this pest.

A report from the Vesicular Stomatitis Subcommittee was presented by Dr. Victor Nettles. A Subcommittee composed of Dr. Victor Nettles (Chair), Dr. M. A. Mixon, Dr. M. D. Salmon, Dr. Pat Smith, and Dr. Tom Walton reviewed an APHIS/ARS Committee report on Vesicular Stomatitis Research Needs that was prepared in January 1983 by Drs. Lonnie King, Jack Hyde, Ralph Bram and Graham Purchase. The Subcommittee concluded that limited progress since that time had been made in regard to natural virus reservoirs, ecological requirements for viral persistence, distribution of endemic foci, and mode/s of transmission. The Subcommittee predicted that we will not be any more effective in combatting the next vesicular stomatitis outbreak than we were the last time.

The Subcommittee encouraged that vesicular stomatitis research be addressed as a priority. In addition, the Subcommittee made the following suggestions:

1. USDA should make an attempt to retrieve the details of unpublished work on vesicular stomatitis that came in the wake of the last series of outbreaks in the western states, (1982–83). The information should be compiled and preserved in some form, possibly in the Foreign Animal Disease Report.

2. Cattle sera banked by the NAHMS program in recent years should be tested to help resolve the question about the possibility of endemic foci in the Western United States.

3. A team of epidemiologists should be pre-designated in order to be able to react to the next VS epizootic in a prompt manner.

4. Communications should be maintained between the USDA and biologics companies with VS vaccines in order that vaccines could be deployed with an absolute minimum start-up time if vaccines are to be used.

5. Laboratory capability to fingerprint various VS virus isolates should be maintained.

Dr. Roger Bram then discussed the facilities of the Plum Island Animal Disease Center and future construction as well as research activities currently underway.

Canada

Dr. William G. Sterritt, Health of Animals Directorate, Agriculture Canada then discussed the foreign animal disease activities in Canada. Canada has remained free of animal diseases foreign to their shores in fiscal year 1988–89 (April 1, 1988 to Nov. 31, 1989).

Lentogenic Newcastle disease virus continues to be sporadically isolated from commercial poultry flocks. In addition, an outbreak of Newcastle
disease in pigeons caused by a virus typed as mesogenic in chickens, occurred in southern Ontario in the summer of 1988.

Bluetongue seroconversion was observed during the summer in sentinel cattle in the southern Okanagan Valley of British Columbia. Testing of cattle in areas adjacent to the Valley revealed that the incursion was confined, as always, to the Okanagan.

Agriculture Canada is collaborating with the federal health department, Health and Welfare Canada to design and construct a new high containment laboratory complex in Winnipeg, Manitoba. Agriculture's component will house the foreign animal disease diagnosis, test development, antigen production and the operation of the exotic diseases course previously conducted at Gross Ile. The facility will give them about 4,300 net square meters of space when it is completed in 1995 at a cost of about $51.8 million Canadian.

Mexico

Foreign animal disease activities in Mexico were discussed by Dr. John Mason, Mexico-United States Commission for the Prevention of Foot-and-Mouth Disease and other exotic diseases of animals.

The activities of the Commission during 1989 were reviewed. Most of its efforts were devoted to the campaign to eradicate the outbreak of viral hemorrhagic disease of rabbits. Apart from this activity the Commission continued with its primary responsibility of conducting surveillance and investigations of cases or outbreaks suspected of being caused by foreign animal diseases. To date in 1989 some 180 routine investigations were carried out. In relation to the outbreak in rabbits some 10,700 reports were investigated.

Israel

A paper on exotic animal diseases of concern to Israel was then presented by Dr. A. Shimshony, Chief Veterinary Official of Israel.

During 1989, six exotic animal diseases have attracted special attention and required preventive or control steps of the Israeli Veterinary Services: American Screwworm, exotic types of FMD, African Horse Sickness, Rinderpest, Salmonella enteritidis in poultry and Lumpy Skin Disease.

In May 1989 the American Screwworm, Cochlyomia Hominivorax, was reported by Libya to the list B diseases of the O.I.E. This myasis has never been recorded in Israel.

Foot and Mouth Disease, type 01, was recorded between the months March and July in 1989 (5 outbreaks in Israel, and 5 in the controlled territories.) In June, an outbreak in a feedlot with 400 fattening calves, adjacent to the Lebanese border, was recorded.

In light of numerous cases of post-vaccinal allergic reactions of the delayed dermal type in dairy cattle, which had been encountered during
1988, it was decided to use BHK and Frenkel-method derived vaccines on alternate years. A field-trial with improved BHK vaccine is envisaged.

African Horse Sickness (A.H.S.) has not been recorded in Israel since 1944. In light of the deteriorating situation in the region, as reflected by the recent epizootics reported by Spain, Portugal, Morocco and the Arab peninsula, a census and registration of all horses is presently being carried out in Israel.

Rinderpest was absent from Israel between 1927 and 1983, when it penetrated from Lebanon and was quickly eradicated. In view of the recent report on rinderpest in Iran, and the continuing unstable situation in Lebanon, a warning has been sent to the practitioners and the cattlemen. Vaccination against rinderpest in Israel has been discontinued since mid 1985.

Salmonella enteritidis: In light of the animal and public health problem related to S. enteritidis in poultry, as reported in recent years in several countries, severe measures are being adopted to prevent its introduction into Israel.

During 1989, 5 imported breeding flocks, including 50,000 birds, were found to be infected and accordingly destroyed. In addition, 2 layer and 2 broiler flocks, which were the progeny of the said imported flocks, were destroyed as well. They included 40,000 birds.

Lumpy Skin Disease: undoubtedly, the most serious veterinary problem in Israel during 1989 was the first outbreak in this country of Lumpy Skin Disease. It involved one village, Moshav Peduyim, in the district Beer-Sheva which borders Sinai. During the first weeks, allergy was suspected to be the cause of the observed skin lesions. However, it was soon realized that the condition had spread to additional farms and that serious economic damage was involved, due to milk-yield loss, inappetance, loss of weight and probably some abortions and even mortalities. On August 19, suspicion of LSD was raised for the first time and the State Veterinary Services were called upon. In attempting to eradicate the disease and prevent the development of an endemic situation, a stamping out policy was adopted, 168 animals were killed and destroyed; this included 63 bovines with clinical symptoms, and 105 young calves. All other 464 animals in the 17 affected and unaffected farms in the village were slaughtered; offal and skins were condemned and destroyed. Severe animal movement restrictions in the Beer-Sheva district were enforced. Ring vaccinations with a locally produced sheep-pox vaccine, which is certified for use in sheep in Israel, were promptly carried out. Restocking of the farms will be considered at least 3 months after the depopulation, which was accomplished on September 29.

Dr. Alex Thierman reviewed the current organization of the International Services of the Animal and Plant Health Inspection Service and discussed progress in some of the Key program areas. He emphasized the importance of facilitating improved communications amongst all groups.
A presentation on the Pan African Rinderpest Campaign was given by Dr. C. Groocock. After the near eradication of Rinderpest from Africa by the JP-15 campaign of 1962–1976 the disease re-established itself over the following decade. Many 100,000's of cattle died. In 1986 the Pan African Rinderpest Campaign (PARC) was initiated in a joint effort to again control rinderpest. PARC is coordinated by the Inter-African Bureau of Animal Resources (IBAR) of the O.A.U. It was mainly funded by the EEC and Technical support was provided by FAO, IAEA and OIE.

PARC set up two regional coordinating offices in Mali and Kenya. Two laboratories for vaccine quality control and disease diagnosis in Senegal and Ethiopia and five emergency vaccine banks in Senegal, Chad, Ethiopia, Kenya and Botswana.

PARC covers 34 countries. Fifty-percent of the funding is used for vaccination campaigns and 50% for the rehabilitation of National Veterinary Services.

Participating governments have to agree to a series of strict conditions which ensure the continued financial and political support of their Veterinary Departments. These negotiations have been difficult and lasted up to two years ago. However, most countries are adopting these conditions.

In phase one of the vaccination campaign there has been considerable success. Of over fifteen countries reporting rinderpest at the start of PARC, only four have reported in 1989 and two since May 1989. As a result of PARC activity, there is a clear possibility of eradicating rinderpest from Africa. However, it will require a sustained effort. Additional International Support by agencies such as USAID who have given excellent support in the past should be encouraged.

Dr. Juan Gay, Director of the Mexico-United States Commission for the Prevention of Foot-and-Mouth Disease and other exotic Diseases of animals presented an excellent overview of the outbreak of Viral Hemorrhagic Disease of Rabbits in Mexico. In the course of their investigations, visits were made to more than 6000 premises, of which some 2000 were positive. This was in an area where rabbits were mostly raised at small backyard farms for family consumption.

A discussion on Emergency Planning was then presented by Dr. Gary P. Combs. Animal Health and Depredation Management Systems (AHDMS) staff has been assigned the task of Emergency planning and risk assessment for the animal side of APHIS, USDA.

The Emergency Planning and Risk Assessment section of AHDMS is composed of four veterinarians and one wildlife biologist.

One of the section's first tasks is to develop a risk assessment process and to review and revise the APHIS FMD action guidelines. Training in foreign animal diseases will be a high priority for the coming year.
FOREIGN ANIMAL DISEASES

This section has already conducted a risk assessment on the impact of Viral Hemorrhagic Fever of rabbits in Mexico on the United States. It has also studied the risk of transporting ticks on wood products from Mexico to the United States.

Dr. D. A. Gregg then discussed this new disease of rabbits, called rabbit hemorrhagic disease syndrome which has been reported in China since 1984. Since 1986 it has been reported in most countries of Europe by such names as necrotic hepatitis of rabbits and X disease. In 1989, the disease spread rapidly through 10 states in Mexico. Domestic rabbits die in 2 to 3 days with few clinical signs except terminal epistaxis and sometimes convulsions. Mortality reaches 80–90%. Primary sites of replication are in the small intestine crypt and villous epithelium, hepatocytes, and splenic lymphocytes. Necrosis is found in many organs with fibrin thrombi suggesting a terminal coagulopathy. The evidence for a parvovirus etiology was also presented. (The full text of this paper appears elsewhere in the proceedings).

Dr. Paul Sutmoller presented a paper pointing out the extreme hazards to many countries to the importation of illegal germ plasm (embryos & semen). He also addressed procedures being developed to allow safe importation. (The full text of this paper appears elsewhere in the proceedings).

Emergency Programs Report
FY 1989
Dr. M. A. Mixson

FOREIGN ANIMAL DISEASE (FAD) INVESTIGATIONS

During the FY 1989 (October 1, 1988 through September 30 1989), a total of 220 Foreign Animal Disease (FAD) investigations were conducted in the United States and Puerto Rico. These investigations included, 117 (53%) suspected vesicular conditions, 43 (20%) Newcastle disease, 4 (2%) avian influenza, 3 (1%) mucosal conditions, 7 (3%) swine septicemic conditions, 12 (5%) encephalitic conditions, and 34 (15%) for screwworms or undesignated conditions.

HOG CHOLERA/AFRICAN SWINE FEVER SURVEILLANCE

Last year, the National Veterinary Services Laboratory (NVSL), Ames, Iowa, tested 24,572 swine blood specimens for hog cholera and African swine fever. The specimens are collected from slaughter establishments in Massachusetts, New Hampshire, New Jersey, Texas, and Puerto Rico. Serological titers for hog cholera were found in samples collected in New Jersey, Puerto Rico and Texas; “however, no virus was isolated and no clinical signs were observed in the herds of origin.”
REPORT OF THE COMMITTEE

VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE (VVND)

In FY 1989, there were 43 FAD investigations in which VVND was suspected. During this period, two outbreaks of exotic Newcastle disease were diagnosed and eliminated in pet birds. There was no involvement with or spread to poultry.

On June 14, 1989, NVSL confirmed exotic Newcastle disease in 17 Amazon parrots and a sun cockatoo from a private bird collector located in Stratford, Connecticut. The owner claimed to have purchased seven young Amazon parrots from a dealer in Houston, Texas. All birds bought and sold by the collector were traced, quarantined and tested for Newcastle. All attempts to trace the source of infection resulted in no further VVND isolations. All birds belonging to the collector and one pet store owner who purchased birds from the collector, were destroyed and both premises cleaned and disinfected. No domestic poultry were involved in the outbreak.

In July, 1989, Fish and Wildlife Service agents of the U.S. Department of Interior seized approximately 115 allegedly smuggled birds on a premises in Spring Valley, California. The seized birds were placed in the U.S. Department of Agriculture bird quarantine station at San Ysidro, California. Afterward several of the birds appeared sick. Veterinary Services collected and submitted samples to NVSL, Ames, Iowa. Subsequently, an exotic Newcastle disease virus was isolated. Those exposed birds, which are on the Endangered Species List, were maintained in isolation at the bird quarantine station. These birds remained quarantined for 120 days and were tested negative for Newcastle disease, before they were released to Fish and Wildlife Service authorities.

On August 2, 1989, NVSL isolated a Newcastle disease paramyxovirus from specimens submitted from pigeons in Columbus, New Jersey. The case was reported after pigeons developed severe central nervous system (CNS) symptoms and many died. Several guinea fowl on the same premises subsequently developed CNS like symptoms. This is believed to be the first case in the United States where the pigeon paramyxovirus spread from pigeons to other avian species.

On September 28, 1989, EP was notified that NVSL had isolated exotic Newcastle disease virus from conures in the U.S.D.A. Quarantine Facility in Mission, Texas. The virus was isolated from pet birds that had been seized by the Texas Border Patrol on a premises in Brownsville, Texas. The premises was placed under State Quarantine and game chickens on the grounds were destroyed and submitted to NVSL for virus isolation. Newcastle disease surveillance around the premises is in progress.

AVIAN INFLUENZA (AI)

AI VIRUS ISOLATIONS

On May 11, 1989, NVSL, Ames, Iowa, reported H5 antibodies in serums
submitted from a Pennsylvania chicken flock. Extensive sampling was conducted in the flock. Serum samples taken from birds in the flock revealed H5N2 antibodies, but no virus was isolated. No clinical signs or abnormal mortality was associated with this flock.

In July, 1989 H7N3 antibodies were detected on a serological test in two broiler-breeder flocks in the Shenandoah Valley, Virginia. One flock was located in Page County and the other in Rockingham. Both flocks were related and were serviced extensively together by the same company. Morbidity and mortality in both flocks remained normal. Sentinel chickens were placed in both flocks with no sero-conversion or virus isolation. Both AI sero-positive flocks were depopulated by the owner and the premises cleaned and disinfected. Extensive testing and surveillance of all production and backyard flocks within a 4 mile radius of each flock was conducted. In addition, all broiler production and breeder flocks in the Shenandoah Valley were tested with no further sero-positive titers found.

Blood samples collected from chickens in the Fort Lauderdale and Miami, Florida, area were found to be positive on serology for AI H5N2 in January and again in August, 1989. Serums, tissues and swabs have been collected from poultry in live bird markets and from poultry dealers in the area with no virus isolation. There have been no reports of clinical signs in chickens or abnormal mortality.

A number of turkey flocks in several states have been positive on serology for AI other than H5N2.

**AI SURVEILLANCE**

AI surveys were conducted at 73 live bird markets in New York City and in New Jersey during March and April, 1989. Swabs were collected from poultry on the premises and specimens sent to NVSL, Ames, Iowa. All samples were negative for avian influenza virus.

**SCREWWORKS**

On September 6, 1989, fly larvae were recovered from an open wound on a horse at the quarantine station in Canovanas, Puerto Rico. The larvae were identified as third instar-screwworm larvae, Cochliomyia hominivorax, by entomologist at both, the Puerto Rico Tick Force and at NVSL, Ames, Iowa. The affected horse was one of three equidae which originated from Puerto Rico and were shipped to Caracas, Venezuela, on August 27 to participate in a sports event there. The horse was returned to Puerto Rico on September 5, 1989, through the International Airport at Carolina, Puerto Rico. The horses were transported from the airport by truck and placed in quarantine at a U.S.D.A. approved quarantine facility at Canovana, Puerto Rico. The truck and aircraft use to transport the equidae were cleaned, disinfected, and treated with Atroban. Emergency operations began on September 11, 1989, with an assembled team from the Commonwealth and APHIS. An extensive information and surveillance campaign
was started with fliers and forms in Spanish. Approximately 1.2 million sterile screwworm flies were released twice a week by ground dispersal methods. This continued for 6 weeks, along with intensive surveillance of livestock and wildlife on the island.  

**EQUINE ENCEPHALOMYELITIS**

Numerous reports of equine with possible encephalomyelitis have been investigated in the southeastern States. Serological tests are suggestive of eastern equine encephalomyelitis, (EEE). This year, NVSL has reported a total of 21 EEE virus isolations from equine in 7 states; 9 from North Carolina, 4 from Maryland, 3 from South Carolina, 1 from Louisiana, 1 from Tennessee, 1 from Michigan, and 2 from Mississippi. The news media reported that the death of one person in Mississippi was due to EEE.

**VESICULAR STOMATITIS—Ossabaw Island, Georgia**

Dr. Victor F. Nettles, Director, Southeastern Cooperative Wildlife Disease Study (SCWDS), Athens, Georgia, reported on July 17, 1989 that a pig on Ossabaw Island, Georgia was observed to have a vesicle on its snout. Specimens were collected and submitted to NVSL, Ames, Iowa. A New Jersey-type vesicular stomatitis virus was isolated. This is the same type of virus that was isolated the year before in sand flies, *lutzomyia shannoni*, that were collected on the island in June, 1988.

**CONTAGIOUS EQUINE METRITIS**

On August 4, 1989, NVSL, Ames, Iowa, reported that a test mare bred to a French warmblood stallion was culture positive for contagious equine metritis. The stallion and the test mare were on a farm in Maryland which is approved to handle imported horses in accordance with Section 92.2 (i) (2) (iv), Title 9, Federal Code of Regulations.

**EXOTIC TICKS**

On May 24, 1989, Veterinary Services, Emergency Programs, was notified by the Area Veterinarian in Charge, Texas, of exotic ticks collected from adult ostriches recently imported through a privately owned USDA approved quarantine facility in Illinois. The ticks were submitted by Texas A&M University to the Smithsonian Institute in Washington, DC, where they were identified as *Amblyomma gemma* and *Hyalomma* species. Subsequently, NVSL, Ames, Iowa, identified ticks found on the adult imported ostriches located on a premises in Ohio, and four premises in Texas as *A. gemma*, *A. lepidum* and *H. Albiparmatum*. All exotic ticks found were on adult ostriches and were males. Both *Amblyomma* species are known vectors of heartwater. The *Hyalomma* species are not known to transmit diseases of livestock.

Since January 1, 1989, approximately 800 ostriches were imported into the United States through USDA approved quarantine stations. Of those
imported, only 25 were adults which were released, and it was this group that was subsequently found to be infested. The major concern was the adult group and one juvenile group which entered under permit at two USDA approved privately owned quarantine stations in Illinois.

On May 26, 1989, quarantine and treatment requirements were established for all ostriches and other ratite imported into the United States through privately owned quarantine stations after January 1, 1989. These requirements involved surveillance, treatment and the placement of State quarantines on all premises for 30 days. The adult ostriches in the group on which exotic ticks were found were treated 3 times with an approved acaricide. All areas where the adults were housed were treated a minimum of 4 times with either chlorpyrifos or coumaphos at a concentration of 0.05 percent at 14 day intervals. Surveillance and quarantine for these premises were established for a minimum of 6 months.

Surveillance on these premises included using sentinel animals, cloth drags, and CO₂ traps along with the examination of wildlife. In addition to sentinel animals, other animals on the premises were examined for ticks every 2 weeks. No more exotic ticks were found.

On July 16, 1989, 2 black rhinoceros arrived at Dallas, Texas, from South Africa, and were inspected for ticks and sprayed with coumaphos. Twenty male ticks were collected and identified by NVSL, Ames, Iowa, as Dermacentor Rhinocerinus, Amblyomma sparsum, and Hyalomma truncatum. On August 29, 1989, the rhinos were treated a second time for ectoparasites. Subsequently, when one of the rhinos became icteric, depressed, anorexic, and reluctant to move, the animal was sedated, examined, and samples were collected. NVSL found antibodies for Babesia bigemina.

SALMONELLA

Emergency Programs is monitoring the spread of Salmonella enteritidis (SE) in poultry through reporting on SE sero-typing by NVSL, Ames, Iowa. These strains are being characterized in terms of plasmid profile and phage-typing on a priority basis. The highest priority has been assigned to isolates with the potential of being SE phage-type 4 (PT4), or of causing the most damage to the poultry industry. These are isolates from poultry with clinical signs of SE, isolates from imported poultry or their progeny, isolates from breeder flocks, and isolates from flocks implicated in human outbreaks. Assistance has been provided to the poultry industry in testing flocks under the Voluntary Model State Program for SE Quality Assurance. This program was developed in conjunction with the Northeastern Council on Avian Disease (NECAD). Emergency Programs has cooperated with State agriculture departments and public health agencies to help resolve SE outbreaks in which poultry flocks have been implicated.

SECRETARY'S ADVISORY COMMITTEE

The Secretary of Agriculture's Advisory Committee on Foreign Animal
REPORT OF THE COMMITTEE

and Poultry Diseases met August 15–17, 1989. The meeting was held at the Plum Island Animal Disease Center, Plum Island, New York and in New Haven, Connecticut. The committee reviewed issues involving the Foreign Animal Disease Diagnostic Laboratory facility needs, diagnostic changes in basic services, global perspectives on foreign animal diseases, environmental concerns during emergency operations, research plans and initiatives involving new technology, and importation of exotic species into the United States. The committee made 24 resolutions and recommendations for the consideration of officials of the U.S. Department of Agriculture. These pertained to the Plum Island Animal Disease Center, animal import standards, importation of exotic species, user fees for import services, foreign travel, international trade, and foreign animal diseases.

NORTH AMERICAN FOOT-AND-MOUTH DISEASE VACCINE BANK

The Commissioners of the North American Foot-and-Mouth Disease Vaccine Bank held a meeting in Little Rock, Arkansas on October 27, 1988. Emergency Programs has assisted in the preparation of solicitation for bids to procure antigen for one million doses of A-81 Argentina 87 Foot-and-Mouth Disease Vaccine Antigen. Two bids were received for the production of the vaccine. One bid was provisionally approved and the inspection of the production facility was completed in September, 1989.

EMERGENCY PREPAREDNESS

STATE FOOD AND AGRICULTURE COUNCIL

The lists were updated with the names and locations of new members to the State Food and Agriculture Council.

REGIONAL EMERGENCY ANIMAL DISEASE ERADICATION ORGANIZATION (READEO)

All four READEO's have been fully staffed and maintained. A State Wildlife Official from each State has been designated as a Wildlife Officer for each of the READEO's.

READEO TRAINING

The Western Region's READEO conducted a training exercise during the week of January 9–12, 1989, in Denver, Colorado. The exercise used the Recorded Emergency Animal Disease Information (READI) system that has recently been reprogrammed using Oracle 5.1B. A simulated disease outbreak was presented to the READEO Officers in an organized task force. All data entry and information retrieval used 80386 microcomputers and simulated VS Form 12-27 data.

MILITARY SUPPORT

A meeting was held in April, 1989, with General Robert E. Viva, United
FOREIGN ANIMAL DISEASES

States Army Veterinary Corps, to discuss and enhance the emergency preparedness of Veterinary Services.

The Military Support to Emergency Animal Disease Programs training course was held April 10–14, 1989 in Hyattsville, Maryland. This was held in conjunction with the U.S. Army and seventeen military veterinarians completed the course.

MALIGNANT CATARRHAL FEVER STUDY

A grant to support a study to develop a test for latent malignant catarrhal fever carriers was awarded to the Zoological Society of San Diego in San Diego, California.

FOREIGN ANIMAL DISEASES TRAINING

FOREIGN ANIMAL DISEASE DIAGNOSTICIAN TRAINING

No Foreign Animal Disease Diagnostician (FADD) training courses were held in FY 1989.

FOREIGN ANIMAL DISEASE SEMINAR

A Foreign Animal Disease Seminar for FADD’s was held at the College of Veterinary Medicine, University of Georgia, Athens, Georgia, during the week of June 20–22, 1989. Thirty Federal and State FADD’s from the Southeast Region attended the course.

PLUM ISLAND ANIMAL DISEASE CENTER TRAINING

Eighteen veterinarians with State universities or State diagnostic laboratories were trained in foreign animal diseases at the Plum Island Disease Center in November, 1988.

WILDLIFE DISEASE SEMINAR

A Wildlife Diseases Seminar for FADD was held in Athens, Georgia, in cooperation with the Southeastern Cooperative Wildlife Disease Study on August 1–4, 1989. Sixteen FAD diagnosticians and one military veterinarian attended the seminar.

PUBLIC AWARENESS

BIOSECURITY POSTERS AND FLIERS

Emergency Programs with the Mid-Atlantic Cooperative Extension (MACE) and Public Awareness developed program aids to increase awareness of biosecurity for poultry operations. Posters and fliers “Good Neighbors Protect Poultry” continue to be made available for distribution. These materials are available to cooperators, the Extension Service and industry representatives as program aids on disease prevention.

Additionally, there are two exhibits about biosecurity for chickens,
turkeys, and game birds available for use at local, state and national poultry meetings. This is in cooperation with APHIS Public Information.

POULTRY BIOSECURITY VIDEO TAPES

Filming of the eight video tapes concerning biosecurity in the poultry industry is complete. The final editing and compiling of the videos is in progress. Each video tape is approximately 10–12 minutes in length. The series involves general poultry biosecurity, broiler flocks, commercial egg-layers, breeder farms, turkey operations, gamebird farms, live poultry markets, and feedmills, rendering plants and poultry transportation. The biosecurity series of video tapes are available to the public and industry groups.

SMUGGLED BIRD CAMPAIGN

An intensive information campaign was conducted with the Public Awareness Staff to inform the public of the problems associated with smuggled birds in an attempt to prevent the introduction of Exotic Newcastle Disease into the United States. Press releases, APHIS fact sheets, brochures on “Importing a Pet Bird” and “Exotic Newcastle Disease and the Pet Bird,” television and radio spots, articles in newspapers and trade journals, etc. were used to show individuals and businesses how to avoid smuggled birds.

PROGRAM AIDS

Program aids for Exotic Newcastle Disease and Rift Valley Fever have been completed and are available for distribution.

FOREIGN ANIMAL DISEASE AWARENESS MEETINGS

Presentations on Foreign Animal Diseases made by the EP Staff during FY 1989, have included: the U.S. Army Veterinary Technicians at the Walter Reed Institute of Pathology, at Colleges of Veterinary Medicine, the Foreign Animal Diseases Course for Teachers of Infectious Diseases, the North American Foot-and-Mouth Disease Vaccine Bank, the Mid-Atlantic Cooperative Extension meeting, the Foreign Animal Diseases Seminar, and other group meetings and courses.

THE FOREIGN ANIMAL DISEASE REPORT

During FY 1989, four Foreign Animal Disease Reports were prepared and sent to FAD Diagnosticians, State Cooperators, state and federal field veterinarians, VS Staff, Area Veterinarians in Charge, Schools of Veterinary Medicine, state diagnostic laboratories, the Secretary's Advisory Committee members, Animal Health Officials of other countries, and other related groups and individuals. The total circulation of each Foreign Animal Disease Report reaches more than 6,700 readers around the world. Emergency Programs (EP) contributed an article for each of the four
FOREIGN ANIMAL DISEASE

publications. These articles covered current EP activities throughout the year.

VETERINARY SERVICES DATA BANK

The Veterinary Services Data Bank (VSDB) is a computer- and micro-
film-based information system providing rapid access to literature cover-
ing diseases of concern to the livestock and poultry industries of the United
States. The VSDB primarily provides technical and scientific information
services to Veterinary Services animal disease programs and to cooperators
in Federal, State and foreign governments. The VSDB has more than
67,300 articles micro-filmed in files covering 43 diseases of livestock and
poultry, entomology, and 6 non-diseases. During FY 1989, the VSDB
processed 234 requests for disease information which involved approxi-
mately 3,000 pages of written information.

A technical review panel conducted a series of meetings and made
recommendations for updating equipment, methods and mapping systems
used in planning and during disease outbreaks.

Efforts are currently underway to transfer the EMERPRO program to a
microcomputer and rewrite the program with Oracle 5.1B.

A directory of veterinary research has been published. This directory is
a selected listing of research projects on and related to animal diseases and
parasites, their diagnosis, prevention, control, epidemiology and animal
welfare. Included are projects conducted or sponsored by U.S.D.A., the
State agricultural experiment stations and land-grant institutions, col-
leges of veterinary medicine in the U.S. and other cooperating State
institutions. Nearly 600 projects are described. The source for all projects
listed in this directory was the Current Research Information System
(CRIS), U.S.D.A.’s computer-based documentation and reporting system
for publicly supported agricultural and forestry research in the U.S.

WORLD ANIMAL DISEASE
SITUATION—1988

Prepared by: Dr. M. J. Gilsdorf
Presented by: Dr. Wesley Garnett

Foot-and-Mouth Disease (FMD): IN SOUTH AMERICA, during 1988,
FMD types O, A, and C were identified in Brazil. Types O and A were
reported in Colombia. Argentina reported type O in 43 herds, and Bolivia
reported type C in 6 herds. In Ecuador, 36 herds were infected with FMD
type O. Paraguay reported 1 herd infected but the type was not specified.
Chile has not reported a case of FMD since August 1987, when a total of 81
outbreaks was reported and 31,386 animals were destroyed.

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IN EUROPE, West Germany reported 4 herds affected with FMD type O, during January 1988. There were 558 animals destroyed. This was the second outbreak of FMD in this area since October 1987. Epidemiological analysis showed that both outbreaks were probably related to vaccine challenge tests being performed in a nearby FMD vaccine plant. No outbreaks have been reported in West Germany since then.

Italy reported outbreaks of FMD Type C during June, July and November of 1988, in swine in the Tuscany region. Since then, Italy has reported over 77 outbreaks from February to July 1989. They have imposed quarantine measures and depopulated over 29,877 cattle and swine. Last reports indicated no improvement in the control of the disease.

Israel reported outbreaks of FMD Type O in the northern districts of Tsefat and Golan. In August 1989 Israel reported an outbreak of FMD type Asia 1, that occurred in June 1989. The outbreak was close to the Lebanese and Syrian border. This is significant since Asia 1 has not been reported in Israel before. FMD Types O and A were also reported in Saudi Arabia.

IN ASIA, Kuwait reported FMD Type O from samples submitted to the World Reference Laboratory, Pirbright, England for subtyping. They were found to be antigenically related to O₁, BFS and O₁ Manissa. Susceptible livestock were being vaccinated with O₁ Manissa monovalent vaccine. The last 20 reported cases were in February, and March, with 1560 exposed animals, 95 affected and 238 animals destroyed.

Iran reported Type O, the Philippines reported Type C, and Nepal reported Type O and Asia 1. Thailand reported FMD during the last half of 1988. Pirbright reported Type O from Bahrain, Turkey, Pakistan, Jordan, Laos, Cameroon, Libya, Bangladesh, Sri Lanka, Yemen and Hong Kong, Type A from Turkey and Nepal, and Type Asia, in Bangladesh. In Africa, SAT₁ was isolated from Zambia, SAT₂ in Zimbabwe, and Type O from Niger. FMD was also reported in Senegal, Chad, and Oman.

To summarize, the number of countries reporting FMD outbreaks during 1988, are:

- **Untyped** (5) Paraguay, Thailand, Senegal, Chad, Oman
- **Type A** (5) Colombia, Brazil, South Africa, Nepal, Turkey
- **Type O** (22) Colombia, Brazil, Argentina, Ecuador, Germany, Israel, Jordan, South Africa, Kuwait, Iran, Nepal, Bahrain, Turkey, Pakistan, Laos, Cameroon, Libya, Bangladesh, Sri Lanka, Yemen, Niger, Hong Kong
- **Type C** (4) Brazil, Bolivia, Italy, Philippines
- **Type A₁** (2) Nepal, Bangladesh
- **SAT₁** (1) Zambia
- **SAT₂** (1) Zimbabwe
- **SAT₃** NONE

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Vesicular Stomatitis (VS): VS was reported in Mexico, Central America, Peru, Ecuador, and Colombia, and on Ossabaw Island off the coast of Georgia. Colombia reported a total of 644 cases of VS types Indiana (IN), and New Jersey (NJ) in 200 outbreaks between October 1988 and May 1989. Cases of IN, and NJ also occurred in Ecuador from January to October 1988. Peru reported 6 outbreaks in swine during January 1988. Costa Rica reported outbreaks in May and July 1989. Venezuela reported 25 outbreaks of NJ between July and November 1988. No additional cases have been reported in Venezuela since then. Panama reported outbreaks of NJ and IN in February, May and July 1988 and outbreaks of NJ in May and June 1989. Twenty outbreaks involving 23 herds were reported in El Salvador between May and August 1989. Guatemala and Honduras reported 6 and 16 outbreaks respectively during the same time period. The serotypes were not given. Nicaragua reported cases in April 1988. Mexico reported VS Type NJ and IN in February 1988. In March and April 1988, outbreaks of type NJ were reported. In May 1989, outbreaks were again reported by Mexico.

Swine Vesicular Disease (SVD): SVD was diagnosed in Italy, and Hong Kong during 1988. No other countries have reported the diseases.

Rinderpest (RP): RP was reported in Ghana in December 1987, and in April through August 1988. Uganda reported 75 deaths due to RP in April 1988. Sri Lanka reported 80 outbreaks during March through July 1988, involving over 7000 animals.

African Horse Sickness (AHS): AHS was reported in South Africa, Namibia, and Zimbabwe in 1988, and in Kenya, South Africa and Namibia in 1989. Spain had an outbreak in November 1988 around the Chiclanra region in the south. Type 4 was isolated. In February, another outbreak of type 4 occurred in the Andalusia region involving 4 horses. In July, August and September 1989, Animal and Plant Health Inspection Service (APHIS), International Services (IS) received reports of several more outbreaks of AHS type 4 in the Andalusia region of Spain. Over 130 animals have died or been sacrificed and 130,000 horses have been vaccinated. Ninety-eight percent of the horses in Andalusia are vaccinated. The government of Spain is planning to vaccinate the entire equine population south of latitude 40. In October 1989, we have received unofficial reports of AHS in Portugal, Saudi Arabia, Yemen, and Morocco.

African Swine Fever (ASF): Cases of ASF were reported in Italy, Spain, and Portugal throughout 1988. During the first half of 1989, Italy had 1 to 9 outbreaks per month involving up to 58 cases. Portugal reported 294 outbreaks in September 1988, involving 11,000 swine. However, the largest number of outbreaks per month reported so far in 1989 was 49 in March with 12,488 swine exposed. Spain has reported between 5 to 26 outbreaks per month during the first 3 months of 1989.

Other countries reporting ASF include Angola and Zaire in 1988, and Namibia, Malawi, and Senegal in 1989.
REPORT OF THE COMMITTEE

Hog Cholera (HC): In The Americas, HC is present throughout Mexico, and Central and South America. Mexico reported 12 outbreaks in February 1989, with 2,286 cases. During the next 3 months, a total of 24 more outbreaks have been reported with 909 cases.

In Europe, HC was reported in Austria, Belgium, Czechoslovakia, France, West Germany, Italy, and Yugoslavia. France had not had a case of HC since April 16, 1987, and was declared free by the OIE on April 16, 1988. However, in November 1988, 1 outbreak was reported. Six cases were diagnosed, 4 animals died, and 92 were destroyed. Austria had outbreaks of HC in January and February 1989 involving 514 swine on 12 premises. In June 1989, Austria declared themselves free of HC. In Africa, HC was reported from Senegal, Madagascar, and on the island of Mauritius. In Asia, HC was reported from Hong Kong, Malaysia, Sri Lanka, the Philippines, Korea, and Taiwan.

Teschen Disease (TD): Only Madagascar and the USSR reported TD since January 1988. Madagascar reported 18 outbreaks in 1988, and 7 during the first 2 months of 1989. The USSR’s last reported case was in March 1989. No outbreaks have been reported since.

Fowl Plague (FP): FP was reported in Benin, Niger, Mauritania, and Senegal during the first half of 1988. The Congo, Gabon, and Tunisia reported outbreaks during the fall of 1988. No outbreaks of FP have been reported in 1989.

Burkina reported 64 deaths during an outbreak in June 1988. The Sudan and Kenya reported cases during the first 4 months of 1988.

Peste des petits ruminants (PPR): Outbreaks of PPR were reported in Oman during January and April, in Mali during February, and in Senegal during March. Togo reported over 22,000 animals affected in July 1988. Cases were also reported in Benin, Ghana, and Mauritania during 1988. During the first 6 months of 1989 cases were reported in the Ivory Coast, Ghana, Oman, Burkina, Senegal, and Ghana.

Contagious Bovine Pleuropneumonia (CBPP): During the first 6 months of 1988, CBPP was reported in the African countries of Burkina, Angola, Mali, Namibia, and Sudan and in Kuwait on the Asian peninsula. Portugal also reported CBPP from January through July 19, 1988, with 29 to 124 outbreaks each month. Guinea reported outbreaks in January through October 1988. During the first half of 1989, outbreaks were reported in Kuwait, Benin, Mali, the Ivory Coast, Burkina, and Kenya.

Rift Valley Fever (RVF): RVF was reported in Senegal during January, March, and April of 1988. Namibia had outbreaks in March and was the only other country reporting RVF in 1988. Kenya reported outbreaks from January through April of 1989.

Lumpy Skin Disease (LS): LS was reported on the continent of Africa and in Madagascar. During the first half of 1988, outbreaks occurred in
FOREIGN ANIMAL DISEASES

Mauritania, Senegal, and Mali in East Africa. In South and Central Africa, LS was reported in the Congo, Angola, Zaire, Uganda, Kenya, Zimbabwe Namibia, South Africa, and Madagascar (12 countries). During the second half of 1988, LS was reported in Niger, Mali, the Congo, Uganda, Kenya, Zimbabwe and Madagascar (7 countries). During the first half of 1989, LS has been reported in 10 African countries: The Ivory Coast, Ghana, Senegal, Egypt, Kenya, the Congo, Zambia, Zimbabwe, South Africa and Madagascar.

**Bluetongue (BT):** In late 1987, and 1988, BT was reported in the United States, Canada, Malaysia, Israel, Namibia, South Africa, and Mauritius. Canada's 2 outbreaks were reported in November 1987, with serological evidence only. Seventeen animals were involved and they were all located in the Okanagan Valley of Western Canada. There was also positive serology reported in Australia. In the first half of 1989, BT was reported from the United States, Malaysia, Israel, Botswana, Kenya, and South Africa.

**Sheep and Goat Pox (SGP):** SGP was reported in 7 African countries; Senegal, Mali, Tunisia, Algeria, Egypt, Togo and Morocco in 1988, and in 4 Asian countries; Kuwait, Oman, Pakistan, Iraq, and in Turkey and Greece in the Mediterranean. In 1989, SGP has been reported in Kuwait, Turkey, Oman, Pakistan, Iraq, Algeria, Morocco, Senegal, Kenya, Burkina, Tunisia, the Ivory Coast and Cyprus.

**Velogenic Viscerotropic Newcastle Disease (VVND):** VVND was reported in Malaysia, Pakistan, the USSR, South Africa, Cyprus, Brazil, Venezuela, Ecuador, and Paraguay in 1988. Malaysia reported outbreaks from March through December with up to 37,000 birds exposed each month. In 1989, cases have been reported from Botswana, Ecuador, Malaysia, Pakistan, South Africa, and the USSR.

**Necrotic Hepatitis of Rabbits (NHR):** NHR (also called Viral Hemorrhagic disease or x disease of rabbits) was added to the OIE list of reportable diseases. The disease was first reported in China in 1984. It was reported in Korea in 1985.

In November 1988, the German Democratic Republic and the Federal Republic of Germany reported that the disease was causing up to 95 percent mortality in their country. The disease has also been reported from Eastern France, Czechoslovakia, Italy and Spain.

In June 1989, Yugoslavia reported 22 outbreaks of VHD. Austria reported the disease in July 1989. Unofficial reports indicate that Switzerland has had outbreaks also. Mexico first reported cases on December 1988. To date 65,420 deaths have been reported on 110,153 premises in 13 states plus the federal district. The latest cases were within 100 miles of the Texas border.

**Bovine Spongiform Encephalopathy (BSE):** BSE has been reported in Great Britain, Northern Ireland, and Ireland. Great Britain has reported
REPORT OF THE COMMITTEE

4606 cases. Seven cases were reported in Ireland. Three of the affected animals were born in the United Kingdom and the rest were located near the border of Northern Ireland. Ireland has banned all cattle imports from the United Kingdom if they were born before July 1988, which is the date the United Kingdom banned the feeding of livestock with protein concentrate containing animal products. The number of cases in Northern Ireland was not received.

Contagious Equine Metritis (CEM): CEM outbreaks were reported in Switzerland and Denmark. The countries considered affected by the OIE are: Belgium, Denmark, France, the Netherlands, Norway, Sweden, Switzerland, and Japan. The United States also considers Austria, Ireland, Italy, the Federal Republic of Germany, and the United Kingdom affected.

ANIMAL DISEASES FOREIGN TO THE U.S.
RECENT RESEARCH REPORTS

Donald O. Morgan, D.V.M., Ph.D., PIADC, NAA, ARS, USDA

This report presents representative research results (late 1988 through mid 1989) on foreign (to USA) diseases, and clinically related domestic diseases, of food animals which have received significant USDA consideration and research effort during the reporting period. A sketchy synopsis of the areas of research along with selected references is presented. The diseases are:

AFRICAN SWINE FEVER (Iridoviridae (ASFV))

Persistent ASFV infections were established in Vero cells, in this case, both the cells and the virus were changed. Specific receptors on Vero cells allow ASFV infection, L cells lack these receptors and do not support ASFV infection. Cell adapted ASFV does not induce the same cell surface proteins as the wild type virus. Considerable genomic diversity is shown by ASFV isolates from different geographic areas. Tick populations infected with ASFV lose the infection after 1 year. Immunoblot and monoclonal antibody procedures for measuring ASFV specific antibodies in serum have been described.

FOOT-AND-MOUTH DISEASE (PICORNAVIRIDAE, APHTOVIRUS (FMDV))

 Transmission of FMDV can occur as a subclinical disease without overt signs of disease. Peptide vaccines are becoming more effective through the coupling of carrier and adjuvant molecules with the defined peptide. The ultrastructure of FMDV shows similarities to other picornaviruses, however, "the canyon" a picornaviridae feature involved in cell attachment is missing from FMDV. Monoclonal antibodies have been used to define the neutralization sites on FMDV and for the antigenic analysis of field isolates. Nucleotide and amino acid sequence analyses are being used...
FOREIGN ANIMAL DISEASES

to study mutation, virulence, cell attachment and the inter-relationships of
field isolates.\textsuperscript{5,9} Antiidiotypes were used to demonstrate common antibody
specificities in antisera from mice, cattle and swine.\textsuperscript{6} The ELISA reaction
in various forms is rapidly becoming the primary diagnostic tool for FMDV
and for matching vaccine strains with outbreak strains.\textsuperscript{4,7,13}

**HEARTWATER** (Erlichieae, Cowdria ruminantium)

Calves show considerable variation in resistance to infection with HW
regardless of whether they are born of HW immune or non-immune dams.\textsuperscript{1}
Clinical pathological studies of HW in calves showed a severe drop in serum
protein, especially in the albumin.\textsuperscript{3} Cowdria ruminantium was shown to
persist in the hemolymph of the soft tick Ornithodoros coriaceus for a period
of at least 2 years.\textsuperscript{2} Cowdria ruminantium was demonstrated in several
tissues of infected Amblyomma.\textsuperscript{4} Clones of C. ruminantium genomic DNA
hold promise as probes specific for the detection of the organism in tissues.\textsuperscript{5}

**HOG CHOLERA** (Togaviridae, Pestivirus (HCV))

The nucleotide sequence of Hog Cholera virus has been elucidated and it
shows considerable homology with BVDV.\textsuperscript{5} Monoclonal antibodies have
been used: to differentiate HCV and BVDV in swine tissues and in vitro
tissue culture;\textsuperscript{2,4,6} to evaluate protective vs non-protective immune re-
sponse of swine to HCV vaccination.\textsuperscript{7} A microcarrier system for the
production of HCV has been described.\textsuperscript{1} Simultaneous vaccination of swine
with attenuated HCV and “killed” FMDV vaccines gave good reponse to
both.\textsuperscript{3}

**RIFT VALLEY FEVER** (Bunyaviridae, Phlebovirus (RVFV))

The disease has been characterized in mosquitoes and young gerbils, the
later can be used as a model for RVFV induced encephalitis without
significant extraneural lesions.\textsuperscript{1,2} Ribavirin and interferon have been
shown to be effective treatments against the ill effects of RVFV infection.\textsuperscript{3}
Heat inactivation is an effective means for insuring the innocuity of
diagnostic reagents.\textsuperscript{6} Nucleotide probes are being developed for use as
RVFV diagnostics.\textsuperscript{4} Attenuated and biosynthetic vaccines both show prom-
ise of effectiveness against RVFV.\textsuperscript{5,7,8} Passive protection shows that a
humoral immune response is sufficient to protect against RVFV infection.\textsuperscript{7}

**RINDERPEST** (Paramyxoviridae, Morbillivirus (RPV))

A computer model of rinderpest epidemiology in wildlife is being used in
Africa.\textsuperscript{5} Rinderpest virus was demonstrated in a wide range of tissues from
fetuses aborted after the deliberate infection of their dams.\textsuperscript{7} Monoclonal
antibodies are being used to analyze rinderpest virus.\textsuperscript{3} Nucleotide probes
are being used to differentiate RPV from PPRV in infected tissues.\textsuperscript{4} An
ELISA for screening large numbers of sera for antibodies to RPV has been
described.\textsuperscript{6} Bovines, porcines and lapins have been protected from rinder-
pest by recombinant vaccinia viruses carrying RPV protein.¹,²,³

**SWINE VESICULAR DISEASE (Picornaviridae, Enterovirus (SVDV))**

The complete nucleotide sequence of the genome of the enterovirus SVDV (H/3'76) isolated from a healthy pig has been determined. A detailed analysis of homology between SVDV and coxsackieviruses shows that non-structural proteins are highly conserved; whereas, the structural proteins are less well conserved.¹

**VESICULAR STOMATITIS (Rhabdoviridae, Vesiculovirus (VSV))**

Vesicular Stomatitis virus is the subject of many molecular biology investigations and the reports of direct veterinary interest are only a small part of the VSV literature. A new vesiculovirus (Malpais Springs virus) has been described and shown to have infected mule deer and pronghorns in the southwestern US.¹ Chandipura virus has been cloned and its G protein expressed as a biologically active molecule in mammalian cells.³ A diagnostic ELISA has been developed for VSV.² Inactivated VSV vaccine was shown to be effective in eliciting an immune response in cattle.⁵ Bovines, porcines and canines have produced high titers of VSV neutralizing antibodies after vaccination by either the subcutaneous or oral routes with a human adenovirus vector carrying the VSV G protein.⁴

**REFERENCES**

**AFRICAN SWINE FEVER**


**FOOT-AND-MOUTH DISEASE**

FOREIGN ANIMAL DISEASES


HEARTWATER


**HOG CHOLERA**


**RIFT VALLEY FEVER**


**RINDERPEST**

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FOREIGN ANIMAL DISEASES


SWINE VESICULAR DISEASE


VESICULAR STOMATITIS


IMMUNIZATION STUDIES ON AFRICAN HORSE SICKNESS

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INTRODUCTION

African horse sickness (AHS) affects equines including horses, donkeys, mules, and zebras. Dogs may be infected with AHS virus (AHSV), but their role in the epidemiology of AHS is not clear. The AHSV, an orbivirus, is thought to be transmitted mainly by Culicoides spp.; it has been shown experimentally to be vectored by mosquitoes. There are nine serotypes of AHSV. Theiler described four types of clinico-pathological AHS:

1) the peracute or pulmonary form; 2) the subacute, edematous form; 3) the mixed form; and, 4) the horse fever form. The first three forms of disease terminate with death. Animals recover from the fourth form.

African horse sickness is endemic in central and southern Africa and occasionally intrudes into northern Africa. As well, AHS has occurred outside of Africa on several occasions as shown in Table 1. The recent outbreaks of AHS in Spain (1987, 1988, and 1989) resulted in notable losses shown in Table 2.

Vaccination with modified live virus vaccines was used in Spain, but questions arose as to the inocuity of the product, especially with regard to the polyvalent vaccine used in 1987 and 1988. A monovalent product was prepared in South Africa for use in Spain in 1988 and 1989. Procedures for diagnosis and control of AHS have recently been reviewed at the Foreign Animal Disease Diagnostic Laboratory (FADDL). This article will discuss diagnostic tests, vaccines, and regulatory considerations of AHS.

Diagnostic Tests

Several diagnostic tests to detect antibody to AHSV have been described including the agar gel immunodiffusion test (AGID), complement fixation (CF), virus neutralization (VN), indirect fluorescent antibody (IFA), indirect ELISA, and an improved ELISA test. The tests have been reviewed recently, and Table 3 shows the noteworthy characteristics of these tests.

The AGID test, the official test used to test sera from animals participating in the 1988 Olympic games, is accurate but insensitive. The CF test reflects recent infection and is group reactive but requires extensive controls and experienced laboratory personnel. The IFA and ELISA are group reactive and accurate, but serum samples from horses recently or
repeatedly inoculated with cell culture vaccines may give background reactions. Virus neutralizing antibody is type specific and persistent in infected and vaccinated animals, but the VN test requires nine separate testing procedures to ascertain that a serum is negative; the microtiter VN test is used at the FADDL.

Isolation of AHSV may be done by intracranial inoculation of washed red blood cells into suckling mice or cell cultures. Both tests require up to one month to confirm a negative result on a sample (Table 4). All the above serological and virological test systems are in use at the FADDL.

Control & Vaccination

Since AHSV is transmitted by insect vectors or direct inoculation of blood, exposure can be minimized by confinement of animals during the peak Culicoides and mosquito feeding times of dusk and dawn. The use of insecticides and repellents and prevention of mechanical transmission by needles and surgical equipment is indicated.

The protection of horses against AHS using a virus passaged in mice was first described in 1934. Some vaccine strains, developed by numerous passages in adult mice, caused encephalitis in horses, particularly when used in polyvalent vaccines. Following vaccination with polyvalent vaccines, encephalitis has been reportedly caused by vaccine strains for AHSV 2, AHSV 7, and with AHS viruses of unknown serotypes. In India, polyvalent vaccine from adult mice caused viremia between 14 and 23 days post vaccination (DPV) and up to 49 DPV in one horse. Certain serotypes of polyvalent vaccines may be overattenuated or cause a poor immune response perhaps due to "interference" between strains. Modified-live viral vaccines have been produced using viruses of mouse origin adapted to cell culture such as monkey kidney stable (MS), hamster kidney, or Vero cells. The cell culture propagation of the viruses resulted in a rapid loss of immunogenicity. Attempts to select "cold-variants" in MS cells yielded strains which were non-immunogenic.

Killed AHS vaccines have been used but are impractical as they impart only a short-lived immunity.

Monovalent components of the adult mouse brain vaccines were received at the FADDL from Onderstepoort in the 1960's. In 1968, viruses were propagated in suckling mice by intracranial inoculation, and 10% tissue suspensions were lyophilized. After 20 years storage at 4°C, the titers were remarkably stable. The minimal recommended dose of adult mouse brain vaccine is 10^4.3 TCID, which is essentially equivalent to the mouse intracranial dose 50% (MICD,). In all cases, the vaccine titers were equal to or greater than the minimal standard.

In an earlier study, horses in the containment laboratory (P3 or greater) at the FADDL were vaccinated and challenged as indicated in Table 5. Seven of the 11 horses vaccinated survived challenge with homologous
virulent AHSV. The vaccines failed to protect 4 horses. The serological
response of the vaccinated horses did not relate to the animals’ ability to
survive challenge (Table 5).

Because of the AHSV 4 outbreak in Spain, additional studies were
undertaken. Horses were obtained from local sources and were conditioned
by worming and holding in quarantine for 3 weeks prior to moving to the
containment facilities at the FADDL. Four horses were vaccinated with the
AHSV 4 vaccine. Twenty-one days later, they were inoculated subcutane-
ously with 1 ml of AHSV 4 from the Spain 1987 outbreak (AHSV 4 Spain
87). The virus was kindly supplied by Spanish regulatory officials as a cell
culture adapted virus having a history of 1 suckling mouse brain passage,
6 MS, and 1 Vero cell passage. The titer of the virus was $10^{4.5}$ TCID,$\text{50}$. As a
control, one horse was not vaccinated and received only the virulent virus.
Horses were observed, and their rectal temperatures were taken daily.
Blood samples were taken at 1 to 7 day intervals throughout the study.
Animals, if found moribund, were euthanized. All animals that died were
necropsied. The AGID, VN, CF, ELISA, and virus isolation (VI) tests were
performed as previously described.25

No antibody was detected by VN, CF, AGID, or ELISA tests in the 4
vaccinated equines 3 weeks after vaccination. Three of the four vaccinates
and the control died following inoculation with virulent AHSV 4 Spain 87
(Table 6). Lesions typical of subacute and mixed AHS were found in all
horses that died. These lesions included subcutaneous and intermuscular
edema, lung congestion with edema, and froth in the bronchi and trachea,
and petechiae in the serosa of the intestines.

Six to 8 days following challenge inoculation, AHS virus was isolated
from the blood of the 3 vaccinates and the control animal, all of which died.
Virus was not isolated from the horse (No. 25) which remained asympto-
matic following challenge inoculation. Complement fixing, VN, and ELISA
antibodies were found in the serum of surviving horse 25 as well as the
serum of horse 28 which died day 13 post challenge inoculation (Table 6).

From the studies on the 4 horses vaccinated with the high adult mouse
brain passage, it evident that the AHSV 4 monovalent vaccine stored at the
FADDL is not efficacious. Earlier serological studies indicated that the type
4 component of polyvalent vaccines27 had not uniformly elicited a VN
antibody response in vaccinates under field conditions. Erasmus16 noted
that the AHSV 4 component of the polyvalent vaccine was replaced because
of a poor antibody response. The components of the improved polyvalent
vaccine were not made generally available.

Our studies are now seeking improved vaccine components by selecting
viruses which provide protection without untoward side effects.

**Regulatory Considerations**

The likelihood of an AHS infected animal being brought into a non-
endemic country increases with the rapid international exchange of horses.
AFRICAN HORSE SICKNESS

Considering that the diagnosis of AHS in a non-endemic country may require at least 2 weeks, a continuing assessment of abbreviated quarantines should continue.

Current U.S. regulation requiring a 60-day quarantine for horses coming from AHS endemic countries is generally satisfactory, considering that the viremic state of vaccinated animals may last up to 49 days. The performance of serological tests other than the AGID test would detect antibody earlier than the AGID test.

Zebras may be infected with AHSV and remain asymptomatic but have prolonged periods of viremia or virus perstivity (at least 40 days). Re-evaluation of the requirements for importing zebras and equines was made following the introduction of AHSV 4 into Spain in 1987. It was concluded that the regulations in place were adequate for the U.S.A.

Following the index case, AHS may spread rapidly in a non-immune equine population. It is prudent to have available, safe, effective AHS vaccines for emergency use.

Future means of control of AHS may employ inactivated or genetically engineered vaccines used with appropriate adjuvants. However, until these vaccines are developed, the modified-live vaccines will have to be used.

SUMMARY

The following areas have been discussed: 1) There are several useful diagnostic tests for detecting antibody to AHSV including CF, VN, and ELISA. The AGID test is accurate but insensitive; 2) AHSV may be isolated in suckling mice or cell cultures; 3) Adult mouse brain vaccines may be useful, but each serotype must be evaluated for safety and effectiveness; 4) The AHSV 4 mouse brain vaccine stored at the FDDL did not induce protection against challenge with virulent AHSV 4 Spain 87 as 3 of 4 vaccinated animals died; and 5) Stockpiles of AHS vaccines for emergency use may represent a means for control of AHS.

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AFRICAN HORSE SICKNESS


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AFRICAN HORSE SICKNESS


Table 1

Incidence of African horse sickness outside of Continental Africa.

<table>
<thead>
<tr>
<th>Year(s)</th>
<th>Area</th>
<th>Serotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1930</td>
<td>Yemen</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35</td>
</tr>
<tr>
<td>1944</td>
<td>Palestine</td>
<td>3</td>
<td>3,17</td>
</tr>
<tr>
<td>1959-60</td>
<td>Iran, West Pakistan, Afghanistan</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>1960-61</td>
<td>IBID plus India, Turkey, Iraq, Syria, Lebanon, Jordan</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>1966</td>
<td>Spain</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>1974</td>
<td>Pakistan</td>
<td>NA</td>
<td>44</td>
</tr>
<tr>
<td>1975</td>
<td>Saudi Arabia</td>
<td>NA</td>
<td>44</td>
</tr>
<tr>
<td>1980-81</td>
<td>Yemen</td>
<td>NA</td>
<td>44</td>
</tr>
<tr>
<td>1987-89</td>
<td>Spain</td>
<td>1</td>
<td>4.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> = serotype not available

Table 2

Some features of the recent outbreaks of African Horse sickness in Spain.

<table>
<thead>
<tr>
<th>Year</th>
<th>Origin of Outbreak</th>
<th>Area Affected</th>
<th>No Horses Affected&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No Horses Vaccinated&lt;sup&gt;b&lt;/sup&gt;</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Zebras from Namibia</td>
<td>Madrid</td>
<td>146 (63D; 83S)</td>
<td>38,000 (P)</td>
<td>4</td>
</tr>
<tr>
<td>1988</td>
<td>Unknown</td>
<td>Andalucia</td>
<td>165</td>
<td>13,500 (P)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(M)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>1989&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Unknown</td>
<td>Andalucia</td>
<td>405</td>
<td>16,607 (M)</td>
<td>18</td>
</tr>
</tbody>
</table>

<sup>a</sup>D = died of AHS; S = Sacrificed
<sup>b</sup>P = Polyvalent vaccine; M = monovalent
<sup>c</sup>number unknown
<sup>d</sup>data as of September 1, 1989.
Table 3
Selected Characteristics of Serological Tests for African horse sickness.

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar gel immunodiffusion</td>
<td>group reactive</td>
<td>insensitive</td>
</tr>
<tr>
<td>Complement fixation</td>
<td>group reactive, detects recent infection</td>
<td>cumbersome</td>
</tr>
<tr>
<td>Indirect fluorescent antibody</td>
<td>group reactive</td>
<td>high background in some horses; subjective reading</td>
</tr>
<tr>
<td>ELISA</td>
<td>group reactive, fast, easily interpreted</td>
<td>high background in some horses</td>
</tr>
<tr>
<td>Virus neutralization</td>
<td>serotype specific, sensitive</td>
<td>requires live virus plus 9 titrations per sample</td>
</tr>
</tbody>
</table>

Table 4
Virus isolation methods for African horsesickness virus.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckling mouse (intracranial inoculation)</td>
<td>sensitive</td>
<td>requires live mice, takes up to 1 month to ascertain negative test</td>
</tr>
<tr>
<td>Tissue culture (VERO, MS cells)</td>
<td>easy to perform</td>
<td>requires tissue culture, sensitivity not clearly documented</td>
</tr>
</tbody>
</table>

Table 5
Summary of vaccine studies on African horse sickness using one injection of high passage mouse brain virus*

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Challenge strain</th>
<th>Vaccine strain</th>
<th>Vaccine titer/dose</th>
<th>Serological response from vaccine</th>
<th>Result of challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29/62</td>
<td>A501</td>
<td>4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NEG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>D&lt;sup&gt;a&lt;/sup&gt;-Subacute</td>
</tr>
<tr>
<td>2</td>
<td>MAT</td>
<td>OD</td>
<td>4.3</td>
<td>NEG</td>
<td>Survived</td>
</tr>
<tr>
<td>3</td>
<td>13/63</td>
<td>L</td>
<td>5.3</td>
<td>POS</td>
<td>Survived</td>
</tr>
<tr>
<td>4</td>
<td>12/60 VRYHEID</td>
<td></td>
<td>4.9</td>
<td>NEG</td>
<td>D-Subacute</td>
</tr>
<tr>
<td>5</td>
<td>30/60 VH</td>
<td></td>
<td>4.9</td>
<td>NEG</td>
<td>2/2 D-Peracute</td>
</tr>
<tr>
<td>6</td>
<td>3887</td>
<td>114</td>
<td>5.5</td>
<td>NEG</td>
<td>2/2 Survived</td>
</tr>
<tr>
<td>7</td>
<td>38932 Karen</td>
<td></td>
<td>5.4</td>
<td>NEG</td>
<td>Survived</td>
</tr>
<tr>
<td>8</td>
<td>10/60 18/60</td>
<td></td>
<td>4.5</td>
<td>NEG</td>
<td>Survived</td>
</tr>
<tr>
<td>9</td>
<td>7/60</td>
<td>60</td>
<td>4.8</td>
<td>POS</td>
<td>Survived</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Abstracted from House & House, 1989; 1 horse unless otherwise noted
<sup>b</sup> = MICD<sub>50</sub> — mouse intracranial dose — 50%
<sup>c</sup> = negative
<sup>d</sup> = positive
<sup>e</sup> = died
**AFRICAN HORSE SICKNESS**

*Table 6*

Data on horses vaccinated with AHSV 4 mouse brain vaccine and control.

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>Antibody Present</th>
<th>Antibody Test</th>
<th>DPVa</th>
<th>Virus Isolation</th>
<th>DPI Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Neg</td>
<td>CF</td>
<td>12</td>
<td>NI</td>
<td>survived</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VN</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ELISA</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
<td>Pos</td>
<td>7,8 died</td>
</tr>
<tr>
<td>27</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
<td>Pos</td>
<td>8 died</td>
</tr>
<tr>
<td>28</td>
<td>Neg</td>
<td>CF</td>
<td>7</td>
<td>Pos</td>
<td>6,8 died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VN</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ELISA</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>NV</td>
<td>Neg</td>
<td></td>
<td>Pos</td>
<td>7,8 died</td>
</tr>
</tbody>
</table>

*a* DPV = days post vaccination  
*b* DPI = days post inoculation with virulent virus  
*c* Neg = negative – all tests  
*d* CF = complement fixation  
*e* day on which titer first detected  
*f* NI = not isolated  
*g* VN = virus neutralization test  
*h* Pos = positive  
*i* day on which virus was isolated  
*j* NV = not vaccinated
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. C. L. Campbell, Tallahassee, FL
Vice Chairman: Dr. R. C. Knowles, Rehoboth Beach, DE

J. B. Anderson, TN; C. Carter Black, GA; H. Gaffney Blalock, SC; L. Coggins, NC; A. M. Creswell, TN; C. A. Gipson, MD; L. H. Greene, CT; R. D. Hobbs, MD; F. M. Jones, FL; P. L. McDonough, NY; C. W. McGinnis, NH; R. Mead, WA; W. D. Miller, VA; M. J. Nolan, KY; S. R. Nusbaum, NJ; M. A. Owen, MA; R. Polen, NJ; L. Schlater, IA; J. D. Smith, KY; D. M. Sofranko, MD; C. E. Starkey, AR; C. D. Vail, CO; H. A. Virts, MD; A. Wallop, D.C.; R. H. Whitlock, PA.

The Committee on Infectious Diseases of Horses convened in Las Vegas, Nevada, on October 30, 1989, some 45 to 50 members and guests in attendance.

A moment of silent prayer was observed in memory of the late Dr. John O’Harra, the first chairman of this committee when it was established in 1964.

Numerous items of consequence to the equine industry were discussed.

Dr. James Pearson presented his annual encephalitis monitoring summary, observing that Venezuelan equine encephalitis appears to no longer be of significance within the United States. In 1988, there have been 71 positive WEE cases and 10 positive EEE cases diagnosed at the NVSL. The CDC has reported an additional 21 cases of WEE, and 51 cases of EEE (Tables 1 and 2). The totals of the NVSL and the CDC cases were: 92 EEE and 61 WEE. In 1989, there were 8 positive WEE cases diagnosed at the NVSL and none reported to the CDC for a total of 8. There were 51 positive EEE cases at the NVSL and 72 reported to the CDC for a total of 123 (Tables 3 and 4, Figures 1 and 2). EEE was diagnosed in most of the Gulf and Atlantic Coast states, plus Michigan and Tennessee. There were more EEE cases over a wider geographic area than in any year since 1973 (Table 5).

The data are shown in accompanying tables.

Dr. Chester Gipson reported on the incidence of equine infectious anemia in the country during FY 1989, a map of which shown in these proceedings depicts individual state status. Of the 831,920 horses tested nationwide, 2,728 were positive — a 0.32 percent infection rate.

Dr. James Pearson discussed comparative results of the agar gel immunodiffusion (AGID) test with those of the competitive-enzyme linked immunosorbent assay (C-ELISA) test since approval of the latter technique in 1987. He noted that discrepancies between the two tests have been observed in less than 7 cases submitted to the NVSL. Most of the samples that produce discrepancies between the tests were also positive by Western
INFECTIOUS DISEASES OF HORSES

blot technique. He stated, while all states now recognize the results of C-ELISA testing for the entrance of horses moving in interstate commerce relative to a negative equine infectious anemia status that there are at least three disadvantages to its use: test reagents are more costly than those of the AGID procedure; more technician time is required, although results can be obtained in less time; and Canada does not recognize the test for the introduction of horses.

Dr. Linda Schlater apprised the Committee that Dr. T. Inzana of the Virginia-Maryland Regional College of Veterinary Medicine has developed the ELISA test for glanders. He plans to present this information at the Conference of Research Workers in Animal Disease, November 6-7, 1989. After this meeting, Dr. Inzana will visit the NVSL (November 8-10) to transfer the technology.

The Diagnostic Bacteriology Laboratory (DBL), NVSL, will test serums from animals (4 ponies, 1 mule, 1 burro) exposed to live Pseudomonas mallei organisms to evaluate the ELISA test during the next few weeks. The DBL also has serums from noninfected ponies and horses tested with intradermal mallein. The serological response to mallein testing can then be evaluated with both complement fixation and ELISA tests.

Dr. Wesley Garnett, USDA, apprised the Committee of the current status of African horse sickness, stating not only are there cases in Spain, but also some related unidentified problems in Portugal and Morocco as well. AHS was reported in South Africa, Namibia, and Zimbabwe in 1988, and in Kenya, South Africa and Namibia in 1989. Spain had an outbreak in November 1988 around the Chiclana region in the south. Type 4 was isolated. In February 1989 another outbreak of Type 4 occurred in the Andalusia region involving 4 horses. In July, August and September 1989, Animal and Plant Health Inspection Service (APHIS), International Services (IS) received reports of several more outbreaks of AHS type 4 in the Andalusia region of Spain. Over 130 animals have died or have been sacrificed and 130,000 horses have been vaccinated. Ninety-eight percent of the horses in Andalusia are vaccinated. The government of Spain is planning to vaccinate the entire equine population south of latitude 40. In October 1989, we have received unofficial reports of AHS in Portugal, Saudi Arabia, Yemen, and Morocco.

Dr. Ralph Knowles discussed an episode in Maryland involving the "Quick du Gavon" stallion which had been imported from Europe this past July after having been submitted to the various mandated CEM culture, scrubbing and treatment requirements prior to exportation from overseas. Upon arrival at an approved quarantine facility at Annapolis, the stallion was again scrubbed and treated prior to breeding two negative test mares following a five day rest period.

The two mares developed contagious equine metritis and the causative organism was isolated at NVSL. Dr. Knowles states, in spite of the
REPORT OF THE COMMITTEE

application of precautionary measures applied prior to debarkation, that this episode emphasizes that warm-blooded stallions coming from Europe can very well constitute a hazard with respect to the importation of CEM.

The Committee was advised of an issue originating this past August with a request from the Ringling Brothers/Barnum and Bailey Shows to import from Italy, a declared contagious equine metritis affected country, a group of 38 stallions for an extended period of two years on a CEM waiver. Under current regulations, temporary entry horses may remain in the United States for up to 60 days under official supervision without having to meet testing, treatment and breeding regimens required for permanent entry horses. Although APHIS denied granting a complete waiver as requested, serious consideration was being given to allow importation without their having to meet country of origin test and treatment rules, permitting the 38 to be imported at which point they were to be cultured with but a sampled ten stallions subjected to the required test breeding of two mares for the detection of CEM. The remaining 28 were to be released to their winter headquarters in Florida, subject to periodic inspection by USDA personnel in efforts to effect proper isolation during the two year period.

Ensuing discussion disclosed that these contemplated departures from CFR-described procedures were not communicated directly from APHIS to affected industry and state regulatory officials, but rather were these involved segments advised through secondary sources. Upon the basis of ensuing comments received from the American Horse Council and other industry representatives voicing opposition to the acceptance of these 38 stallions without all completing the CEM requirements for permanent entry, APHIS receded from its position of sample test breeding upon arrival, extending this requirement to all of the animals.

As a matter of update, in response to an inquiry from this Committee's Chairman in public and private audience two days ago as to whether consideration would be given to requiring these stallions to be cultured and treated prior to leaving the country of origin as requested by industry groups, he was assured by the Administrator of APHIS that it certainly would. As a matter of fact, however, it was disclosed in Committee discussions that at least one-third of these stallions have been brought into the United States more than two weeks ago.

It is not the function of a committee report to postulate and comment upon what conclusions might be reached by an audience presented with the foregoing facts; however, the Committee had some serious misgivings in the entire handling of this matter and to this end has submitted resolutions to be considered by the general assembly later this week.
Eastern Equine Encephalitis Cases
United States, 1989

Equine Cases

Human Cases

Fatal Case

Cases

Mar Apr May Jun Jul Aug Sep Oct
### Table 1.

**WESTERN EQUINE ENCEPHALITIS — 1988**

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
<th>State</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arizona</td>
<td>1</td>
<td>Missouri</td>
<td>1</td>
</tr>
<tr>
<td>Colorado</td>
<td>7</td>
<td>Montana</td>
<td>27</td>
</tr>
<tr>
<td>Idaho</td>
<td>1</td>
<td>Nebraska</td>
<td>1</td>
</tr>
<tr>
<td>Illinois</td>
<td>1</td>
<td>New Mexico</td>
<td>5</td>
</tr>
<tr>
<td>Iowa</td>
<td>4</td>
<td>Oklahoma</td>
<td>7</td>
</tr>
<tr>
<td>Kansas</td>
<td>6</td>
<td>South Dakota</td>
<td>8</td>
</tr>
<tr>
<td>Michigan</td>
<td>2</td>
<td>Texas</td>
<td>5</td>
</tr>
<tr>
<td>Minnesota</td>
<td>14</td>
<td>Wisconsin</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wyoming</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>92</strong></td>
</tr>
</tbody>
</table>

### Table 2.

**EASTERN EQUINE ENCEPHALITIS — 1988**

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
<th>State</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>3</td>
<td>Louisiana</td>
<td>2</td>
</tr>
<tr>
<td>Arkansas</td>
<td>3</td>
<td>Mississippi</td>
<td>3</td>
</tr>
<tr>
<td>Florida</td>
<td>43</td>
<td>North Carolina</td>
<td>3</td>
</tr>
<tr>
<td>Georgia</td>
<td>2</td>
<td>Virginia</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>61</strong></td>
</tr>
</tbody>
</table>

### Table 3.

**WESTERN EQUINE ENCEPHALITIS CASES**

**JANUARY 1 – OCTOBER 1, 1989**

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
<th>State</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>1</td>
<td>Oregon</td>
<td>1</td>
</tr>
<tr>
<td>Montana</td>
<td>3</td>
<td>South Dakota</td>
<td>1</td>
</tr>
<tr>
<td>North Dakota</td>
<td>1</td>
<td>Washington</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE

Table 4.

EASTERN EQUINE ENCEPHALITIS
JANUARY - OCTOBER 1, 1989

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
</tr>
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<tbody>
<tr>
<td>Alabama</td>
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<tr>
<td>Florida</td>
<td>36</td>
</tr>
<tr>
<td>Georgia</td>
<td>10</td>
</tr>
<tr>
<td>Louisiana</td>
<td>12</td>
</tr>
<tr>
<td>Maryland</td>
<td>7</td>
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<td>Michigan</td>
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</tr>
<tr>
<td>Mississippi</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Jersey</td>
<td>4</td>
</tr>
<tr>
<td>North Carolina</td>
<td>18</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>2</td>
</tr>
<tr>
<td>South Carolina</td>
<td>10</td>
</tr>
<tr>
<td>Tennessee</td>
<td>1</td>
</tr>
<tr>
<td>Vermont</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>States combined</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virginia</td>
<td>3</td>
</tr>
</tbody>
</table>

Total: 123

Table 5.

EQUINE ENCEPHALITIS POSITIVE CASES

<table>
<thead>
<tr>
<th>Year</th>
<th>WEE</th>
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<th>Cases Submitted To NVSL</th>
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<tbody>
<tr>
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<td>27</td>
<td>44</td>
<td>397</td>
</tr>
<tr>
<td>1981</td>
<td>328</td>
<td>42</td>
<td>805</td>
</tr>
<tr>
<td>1982</td>
<td>27</td>
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<td>1983</td>
<td>106*</td>
<td>131*</td>
<td>426</td>
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<tr>
<td>1984</td>
<td>19*</td>
<td>121*</td>
<td>274</td>
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<td>1985</td>
<td>13*</td>
<td>64*</td>
<td>296</td>
</tr>
<tr>
<td>1986</td>
<td>45*</td>
<td>96*</td>
<td>363</td>
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<tr>
<td>1987</td>
<td>177*</td>
<td>88*</td>
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<tr>
<td>1988</td>
<td>52*</td>
<td>61*</td>
<td>290</td>
</tr>
<tr>
<td>1989**</td>
<td>8*</td>
<td>123*</td>
<td>293</td>
</tr>
</tbody>
</table>

*Includes the positive cases reported by CDC

**Through October 1, 1989
Eastern Equine Encephalitis
Human and Equine Cases by County
United States, 1969

- Equine Case(s)
- Human Case
ENZYME-LINKED IMMUNOSORBENT ASSAYS OF RUMINANT PARATUBERCULOSIS USING LIPOARABINOMANNAN AND A AND D PROTEIN ANTIGENS

E. A. Sugden, Ph.D., K. H. Nielsen, Ph.D., B. W. Brooks, Ph.D., M. D. Henning, B.Sc., A. Michaelides, B.Sc., S. Balsevicius, and N. L. Lopes

SUMMARY

Lipoarabinomannan (LAM) and two protein antigens (A and D) from a laboratory strain of *Mycobacterium paratuberculosis* have been purified and evaluated in both indirect and competitive enzyme-linked immunosorbent assay (ELISA) on sheep and cattle sera. For both ruminant species, indirect LAM-ELISA exhibited approximately a 2-fold increase in sensitivity as compared to the complement fixation test (CFT). In the case of sheep sera, both indirect A-ELISA and D-ELISA using Nunc high binding polystyrene plates exhibited sensitivities of 77% and 82%, respectively, which were 3-fold greater than for the CFT. Basing specificity on minimal disease flock (MDF) sheep resulted in values for indirect LAM-ELISA, A-ELISA, and D-ELISA of 95%, 93%, and 96%, respectively. A combination of ELISA results with each other or with agar gel immunodiffusion using D antigen (D-AGID) resulted in further increases in specificity. Using anti-A and anti-D mouse monoclonal antibodies in competitive ELISA tests on cattle sera resulted in a much improved differentiation of infected and immunized animals from minimal disease herd (MDH) animals with an increase in specificity to 100%.

INTRODUCTION

With the goal of improving the sensitivity and specificity of serological techniques for ruminant paratuberculosis, research in this laboratory has focussed on the purification of a polysaccharide antigen, lipoarabinomannan (LAM), and two protein antigens, designated A and D, and the evaluation of these in both indirect and competitive ELISA tests. The A and D components of culture filtrates and sonicates of a laboratory adapted strain of *Mycobacterium paratuberculosis* were found to be of value in the serodiagnosis of sheep paratuberculosis in agar gel immunodiffusion (AGID) and crossed immunoelectrophoresis (CIE) (Brooks et al, 1988). The detection of D component precipitins (D-AGID) and the use of LAM antigen in ELISA (LAM-ELISA) resulted in a 2-3 fold more sensitive detection of a humoral response to paratuberculosis than was possible using polysaccharide antigen in a complement fixation test (CFT) (Brooks et al, 1988; Sugden et al, 1989).

With its ability to analyse high numbers of samples through automation, ELISA testing using purified LAM, A, and D antigens should be of advantage, particularly where improved sensitivities and specificities can be realized.
ENZYME-LINKED IMMUNOSORBENT ASSAYS

MATERIALS AND METHODS

Antigens — Polysaccharide antigen for use in CFT and LAM antigen for use in ELISA were extracted and purified from a laboratory strain (V) of *M. paratuberculosis* using ultracentrifugation, Concanavalin A Sepharose chromatography, and DEAE Sephacel chromatography (Sugden et al., 1987). Alternatively, culture filtrates and sonicates of a culture of strain C-286 were used in AGID to detect antibodies against the A and D components (Brooks et al., 1988). The A antigen was precipitated by the addition of solid ammonium sulphate (25% w/v) and purified by subsequent chromatography on a DEAE 5PW high pressure liquid chromatography (HPLC) and a Phenyl 5PW HPLC column. In the case of D antigen, the ammonium sulphate-precipitated protein was passed through Sephacryl S-200 and the excluded peak was chromatographed on DEAE Sephacel followed by passage of high salt eluted material through a column of Concanavalin A Sepharose 4B. The purification of D antigen was aided by a digestion of the ammonium sulphate precipitate with proteinase K prior to passage through Sephacryl S-200. The purified preparations of A and D antigens yielded single bands on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and were seen to give rise to single rockets in CIE. Details will be published elsewhere.

Sera — Sheep sera were from 22 infected animals culled from an Ontario commercial flock defined on the basis of positive histopathology and/or positive culture, and 41 animals culled from the same flock and designated paratuberculosis-free on the basis of performance, serology (CFT and AGID), histopathology, and culture (Brooks et al., 1988). Sera was also taken from a minimal diseases flock (MDF) of 58 animals. Cattle sera were from a group of 15 animals exhibiting heavy/intermittent shedding or had been immunized with whole cells or polysaccharide antigen from *M. paratuberculosis*, and 78 minimal disease herd animals.

Indirect ELISA on high binding plates — For the indirect ELISA results depicted in Table 1, purified LAM, A, and D antigens were passively coated overnight at room temperature on high binding polystyrene plates (Nunc Immunoplates, 4-39454) in carbonate buffer at pH 9.6 (Nielsen and Wright, 1984) at 1 ug/ml, 1 ug/ml, and 0.1 ug/ml, respectively. After 4 washes with 0.01 M Tris, pH 8.0, 0.15 M NaCl, 0.05% Tween 20 (TBS-T), 200 ul of sheep serum diluted 1/200 (LAM antigen) or 1/50 (A and D antigens) with TBS-T was incubated in the plate for 3 hours. After another 4 washes with TBS-T, 200 ul mouse anti-bovine IgG, McAB (M23) (cross-reacts with ovine IgG,) conjugated with alkaline phosphatase was incubated in the plate at an appropriate dilution for 1 hour. The hydrolysis of p-nitrophenyl phosphate in a diethanolamine buffer (pH 9.8) containing magnesium was subsequently monitored at 405 nm according to a modification of a kinetic approach (Barlough et al., 1987).

Indirect and Competitive ELISA tests on low binding plates — For the indirect and competitive ELISA test results depicted in Table 2, LAM, A,
and D antigens were coated as above, but on low binding polystyrene plates (Nunc Microwell 2-69620). After 4 washes with TBS-T the indirect ELISA required the incubation of 200 UL of 1/100 dilutions of cattle serum in the plate for 2 hours. After another 4 washes with TBS-T, 200 ul mouse anti-bovine IgG1 McAB (M23) conjugated with horseradish peroxidase (HRPO) was incubated in the plate at an appropriate dilution for 1 hour. The hydrolysis of H2O2 was subsequently monitored by detecting the OD changes of 2,2'-azinobis(3-ethylbenzthiazoline sulfonic acid) diammonium sat (ABTS) at 414 nm using the timing protocol of Wright et al 1985. For the competitive ELISA tests, after 4 washes of the antigen coated plates, 100 ul of cattle serum diluted 1/50 with TBS-T and 100 ul of appropriately diluted mouse anti-D McAB was incubated in the plate overnight at room temperature. Binding of the McAB to the D antigen was detected by a 1 hour incubation with goat anti-mouse polyclonal antibody conjugated with HRPO (Bethyl Labs), followed by monitoring of the enzymatic hydrolysis of substrate as for the indirect ELISA.

Data interpretation — For all ELISA tests the mean OD value of the MDF (sheep) or the MDH (cattle) + 2 standard deviations (SD) from this value was taken as the cut off. Sensitivity was defined as the percentage of infected (and immunized in the case of cattle) animals with ELISA OD values greater than or equal to the cut-off. Specificity was defined as the percentage of MDF (sheep) or MDH (cattle) with OD values less than the cut-off. For test combinations (Table 1) sensitivity was defined as the percentage of infected sheep with positive OD values for both tests whereas specificity was defined as the percentage of MDF sheep showing OD values less than the cut-off in at least one of the tests.

CFT and AGID tests were performed according to de Lisle et al 1980, and Brooks et al 1988, respectively.

RESULTS AND DISCUSSION

Table 1 illustrates approximately a 2-fold increase in sensitivity for indirect LAM-ELISA as compared to the CFT. Both indirect A-ELISA and indirect D-ELISA reveal sensitivities of 77% and 82%, respectively, equivalent to that of the D-AGID, which were 3-fold greater than that of the CFT. It is believed that the use of high binding plates is necessary to achieve these high sensitivities, given that the use of low binding plates show lesser sensitivities, particularly in the case of indirect A-ELISA (Table 1) (the data for indirect ELISA tests for sheep sera in low binding plates is not shown). It is important, in any event, to indicate precisely the type of plates used in ELISA tests. Specificities in Table 1 revealed values of 95%, 93%, and 96% for indirect ELISA tests using LAM, A, and D antigens, respectively. Combining the results from two tests resulted in further increases in specificity to 98-100% (Table 1) since it is unlikely that animals not exposed to Mycobacteria would exhibit positive results in both.

It was further determined that indirect A-ELISA and indirect D-ELISA
showed positive OD values for 39% and 46% of the 41 paratuberculosis-free animals from the commercial flock, respectively, as compared to only 5% of these being positive by indirect LAM-ELISA. It cannot be ruled out that some of these animals were true positives where infectivity was too low to be detected by histopathology and/or culture.

In Table 2 is depicted the sensitivities and specificities of competitive ELISA tests as compared to indirect ELISA tests for cattle. The competitive ELISA on cattle sera exhibited a more marked differentiation of the infected/immunized animals from MDH animals when compared to indirect ELISA. It could be that the infected/immunized animals have high affinity anti-D antibody which competes well with the anti-D McAB. This is in contrast to MDH cattle which may have similarly reacting antibody of lesser affinity which would be easily detected in indirect ELISA but would compete poorly with the anti-D McAB in the competitive ELISA.

The availability of highly specific antigens for the serodiagnosis of paratuberculosis remains elusive. Alternatively, strategies such as antigen purification (Abbas et al, 1983) and the pre-absorption of sera with M. phlei (Yokomizo et al, 1983) prior to ELISA have improved sensitivity and specificity. This study reports similar improvements using purified antigens in ELISA, combining test results, and particularly using monoclonal antibodies in competitive ELISA.

In lieu of a specific antigen, non-specific antigens such as LAM described here may be successively employed in an ELISA screening test, pinpointing possible problem sheep flocks or cattle herds. This is because of the strong response of ruminants to this polysaccharide, particularly in the case of high bacterial load (Sugden et al, 1989). A secondary evaluation of positive reactors can then be carried out. Such a scheme was fundamental to the planning of an epidemiological study of paratuberculosis in Ontario dairy cattle (McNab et al, 1988).

REFERENCES


Nielsen, K. and Wright, P. F. Enzyme immunoassay and its application to the detection of bovine antibody to Brucella abortus (Agriculture Canada/Animal Diseases Research Institute, Nepean, Ontario), 1984.


ENZYME-LINKED IMMUNOSORBENT ASSAYS

Table 1. Indirect ELISA, CFT, D-AGID, and test combinations for detection of antibody to *M. paratuberculosis* in sheep.¹

<table>
<thead>
<tr>
<th>Antigen-test</th>
<th>Sensitivity</th>
<th>Specificity²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFT</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>D-AGID</td>
<td>82</td>
<td>100</td>
</tr>
<tr>
<td>LAM-ELISA</td>
<td>50</td>
<td>95</td>
</tr>
<tr>
<td>A-ELISA</td>
<td>77</td>
<td>93</td>
</tr>
<tr>
<td>D-ELISA</td>
<td>82</td>
<td>96</td>
</tr>
<tr>
<td>LAM-ELISA + D-AGID</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>LAM-ELISA + A-ELISA</td>
<td>45</td>
<td>98</td>
</tr>
<tr>
<td>LAM-ELISA + D-ELISA</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>A-ELISA + D-ELISA</td>
<td>68</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ — Nunc Immunoplate 4-39454 (high binding) plates

² — Specificity was based on the minimal disease flock (MDF) cut-off = MDF mean + 2SD

Table 2. Indirect and Competitive ELISA, CFT, and AGID for detection of antibody to *M. paratuberculosis* in cattle.¹

<table>
<thead>
<tr>
<th>Antigen-test</th>
<th>Sensitivity</th>
<th>Specificity²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFT</td>
<td>25</td>
<td>93</td>
</tr>
<tr>
<td>D-AGID</td>
<td>11</td>
<td>99</td>
</tr>
<tr>
<td>LAM-ELISA (I)</td>
<td>65</td>
<td>85</td>
</tr>
<tr>
<td>A-ELISA (I)</td>
<td>24</td>
<td>88</td>
</tr>
<tr>
<td>A-ELISA (C)</td>
<td>29</td>
<td>100</td>
</tr>
<tr>
<td>D-ELISA (I)</td>
<td>88</td>
<td>48</td>
</tr>
<tr>
<td>D-ELISA (C)</td>
<td>92</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ — Indirect (I) and Competitive (C) ELISA using Nunc Microwell 2-69620 (low binding) plates

² — Specificity was based on the minimal disease herd (MDH) cut-off = MDH mean + 2SD

¹Agriculture Canada, Animal Diseases Research Institute, NEPEAN, P.O. Box 11300, Station H, Nepean, Ontario, Canada K2H 8P9

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

Chairman: Dr. Robert L. Jones, Fort Collins, CO
Vice Chairman: Ms. Diana L. Whipple, Ames, IA

J. M. Arnoldi, MD; M. T. Collins, WI; T. Conger, AR; A. M. Craig, OR; C. L. Crum, ME; C. F. Emerick, WA; M. A. Essey, MD; J. L. Funk, IL; S. B. S. Hurley, WI; H. M. Lefler, CA; A. M. Lewis, IA; C. W. McGinnis, NH; A. R. McLaughlin, WI; J. B. Payeur, IA; B. S. Perryman, NC; C. O. Thoen, IA; R. Velure, ND; R. H. Whitlock, PA; B. Widger, NY; E. Y. Williams, CO.

The Committee on Johne's Disease met in Las Vegas, Nevada on October 31, 1989 with 12 members and 30 to 40 guests in attendance. Reports describing recent work on diagnostic procedures were presented.

Dr. E. A. Sugden from Animal Disease Research Institute, Agriculture Canada, Nepean, Ontario described enzyme immunossays using lipoarabinomannan and A and D protein antigens for serodiagnosis of ruminant paratuberculosis. Purified antigens were prepared for consistency in the assays. These antigens appear to be dominant antigens that stimulate antibody production in proportion to bacterial load in the animal. The manuscript describing this work is included in the proceedings.

The efficacy of various serologic tests for the diagnosis of Johne's disease was discussed by Dr. Donald Sockeyt, University of Wisconsin. He and co-workers are characterizing the diagnostic accuracy of AGID, ELISA, and CF tests for use as tools in epidemiological investigations. Mr. William D. Richards presented preliminary results of a study to develop an ELISA for detection of antibodies to Mycobacterium paratuberculosis in milk from cows.

A rapid sensitive and specific assay for detection of M. paratuberculosis in feces using a DNA probe was described by Dr. Cal Vary. A 450 base-pair insertion sequence which is unique to M. paratuberculosis, has been identified. This sequence (IS900) is present in multiple copies of the genome of M. paratuberculosis but has not been found in other mycobacteria. A primer sequence has been identified for use in a polymerase chain reaction (PCR) amplification step. A diagnostic kit has been developed using enzyme labeled probe and is expected to be released within the next few months. Preliminary evaluations indicate the DNA probe test may provide up to 30% more positive results than fecal cultures. The lower limit of detection appears to be between $10^3$ to $10^4$ organisms per gram of feces. The assay offers the advantages of being rapid, very sensitive and specific, and is able to detect both viable and nonviable organisms.

Dr. Robert Whitlock, University of Pennsylvania, discussed various aspects of culture techniques for Johne's disease. He characterized the problems with bacterial and fungal contamination of cultures and recommended use of a minimum of four tubes of media per isolation attempt and
JOHNE'S DISEASE

centrifugation as part of the inoculum processing. The manuscript describing this work is included in the proceedings.

Dr. Sang Shin from Cornell University described new methods for reducing bacterial and fungal contamination in fecal cultures for *M. paratuberculosis*. By pre-incubating the inoculum in BHI broth containing a mixture of antimicrobials and adding antimicrobials (vancomycin 50µg/ml, nalidixic acid 50µg/ml, and amphotericin B 50µg/ml) to Herrold's egg yolk agar, the contamination rate and resulting invalidation of culture attempts was reduced to less than 2 percent.

Development and evaluation of a slide latex agglutination test for identification of primary isolates of *M. paratuberculosis* without additional subculturing was described by Mr. Jerry Jarnagin, National Veterinary Services Laboratories.

Dr. Robert Whitlock presented a preliminary summary of the regulations and laboratory diagnostic services available in each of the states. This activity will be completed during the next year.

A proposed document describing culture methods for isolation of *M. paratuberculosis* from feces was discussed by Ms. Diana Whipple, National Animal Disease Center. Further detail, new developments and review of the literature will be added to the document in preparation for final committee approval and publication.
LABORATORY CULTURE TECHNIQUES FOR JOHNE'S DISEASE: A CRITICAL EVALUATION OF CONTAMINATION AND INCUBATION TIMES

Robert H. Whitlock, D.V.M., Ph.D
Anne E. Rosenberger, B.S.
Pamela A. Spencer, Sc.M.

From: Department of Clinical Studies, New Bolton Center School of Veterinary Medicine, University of Pennsylvania
382 West Street Road
Kennett Square, PA 19348

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Pennsylvania Department of Agriculture for their support and funding of the Collaborative Project on Johne's disease.

Many veterinary diagnostic laboratories provide a service to culture fecal specimens for *Mycobacterium paratuberculosis*. However, few laboratories have critically evaluated the observations concerning contamination rates, both fungal and bacterial, and the time for *M. paratuberculosis* to appear on the solid egg yolk based medium (Herrold's). The purpose of this report is to describe the observations concerning repeated visual evaluation of the culture tubes during a 16 week incubation period.

METHODS

During the period September, 1987 to March, 1989, approximately 4688 fecal samples, primarily from herds with a known history of Johne's disease, were brought to the University of Pennsylvania — New Bolton Center laboratory to be cultured for *Mycobacterium paratuberculosis*. The cultures were visually evaluated every two weeks through 16 weeks post inoculation for evidence of bacterial or fungal contamination in addition to colonies compatible with *M. paratuberculosis*.

Most samples arrived at the laboratory the same day obtained from the animals and were kept at room temperature overnight. The next morning, a 2gm aliquot was taken from each specimen and placed in a 50ml plastic tube with 40ml of water. Following rigorous mixing, the sample was allowed to sediment for 30 minutes to remove the coarse fibers. Then, a 5ml aliquot was removed from just below the surface and transferred to a second tube containing 35ml 0.9% HPC (final concentration of HPC 0.75%). After 24 hours of decontamination, the tube was centrifuged for 30 minutes at 1000 G., the supernatant removed and the pellet resuspended in 1ml
LABORATORY CULTURE FOR JOHNE'S DISEASE

water containing 100ug Amphotericin B. Each of the four tubes was inoculated with 0.15ml of the suspension. The remaining suspension was transferred to a 1.0ml tube and frozen at -70°C. In the event two or more of the four original tubes became contaminated, the frozen suspension was thawed and 2 or 3 new tubes were inoculated with 0.1ml of the previously frozen suspension.

After the tubes were inoculated, they were placed in a 37°C walk-in incubator and then visually evaluated at two week intervals for 16 weeks. The evaluation included assessment for fungal and bacterial contaminants and for moist, raised, gray-white colonies consistent with Mycobacterium paratuberculosis. Since each of the four culture tubes contained mycobactin J, colonies consistent with M. paratuberculosis were subcultured on Herrold's egg yolk medium without mycobactin. If the organisms failed to grow on this medium, but were acid-fast and morphologically consistent with M. paratuberculosis, they were considered to be M. paratuberculosis.

When fungal and/or bacterial contaminants had overgrown the culture tube, rendering it unreadable, the tube was discarded. If the contamination appeared on two or more tubes, and loss of the whole culture was inevitable, the culture was reset using the frozen inoculum.

The visual observations for each tube (18,752) were recorded in a notebook with a separate notation for each of the 8 biweekly observations. These data were then transferred to a database program (R-BASE) and evaluated with the statistical program package SAS using a microcomputer.

RESULTS

The first appearance of M. paratuberculosis compatible colonies on any one of the four culture tubes per sample according to time of appearance, from 2 to 16 weeks, is shown in Table I. Losses of one or more tubes to bacterial or fungal contamination at 2 week intervals are also shown.

DISCUSSION

Over 98% of the cultures yielding M. paratuberculosis were positive by 12 weeks with an additional 2% apparent at the 14 week reading and no further positives recorded at 16 weeks. Thus, laboratories should be encouraged to report their results after the 12 week reading. Since 90% of the cultures with an average of 6 or more colonies per tube were detected by 8 weeks of incubation, an interim report following the 8 week reading may be very helpful to the farmer and his veterinarian in determining which cows to cull. The so-called low shedders with less than 6 colonies per tube are the sample cohort that requires the longest incubation time to become positive (i.e, up to 14 weeks [Table 3]).

Bacterial and fungal contamination represent major deterrents to reducing the number of tubes per culture. Approximately 58% of the tubes
contaminated with bacteria and 54% of the tubes contaminated with fungi had overgrown the media by the 4 week reading rendering the culture tubes invalid. Cultures with 2-3 contaminated tubes were reset utilizing the previously frozen inoculum. The overall reset rate was 9.6% or 450 of the 4688 cultures. This procedure salvaged 67% of the reset cultures and yielded 18 positive cultures, or 4.3% of the 421 total positive cultures.

Reduction in the number of tubes per culture, from 4 with mycobactin to 3 with mycobactin and one without, could result in a reduction in test sensitivity of approximately 8% since 33% of the positive cultures were positive on only one of the 4 tubes and given the likelihood of contamination of at least 1 tube. Considering the current state of the art concerning mycobacterial cultures, further attention should be given to reducing bacterial and fungal contamination. Individual farms seem to influence the contamination rate more than does season. The seasonal effect is difficult to quantify since most farms are cultured once, or at most twice per year, and usually in the same month each year.

Table 1

<table>
<thead>
<tr>
<th>Week</th>
<th>Number of Cultures (+)</th>
<th>% Positive</th>
<th>Cultures with Fungal Contaminants*</th>
<th>Cultures with Bacterial Contaminants*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>367</td>
<td>780</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1.0</td>
<td>404</td>
<td>317</td>
</tr>
<tr>
<td>6</td>
<td>93</td>
<td>22.1</td>
<td>217</td>
<td>179</td>
</tr>
<tr>
<td>8</td>
<td>190</td>
<td>45.1</td>
<td>128</td>
<td>126</td>
</tr>
<tr>
<td>10</td>
<td>86</td>
<td>20.4</td>
<td>117</td>
<td>149</td>
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<tr>
<td>12</td>
<td>40</td>
<td>9.5</td>
<td>74</td>
<td>118</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>1.9</td>
<td>41</td>
<td>101</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>421</td>
<td>100%</td>
<td>1348</td>
<td>1770</td>
</tr>
</tbody>
</table>

*One or more of the 4 tubes was overgrown with contaminants and was discarded as unreadable.

Fungal and bacterial contamination were present in 28.8% and 37.8% of the cultures respectively. The relative number of contaminated tubes for each culture is shown in Table 2.
LABORATORY CULTURE FOR JOHNE'S DISEASE

Table 2

<table>
<thead>
<tr>
<th>Tubes Contaminated per Culture</th>
<th>Fungal Contamination (cultures)</th>
<th>%</th>
<th>Bacterial Contamination (cultures)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3340</td>
<td>71.2</td>
<td>2918</td>
<td>62.2</td>
</tr>
<tr>
<td>1</td>
<td>684</td>
<td>14.6</td>
<td>929</td>
<td>19.8</td>
</tr>
<tr>
<td>2</td>
<td>320</td>
<td>6.8</td>
<td>452</td>
<td>9.6</td>
</tr>
<tr>
<td>3</td>
<td>185</td>
<td>3.9</td>
<td>223</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>159</td>
<td>3.4</td>
<td>166</td>
<td>3.5</td>
</tr>
<tr>
<td>Totals</td>
<td>4688</td>
<td>100%</td>
<td>4688</td>
<td>100%</td>
</tr>
</tbody>
</table>

Heavy shedders (at least one tube with colonies too numerous to count) constituted 22.1% of the total positive cultures (92 of 421). The average number of colonies per positive culture was evaluated with respect to the minimum time to detection. These results are shown in Table 3.

Table 3

Time to first appearance of *M. Paratuberculosis* by average colony count (positive cultures)

<table>
<thead>
<tr>
<th>Average Colony Count</th>
<th>Weeks of Incubation</th>
<th>Total (+) Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 6 8 10 12 14 16</td>
<td></td>
</tr>
<tr>
<td>≥ 5</td>
<td>*</td>
<td>0 11.1 44.6 27.5 13.8 3.0 0.0</td>
</tr>
<tr>
<td>6-10</td>
<td>*</td>
<td>0 34.8 43.5 13.0 8.7 0.0 0.0</td>
</tr>
<tr>
<td>11-20</td>
<td>*</td>
<td>0 37.5 50.0 12.5 0.0 0.0 0.0</td>
</tr>
<tr>
<td>≥ 20</td>
<td>*</td>
<td>3.5 43.4 46.0 6.2 0.9 0.0 0.0</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.9 32.2 46.0 14.8 5.9 0.8 0.0</td>
</tr>
</tbody>
</table>

*The numbers in each row represent the percentage of cultures with a given average colony count detected at a specific biweekly reading.

Only 113 (26.8%) of the 421 positive cultures had an average of more than 20 colonies per tube, while 269 (63.9%) had 5 colonies or less per tube.

Bacterial or fungal contamination necessitated the resetting of 450 cultures. All resets were done at either 2 or 4 weeks post original inoculation. Of the 450 reset cultures, 108 reset cultures were fully readable (4 valid tubes) at 16 weeks, while 68, 76, and 51 tubes had 3, 2, or 1 tube readable, respectively. A total of 303 of the 450 (67%) reset cultures were readable at 16 weeks with one or more valid tubes. Eighteen of the reset cultures were positive, with 6 of the 18 classified as heavy shedders.
REFERENCES


REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

Chairman: Dr. J. R. Cole, Tifton, GA
Vice Chairman: Mr. J. Finnell, White Hall, IL

R. W. Behan, SD; B. O. Blackburn, IA; C. A. Bolin, IA; S. L. Diesch, MN; J. C. Frantz, NE; J. W. Glosser, DC; R. F. Hall, GA; L. E. Hanson, IL; C. M. Hibbs, NM; P. B. Kimsey, MA; C. A. Kirkbride, SD; A. D. Liggett, GA; D. A. Miller, IA; R. L. Morter, IN; R. M. Nervig, IA; H. L. Rubin, FL; J. G. Songer, AZ; A. B. Thiermann, MD; D. N. Tripathy, IL; D. L. Weiss, IA; J. M. Williams, MO.

The USAHA Committee on Leptospirosis met on October 31, 1989 with 21 members and guests in attendance.

Mr. Mark Wilson of the Diagnostic Section (APHIS-NVSL) of the National Reference Center for Leptospirosis updated the committee on the 1988–1989 activities of the section. During the year, the section performed 81,685 serologic tests and examined 1,214 specimens with the fluorescent antibody (FA) test and/or culture techniques. Four isolates were serotyped. Over 1400 diagnostic reagents (antigen, antisera, FA conjugates, and media) were provided to other laboratories. Sixty requests for consultation were received from 28 states involving 5 animal species. Six people (3 from state laboratories and 3 from other countries) were trained in various diagnostic procedures. Compared to the previous year, the section experienced a 20% increase in serologic tests, a 28% decrease in submissions for isolation, a 13% decrease in requests for reagents. Results of the microscopic agglutination test (MAT) check test sent to 45 laboratories on September 18, are scheduled to be returned to participating laboratories by December 15. Effective November 1, 1989, the section will no longer routinely perform the MAT on diagnostic sera. The NVSL will continue to provide serotyping of isolates, procedural protocols, reagents, consultation, and training.

Dr. Carole A. Bolin of the Research Section (ARS-NADC) of the Center reported on areas of research conducted during the past year. These are: 1) Experimental vaccines containing serovar hardjo type hardjo-bovis failed to protect heifers from challenge — exposure 2, 3, or 4 months after vaccination; 2) Antibodies from vaccinated cattle that were not protected from challenge exposure were used in a passive immunization experiment in hamsters. Antibody from vaccinated cattle protected hamsters from hardjo-bovis infection. Therefore, hamsters appear to be a poor model for improvement of hardjo vaccines; 3) Leptospira interrogans serovar bratislava was isolated from placentae, stillborn, and weak pigs from herds with a history of reproductive failure; 4) Leptospires were isolated from 12% of 153 adult swine kidneys collected at slaughter. These isolates are predominately serovar bratislava, however, there was one serovar hardjo isolate; and 5) Restriction endonuclease analysis of isolates of serovar bratislava indicates that the genetic types of bratislava in the United States are different from those found in Europe.
Ms. Barbara Smith, University of Kentucky, presented a paper entitled "Diagnosis and incidence of leptospiral infection in aborted and stillborn horses." She reported on the leptospirosis outbreak which occurred from October 1988 through April 1989 in horses located in central Kentucky. The following observations and findings were presented: 1) The multivalent leptospira fluorescent antibody conjugate supplied by NVSL successfully demonstrated leptospires in 14 of 15 equine fetal tissues; 2) Fluids of 12 of 15 cases of leptospiral induced equine abortions/stillbirths had MAT titers of 1:800 or greater; 3) Fluids from all 460 fetal/stillborn horses not diagnosed with leptospirosis were negative by MAT; 4) In contrast to the mares serum, fetal/stillborn fluids were usually positive to only one serovar; 5) Serum from 13 of 14 mares with leptospira-induced abortions/stillbirths had MAT titers ≥ 1:6400 to one or more serovars; 6) When mares serum was obtained on FAT and MAT negative cases, 24 of 27 had leptospiral MAT titers of less than 1:6400 to all serovars; 7) Leptospirosis was diagnosed in 15 of 594 cases (2.5%) of equine abortions/stillbirths in central Kentucky; 8) Leptospires isolated have been identified as serovar *pomona/kennewicki*; and 9) The disease was sporadic — a single case on 13 farms and 2 cases on 1 farm.
HOST PREFERENCE OF BOOPHILUS MICROPLUS IN PUERTO RICO AND ITS IMPLICATIONS FOR AN ERADICATION PROGRAM

R. L. Crom, D.V.M. and J. V. Duncan, D.V.M.
Veterinary Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
San Juan, Puerto Rico
May, 1989

BACKGROUND

The Puerto Rico Tick Eradication Program (TEP) is a cooperative program of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) and the Commonwealth of Puerto Rico Department of Agriculture (PRDA). Its goal is to eradicate the tropical cattle tick, Boophilus microplus, from Puerto Rico.

The Boophilus eradication protocol requires that all potential host animals on all premises receive pesticide treatments at 21-day intervals for a period of 231 days, or longer if Boophilus ticks are still detected. All animals are given a "pre-release" scratch prior to release of the premises from treatment status, as well as two more scratches during a post-treatment period of at least 120 days, to assure that the premises is truly Boophilus-free. Once declared so, periodic surveillance scratches are performed to assess tick status. This entire process on each premises is tracked by a computerized database management system.

As the name Boophilus suggests, these "cattle-loving" ticks prefer a bovine host.¹ Cattle, however, are not present on about 13,000 of the premises on mainland Puerto Rico. About 70,000 animals are located on these premises which have no cattle, which we refer to as "non-bovine" premises. These horses, goats, and sheep, comprise about 15 percent of all potential host animals on the mainland.

Thus, non-bovine premises are a large part of the tick eradication program. If Boophilus microplus is rarely present on such premises, and if it is strongly associated with the presence of bovine as compared to non-bovine host species, there could be significant implications for the eradication program, especially in the areas of pesticide usage and manpower allocation.

The purpose of the study reported here was to estimate the prevalence of Boophilus microplus infestation on non-bovine premises as well as the strength of the association between Boophilus microplus infestation and the presence of various host species.

METHODS

Data collected from premises in the Arecibo (northwest) and Juncos
CROM, DUNCAN

(east) regions of the TEP, and maintained in the TEP database management system, were analyzed. These included results of scratches performed at premises in pre-release, post-treatment, or surveillance status.

Prevalence estimates were made from analyzing data collected from all non-bovine premises scratched in any of the three statuses during a two-year period from May, 1987 through April, 1989. For each status, premises were classified as either *Boophilus*-positive or negative for the entire period.

The strength of the association between *Boophilus microplus* infestation and the presence of various host species on a premises was estimated through analysis of surveillance scratch data collected between 1986 and 1989. Premises where *Boophilus* had been found on at least one animal were compared to premises where *Boophilus* was never found. Various attributes of the premises, including presence or absence of various host species, were determined as of the date of the most recent positive scratch or, if never positive, the last negative scratch.

Odds ratio (OR, an estimate of relative risk) is a measure of the strength of an association between detection of *Boophilus microplus* infestation on a premises and a particular premises attribute.

RESULTS

*Boophilus* ticks were found on 26 of 5,509 non-bovine premises in surveillance status, 4 of 2,322 in post-treatment status, and 2 of 1,752 in pre-release status. Thus, the estimated prevalence of *Boophilus microplus*-infested non-bovine premises in surveillance status was 4.7 per 1000 (95% confidence interval [CI], 2.9–6.5), significantly higher than the 1.7 per 1000 (95% CI, 0.5–4.4) and the 1.1 per 1000 (95% CI, 0.1–4.1) in post-treatment and pre-release status, respectively, during the two-year study period (Table 1).

Among the premises included in surveillance status were 2,054 newly created premises which were being scratched for the first time and had never received pesticide treatments from the TEP. Of these, 15 were found to have *Boophilus*, for an estimated prevalence of 7.3 per 1000 (95% CI, 3.6–11.0). This was significantly higher than the prevalence for other premises in surveillance status which were re-infested after pesticide treatments (3.2 per 1000; 95% CI, 1.3–5.1).

To estimate the association of *Boophilus microplus* and various host species, attributes of 4,189 premises in surveillance status which were found *Boophilus*-positive were compared to those of 12,035 premises found *Boophilus*-negative. Of the *Boophilus*-infested premises, 99.5 percent (4,159/4,189) had bovines present, significantly more than the 54.6 percent (6,573/12,035) of negative premises which had bovines (OR, 115.2 \( p < 10^{-8} \)) (Table 2).
HOST PREFERENCE OF BOOPHILUS MICROPLUS

*Boophilus*-positive premises had a median of 4.0 animals per premises, while negative premises had 3.0. When premises were stratified by the number of animals present, as shown in Table 3, the odds ratio for the association of *Boophilus* and the presence of bovines increased from 94.6 to 127.7 as the number of animals on the premises increased. These odds ratios, however, were not significantly different from one another.

The associations between *Boophilus* and various host species on single species premises are shown in Table 4. *Boophilus* infestation was significantly associated with bovines-only premises as compared to either equines-only (OR, 75.4; \(p < 10^{-8}\)) or ovines/caprines-only premises (OR, 224.4; \(p < 10^{-8}\)). It was also significantly associated with equines-only premises as compared to ovines/caprines-only premises (OR, 3.2; \(p < 0.0056\)). When bovines were present on the premises, however, the presence of equines was not significantly associated with *Boophilus* infestation (OR, 1.02; 95% CI, 0.89–1.16; \(p < 0.80\)).

Of the *Boophilus*-positive premises, 1,335 were newly created premises which had never received TEP treatments. Of these, 99.0 percent (1,321) had bovines. Of 4,058 such premises which were *Boophilus*-negative, only 43.4 percent (1,763) had bovines (OR, 122.8; 95% CI, 70.8–217.3; \(p < 10^{-8}\)). This was not significantly different than for premises in surveillance status which had previously been treated.

**DISCUSSION**

The estimated prevalence of *Boophilus microplus*-infested non-bovine premises in the TEP was very low. Premises in surveillance status had the highest prevalence, due in part to the newly created premises included in this category which had never been treated by the TEP. Even at that highest rate, 7.3 per 1000, we would expect only 95 non-bovine premises in Puerto Rico to be infested with *Boophilus* ticks, whether they have ever been treated or not.

The association between the detection of *Boophilus* and the presence of bovines was very strong. It was strong among all sizes of premises and among both re-infested premises and new premises which had never been treated by the TEP. When bovines were not present, the presence of equines was significantly associated with *Boophilus* infestation but the association was an order of magnitude smaller than that for the presence of bovines (OR, 3.2 vs. 75.4). This association was only seen when no bovines were present.

Our findings do not suggest that non-bovine species rarely host *Boophilus* ticks. Surveys on other Caribbean islands have shown that despite self-grooming, goats and sheep may commonly be found with *Boophilus*. The associations we found indicate that, in Puerto Rico, the presence of bovines on a premises is a strong risk factor for *Boophilus* infestation. These associations reflect the host preference of *Boophilus microplus*, as well as
other factors such as animal movement patterns in Puerto Rico. Therefore, premises with bovines should be the primary focus of our efforts in Puerto Rico, although no premises can be ignored in an eradication program.

As a result, a change in our *Boophilus* eradication protocol was proposed and recently implemented. The new protocol calls for non-bovine premises with no history of *Boophilus* to receive a status evaluation scratch. If this scratch finds *Boophilus* ticks or bovines on the premises, a complete 231-day treatment series will be given. If the scratch finds no *Boophilus* and no bovines, the premises will be advanced to post-treatment status for two additional scratches before being declared free of *Boophilus*. Thus, this protocol does not ignore non-bovine premises, but calls for pesticide treatments only if and when *Boophilus* is found on them.

Since implementation, a status evaluation scratch has been reported for 591 premises. Of these, 200 were found vacated and five had bovines. Of 386 non-bovine premises examined thus far, none had *Boophilus* detected. The cost of doing a complete treatment series at those premises would have totaled over $60,000 for pesticide, labor, equipment, and vehicles. That money can now be spent to treat premises at higher risk of having a *Boophilus* infestation.

When developing a protocol for a tick eradication or control program, the host preference of the tick should be considered. If the preference for a host is strong, and other species rarely host the tick when the preferred host is not present, then the protocol may be more efficiently aimed at treating those premises which have the more preferred host. It should be realized that for such a protocol to succeed, a record keeping system must be in place which can accurately track treatments and scratch results for all premises within the program.

REFERENCES

HOST PREFERENCE OF *BOOPHILUS MICROPLUS*

Table 1. The estimated prevalence of *Boophilus microplus*-infested non-bovine premises, per 1000. By status of premises within the TEP, Puerto Rico, May, 1987 through April, 1989.

<table>
<thead>
<tr>
<th>Status of Premises</th>
<th>Number Positive</th>
<th>Number Scratched</th>
<th>Prevalence per 1000 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Release</td>
<td>2</td>
<td>1,752</td>
<td>1.1 (0.1–4.1)</td>
</tr>
<tr>
<td>Post-Treatment</td>
<td>4</td>
<td>2,322</td>
<td>1.7 (0.5–4.4)</td>
</tr>
<tr>
<td>Surveillance</td>
<td>26</td>
<td>5,509</td>
<td>4.7 (2.9–6.5)</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Premises Where <em>B. microplus</em> Detected</th>
<th>Premises Where <em>B. microplus</em> Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Bovine</td>
<td>Non-Bovine</td>
</tr>
<tr>
<td>4,159</td>
<td>6,573</td>
</tr>
<tr>
<td>30</td>
<td>5,462</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Odds Ratio 95% CI p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>115.2</td>
</tr>
<tr>
<td>79.4–168.3</td>
</tr>
<tr>
<td>&lt; 10⁻⁸</td>
</tr>
</tbody>
</table>

Table 3. Association between *Boophilus microplus* infestation and the presence of bovines on premises. By number of animals on premises in surveillance status of the TEP, Puerto Rico, 1986–1989.

<table>
<thead>
<tr>
<th>Premises Where <em>B. microplus</em> Detected</th>
<th>Premises Where <em>B. microplus</em> Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Animals</td>
<td></td>
</tr>
<tr>
<td>Non-Bovine</td>
<td>Non-Bovine</td>
</tr>
<tr>
<td>1–2</td>
<td>1,339</td>
</tr>
<tr>
<td>3–6</td>
<td>1,277</td>
</tr>
<tr>
<td>7+</td>
<td>858</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Odds Ratio 95% CI p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.6</td>
</tr>
<tr>
<td>57.4–158.2</td>
</tr>
<tr>
<td>&lt; 10⁻⁸</td>
</tr>
<tr>
<td>112.6</td>
</tr>
<tr>
<td>51.9–258.4</td>
</tr>
<tr>
<td>&lt; 10⁻⁸</td>
</tr>
<tr>
<td>127.7</td>
</tr>
<tr>
<td>58.1–350.1</td>
</tr>
<tr>
<td>&lt; 10⁻⁸</td>
</tr>
</tbody>
</table>
Table 4. Association between *Boophilus microplus* and the presence of various host species on premises where only those species were found. Premises in surveillance status of the TEP, Puerto Rico, 1986–1989.

<table>
<thead>
<tr>
<th>Premises Where B. microplus Detected</th>
<th>Premises Where B. microplus Not Detected</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovines Only</td>
<td>Bovines Only</td>
<td>75.4</td>
<td>47.7–120.7</td>
<td>&lt; 10^-8</td>
</tr>
<tr>
<td>Equines Only</td>
<td>Equines Only</td>
<td>224.4</td>
<td>118.6–527</td>
<td>&lt; 10^-8</td>
</tr>
<tr>
<td>Cap/Ov Only</td>
<td>Cap/Ov Only</td>
<td>3.2</td>
<td>1.4–8.0</td>
<td>&lt; 0.0056</td>
</tr>
</tbody>
</table>
REPORT ON THE COMMITTEE ON
PARASITIC DISEASES AND PARASITICIDES

Chairman: Dr. M. G. Scroggs, Amarillo, TX
Vice Chairman: Dr. Tom Holt, Scotia, NY

L. G. Biehl, IL; R. E. Bohlender, NE; R. A. Bram, MD; A. R. Burgess, WY; J. E. Christy, IL; R. O. Drummond, TX; E. G. Fox, CA; D. A. Gable, VA; R. Gadd, SD; B. Gallagher, SD; T. J. Galvin, MD; S. C. Gartman, TX; D. C. Gigstad, MD; C. H. Graham, MO; F. Gvillo, CA; R. Hack, DE; G. W. Hausman, IA; J. W. Holcombe, TX; R. D. Jones, SD; D. Kimbrell, AR; L. F. Moore, KS; J. E. Novy, MD; R. E. Omohundro, CO; R. L. Pyles, NM; T. P. Siburt, VA; P. L. Smith, CA; J. M. Vetterling, CO; D. D. Wilson, MD; G. L. Zimmerman, OR.

This committee met at 1:30 p.m. on Monday, October 30, 1989, at the Riviera Hotel, Room 3, Convention Center, Las Vegas, Nevada.

The meeting was called to order by Dr. Scroggs, Chairman. Thirty-five persons were in attendance, 17 of which were Committee members.

Dr. Leroy Biehl, Extension Veterinarian, University of Illinois, discussed parasiticides presently available for use in swine to control external parasites. Methods of application techniques as well as withdrawal times were discussed. Dr. Biehl feels that mange could be eliminated from the swine farm if a concerted effort was put forth by the swine producer using the available parasiticides systematically.

Dr. Roger Drummond, Veterinary Entomology Consultant, gave an update on the tropical bont tick project in the Caribbean. Bayticol, an insecticide produced by Bayer, has been found to be effective on the bont tick but a decision in April by an environment assessment group not to use Bayticol has prevented its use. This decision was based on the fact that Bayticol has not been approved for use in the United States.

Dr. Roger Drummond presented a resolution concerning the tropical bont tick project commending USDA-OICD for the information and evaluation component of the project but expressed concern at the lack of progress by USDA-APHIS and USAID in starting the pilot eradication program on Antigua. The resolution also strongly urges USDA-APHIS and USAID to meet and agree on the actions necessary to initiate as soon as possible the pilot tropical bont tick eradication program on Antigua. This resolution was passed and is referred to the Resolutions Committee.

Dr. Dave Wilson, Senior Staff Entomologist, APHIS, reported on the incidence of exotic ticks found on imported ostriches this year. The ostriches were found to be infested with three species of exotic ticks, two of which are known vectors of heartwater, a serious disease of ruminant animals. Shipments of ostriches reached Illinois, Ohio, Oklahoma and Texas. Premises receiving the ostriches were quarantined and both the ostriches and premises treated with acaricides at appropriate intervals. Premises receiv-
ing juvenile ostriches since January 1, 1989 were similarly treated. Surveillance activities, including the use of sentinel animals and wildlife surveys, indicate that the establishment of exotic ticks in the United States has not occurred. In addition, imports of ostriches and other ratites have been prohibited in order to prevent further introduction and dissemination of exotic ticks and other potential harmful ectoparasites.

A presentation on Ivomec Pour-On was given by Dr. Jim Cox, a project leader for MSDRL, Merck & Co., Inc. This product has not been approved by FDA at the time of the committee meeting but is expected to have claims against various internal parasites as well as external parasites, including grubs, sucking and chewing lice, two mange mites and horn flies.

Dr. Doug Armstrong, Norden Laboratories, made a brief presentation on the safety and efficacy properties of albendazole. The product is marketed as Valbazen and has activity against various gastrointestinal round worms and tapeworms plus liver fluke.

A couple of years ago, this committee submitted a resolution requesting various agencies and companies to find and develop a vaccine for the prevention of trichomoniasis. Dr. John Schnackel, Ft. Dodge Laboratories, presented information on their vaccine for this disease. The vaccine has been conditionally approved while further field testing is being conducted.

A status report on the Puerto Rico Tick Eradication Program was given by Dr. Randall Crom, APHIS. "Hugo" caused a delay in the program on the East end of the island. The entire island is now under the program. Eight thousand six hundred thirty-three (8,633) premises with 65,195 animals have been treated. A new protocol for non-bovine premises has been implemented. Quality control units have been established at each regional office.

Dr. S. E. Kunz, ARS, reported on various projects being conducted by the Kerrville laboratory. These included tick control along the Southern border of Texas and the Caribbean, development of a grub vaccine and the pyrethroid resistant horn fly situation.

Dr. J. Novy, APHIS, reported that the New World screwworm had been found in Libya. Measures have been taken to control the spread of this pest on the continent of Africa.

Dr. Gary Zimmerman, Veterinary Parasitologist, Oregon State University, gave a very informative and interesting presentation on controlled release technology being developed for various drugs to control parasites. He also gave a brief update on the status of Nematodirus battus, a parasite of sheep. This parasite has been found in various areas of Oregon.

It is suggested that information on Cryptosporidiosis be presented to the committee in 1990 to determine if this is a concern and what action should be taken to control the situation.

This committee adjourned at approximately 4:30 p.m. after a very informative meeting.
MARKET HOGS VERSUS ROASTER PIGS: ARE THERE RESIDUE DIFFERENCES?

Janice Webb, D.V.M.
USDA/FSIS/Science
Residue Evaluation and Planning Division
300 12th Street, S.W., Room 602
Washington, D.C.

During the spring of 1988 when sulfamethazine received national attention as a possible carcinogen, Food Safety Inspection Service and FDA jointly intensified efforts to identify and educate producers who sent sulfamethazine adulterated animals to slaughter. These efforts were coordinated, to some extent, by the Inter-Agency Resident Control Group (IRCG) in Washington, D.C. The IRCG is composed of representatives from FSIS, FDA, APHIS, and EPA who meet on a regular basis to review, discuss, and project corrective measures to ensure residue control in meat and poultry.

As a result of the IRCG focus on swine, another potential problem was brought to the forefront: the use of carbadox in pigs intended to be used for feeder pigs, but purchased and slaughtered for use as roaster pigs. The problem is compounded by roaster consumption among ethnic groups who usually consume the entire muscle mass as a non-comminuted product, and thereby receive a much higher dose of an adulterant than one would expect if only portions of an adulterated carcass were consumed. Carbadox is classified by FSIS as an A-3 compound which means it is a compound with a high health hazard potential and a moderate potential for the likelihood of residue occurrence. It is approved for use in swine weighing less than 75 pounds to prevent or treat dysentery, enteritis, and for increased feed efficiency and weight gain. It has a 10-week withdrawal period; the parent compound is a liver carcinogen.\(^1\)

The swine slaughter class is divided into market hogs, boars or stags, and sows. FSIS has no roaster pig slaughter class, nor criteria for defining such a class. After consultation with USDA's Extension Service, the criteria were set as such:
1. 2–4 months of age
2. 40 to 125 pounds

In September and October of 1988, the Residue Evaluation and Planning Division (REDP) of FSIS conducted a cursory study (26 samples from 2 slaughter establishments) to identify the potentiality of roaster carcasses being adulterated with carbadox. In an effort to gain as much information as possible about this subclass of market hogs, the samples were also analyzed for antibiotic and sulfonamide residues. The results are as follows:
1. One sample was positive for the antibiotic chlortetracycline at 0.11
ppm. The tolerance level is 1.0 ppm.

2. One sample was positive for carbadox at 0.03 ppm. The tolerance level for carbadox in edible tissue is 0.03 ppm.

3. None of the samples were positive for sulfonamides.

In March of 1989, more than 400 roaster pigs were presented for slaughter at an establishment in California. A significant number of these were found to have sulfonamide residues beyond that acceptable for human food. REDP requested that 3 carcasses also be tested for carbadox. One of the three was violative, i.e., contained 0.32 ppm. These two small sample groups, the 26 collected in 1988 and the 3 analyzed in early 1989, indicated a potential problem of carbadox adulteration in roasters. Because of the small number of samples, however, sound conclusions could not be drawn.

In June, July, and September, 1989, REPD sampled another 167 roasters from 12 federal or Talmadge-Aiken slaughter establishments. Positive and/or violative results are given in the following table.

Table 1. Residue Data from 1988–1989 Roaster Pig Surveillance Program. Units are given in parts per million.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Antibiotic *</th>
<th>Sulfonamide *(kidney)</th>
<th>Carbadox *(liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*(kidney)</td>
<td>*(muscle–m)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>*(liver–l, kidney–k)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>.19 (m)</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>.550</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>.226</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>.56 (m)</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>.070</td>
</tr>
<tr>
<td>6</td>
<td>Streptomycin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(.47) k</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>.13 (1)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.05 (m)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>.50 (m)</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>ND</td>
<td>.025</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>ND</td>
<td>.096</td>
</tr>
</tbody>
</table>

ND = none detected * = organ(s) tested

Tolerance concentrations in edible tissue of market hogs are: 0.10 ppm sulfamethazine in muscle, and 0.03 ppm carbadox in liver. Streptomycin has a 0.5 ppm in muscle, and an administrative tolerance of 2.0 ppm in kidney.

As one can see, the 10 samples resulted in a positive but non-violative streptomycin, 3 sulfonamide violations in muscle and 1 violation in liver, 4 violations and 1 non-violative level of carbadox in liver.

When one compares the results of this study to the data generated in the 1988 National Residue Monitoring Program, distinct differences are noted.
MARKET HOGS VERSUS ROASTER PIGS

between market hogs and roaster pigs. Table Two contains this comparison of violations by target tissue.

Table 2. Residue from 1988 National Residue Monitoring Plan (market hogs) and 1988–1989 Roaster Pig Surveillance Program. (Figures are number of violations/number of samples analyzed).

<table>
<thead>
<tr>
<th></th>
<th>Market Hogs</th>
<th>Roaster Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics (kidney)</td>
<td>9/1035</td>
<td>0/193</td>
</tr>
<tr>
<td>Sulfonamides (liver)</td>
<td>21/1266</td>
<td>4/193</td>
</tr>
<tr>
<td>Carbadox (liver)</td>
<td>2/230</td>
<td>4/193</td>
</tr>
</tbody>
</table>

There are sulfonamide and carbadox residues associated with the roaster pig sub-slaughter class. For this reason, the 1990 National Residue Monitoring Plan will most likely contain an expanded surveillance program to aid in the control of carbadox residues. In 1991, REPD may institute roaster pigs as an official slaughter class.

REFERENCES


Acknowledgement:

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REPORT OF THE COMMITTEE ON PHARMACEUTICALS

Chairman: Dr. G. D. Lindsey, Indianapolis, IN
Vice Chairman: Dr. D. O. Farrington, Whitehouse Sta., NJ

D. A. Armstrong, NE; L. G. Biehl, IL; B. J. Bilas, DE; W. B. Bixler, VA; R. E. Bohlender, NE; M. L. Crandall, VA; J. E. Fox, GA; D. A. Gable, VA; R. A. Gessert, MI; D. D. Gingerich, IA; J. A. Gloyd, IL; C. H. Graham, MO; J. R. Harr, NY; G. W. Hausman, IA; J. S. Hayden, MO; W. A. Knapp Jr., NC; L. Leach, VA; K. B. Meyer, IN; G. A. Mitchell, MD; J. F. Mock, NJ; L. F. Moore, KS; T. A. Neuzil, IA; P. W. Reeves, MO; R. Schultz, IA; M. G. Scroggs, TX; M. Sharar, MD; T. K. Shotwell, TX; T. P. Siburt, VA; R. F. Taylor, NJ; J. Webb, DC.

The committee met at 1:30 PM on Tuesday, October 31, 1989 at the annual meeting of the U.S.A.H.A. in Las Vegas, Nevada. Eighteen committee members and 11 guests were in attendance.

The committee continued its emphasis on means of minimizing violative drug residues in livestock and poultry offered for slaughter as well as in milk and eggs. Milk, meat and eggs are reported to represent 52% of the gross national product of the U.S. Agricultural industry. While not considered to be a consumer safety problem, occasional reports of residues in excess of officially recognized safe tolerance levels tend to undermine consumer confidence in the safety of food derived from animals as well as the ability to maximize export sales of such products.

The committee feels that the U.S.A.H.A. with its broad membership base of multiple species production groups, academia, the veterinary profession, state and federal regulatory agencies and industrial service groups including the pharmaceutical and meat packing industries, provides a unique forum for addressing the complexity of issues affecting violative drug residue elimination.

As with any industry, adequate production quality assurance programs are needed in every production unit to assure that every drug product is used responsibly and that prescribed preslaughter withdrawal or premarketing withholding periods are observed.

Considerable progress is being made in the development of such quality assurance programs in larger production units. Most species producer organizations are actively engaged in related program development and education. However, much remains to be done in order to achieve adequate production quality assurance in smaller production units.

The committee believes that few, if any, livestock or poultry producers knowingly or willingly contribute to the drug residue problem. Rather, they appear not to fully understand the characteristics of drug products used in their operations and their potential to result in violative residues. Thus, a continuing emphasis on producer awareness and education is needed.
Wide availability and uncontrolled distribution of unapproved and improperly labeled animal drug products are also believed to be major contributors to the problem.

Dr. Leroy Biehl, University of Illinois Extension Veterinarian reviewed the progress of a pilot voluntary sulfonamide residue certification program involving the FDA, FSIS, APHIS, national and state pork producer organizations practicing veterinarians and the University Extension Service. The NPPC provides funding for the testing and farm inspections by veterinarians. The program features a method of premarket testing, farm surveys and field monitoring on a regular basis. The objective is to assure the marketing of swine free of violative sulfonamide drug residues. The owner is assured of no further residue testing at the slaughter plant.

The program is limited to producers using sulfamethazine in their herds. Since the use of that drug has dropped precipitously, only a limited number of producers have participated. The pilot program indicates that an on-farm testing certification program is a feasible method to prevent residues. It is the committees hope that such a model program might be expanded to include certification of freedom of residues of all drugs and be more widely adopted by the swine industry and perhaps in other species.

Two studies conducted in 1988 and 1989 by the Residue Evaluation and Planning Division of FSIS, to determine if antibiotics, sulfonamides, and carbadox were problems in roaster pigs, i.e., loosely defined as pigs 2–4 months of age and 40–125 lb. in size, were discussed by Dr. Janice Webb, Residue Staff Officer, FSIS. Results indicated that, when compared to market hogs, antibiotic residues are less frequent, sulfonamide residues are at least as frequent and carbadox residues appear to be more frequent in roaster pigs. During 1990, an extended surveillance program for roaster pigs will continue and could possibly result in the eventual creation of a subslaughter class of roaster pigs. Cull dairy animals were also discussed in two aspects, bob veal and culled adult animals, specifically cows. The violation rate in bob veal has decreased from 7% in 1978 to about 2-1/2% in 1989. A task force met in Washington D.C. recently to review the bob veal testing program. Task Force recommendations will be complete by the end of the year. Afterwards, a Docket Committee will be formed to write the final CAST rule. FSIS has conducted further testing of in-plant STOP positive samples. Sulfamethazine, sulfadimethoxine, and gentamycin residues were found. This will most likely lead to the development of a new in-plant test which will detect both sulfonamides and antibiotics.

The status of the FSIS Residue Memoranda of Understanding (MOU) Program was also reported by Dr. Webb. MOUs are voluntary programs developed by an individual producer or company, in agreement with FSIS, to aid in the prevention of adulterated animals presented for slaughter. The purpose of MOUs is to prevent the occurrence of residues pre-slaughter. Currently, by poundage, 40% of turkeys, 20% of broilers, 3% of fed cattle, and 0.1% of hogs are produced under MOUs.
Dr. Webb reported that production residues are generally caused by misuse of a compound, i.e., withdrawal times or label directions are not followed. Environmental residues are generally caused by fomites, contaminated feed & bedding, are inadvertent and their distribution is relatively small. Changes in the National Residue Monitoring Plan were described to help identify environmental residues on a more consistent basis.

Dr. Joe Lyons, Western Regional Residue Staff Officer, discussed current residue screening methods used by FSIS. Rapid tests, which can be conducted on-the-spot in slaughtering or processing plants, are becoming more widely used in meat and poultry inspection. Rapid tests can detect illegal residues from drugs and chemicals, determine what species is present in a product, or reveal if a product has been cooked or canned properly. They are much less expensive and faster than laboratory tests and their use has significantly improved the ability of FSIS to ensure a safe, accurately labeled meat and poultry supply. The residue tests include:

STOP or Swab Test On Premises. Implemented in 1979, this was the first rapid test developed by FSIS. It detects the presence of antibiotics. Originally, it was used only on dairy cows; now it is used on all species.

The test is conducted by taking a swab sample from the kidney of the animal carcass and placing the swab on an agar plate that contains bacteria. If the area around the swab is clear after 24 hours, antibiotics are present and have killed the bacteria. Further laboratory confirmation is required before the carcass is condemned.

CAST or Calf Antibiotic and Sulfa Test. This test detects the presence of antibiotics and sulfa drugs. CAST is used only in bob veal calves, which are those under 150 pounds and less than three weeks old. Sulfa drugs are used more frequently in bob veal calves than in older cattle, and CAST is sensitive to the presence of sulfa residues. CAST is conducted the same as STOP. Unlike the other rapid residue tests, however, CAST does not require laboratory confirmation of the result. Any violation found under CAST results in immediate condemnation of the calf.

SOS or Sulfa On Site. Used since April 1988, SOS is a swine urine test. It detects the presence of violative levels of sulfamethazine, an antibiotic that preliminarily has been linked to tumors in mice and rats. SOS is in use in 100 of the largest swine slaughtering facilities. It is a chemical separation test that separates any sulfa compounds present from the urine. If sulfa is present, it will show up under an ultraviolet light when sprayed with a fluorescent compound.

CUT or Cattle Urine Test. Recently developed and being tested on a limited basis, CUT can detect the presence of chloramphenicol in cattle. Chloramphenicol, an antibiotic, has been banned for use in food animals as it can cause aplastic anemia in susceptible people. The test is conducted by placing a urine sample on a card that has been pretreated with antibodies.
PHARMACEUTICALS

If chloramphenicol is present, the resulting reaction causes the card to remain white instead of changing color. Ultimately, CUT will be used by the FSIS nationwide.

As a result of a recommendation by the Pharmaceutical Committee at its 1988 meeting, the USAHA requested that the director of FDA's Center for Veterinary Medicine (CVM) consider adopting more flexible labeling for antimicrobial injection drugs bearing the veterinarian prescription legend. Dr. Donald Gable of CVM reported that a joint AVMA-Animal Health Institute working Committee of Flexible Labeling was established in June 1989. This committee is chaired by Dr. Samuel Strahm, president of the AVMA. Membership includes AVMA members and staff personnel, AHI members and staff personnel and an advisor from the CVM.

The charge of the committee is to describe the scientific data requirements and their rationale as well as to identify changes needed in the regulatory process that would allow revisions in the labeling criteria for animal drugs used under the supervision of a veterinarian. Changes proposed will be consistent with measures for protection of both human and animal health. The committee will be attentive to the unique role occupied by the veterinarian in the diagnosis and treatment of animal species. Such revisions would allow the veterinarian flexibility in the clinical environment. The working committee has held two general meetings, in June and September, 1989. Staff personnel from AVMA, AHI and CVM and respective legal councils met in August 1989 to review legal considerations.

Dr. Gable who is CVM's advisory member to the committee, provided an overview of the complexity of implementing these procedures within existing programs and policies for the pre-clearance review of new animal drug products.

A status report on animal drug bound tissue residue considerations was provided by Dr. Gable. He reported the CVM will publish a Federal Register notice outlining how they propose to evaluate bound tissue residues of carcinogenic animal drug products. Specific studies will be required to provide data to document the inactivity or unavailability of part or all of the total tissue residues. Provided such data, the CVM will discount an appropriate percentage of the total tissue residues in the establishment of the safe concentration (tolerance) for the product.

Dr. Gable reported on the status of agency consideration of sulfonamide residue safety and related residue tolerances. Issues are 1) the tolerance in tissues based upon the NCTR chronic bioassay studies, and 2) the scientific data base supporting the secondary mechanism for carcinogenesis and the biological difference between animal species, including man.

It is highly probable that the NCTR chronic bioassay data will require a lowering of the tolerances in the tissues of food-producing animals. The tolerance for sulfamethazine may be as a low fraction of 1.0 part per billion (ppb). The present tolerance is 100 ppb (0.1 ppm).
Dr. Gable expressed the opinion that there is a strong scientific base supporting a secondary mechanism for carcinogenesis. The biological difference between animal species is irrefutable. He stated that the acceptance of secondary mechanisms of carcinogenicity is supported by knowledgeable non-regulatory and regulatory scientists. He made specific annotation of the Environmental Protection Agency's position on the toxic compounds and the recent affirmation by the Joint Expert Committee on Food Additives of the World Health Organization (WHO) and Food Agriculture Organization (FAO) of a tolerance of 100 ppp (0.1 ppm) for the marker residue and 300 ppb (0.3 ppm) for total residues of sulfamethazine in the tissues of food producing animals.

Dr. Gable provided a summary of new animal drug approvals by CVM during FY1989. Two new entities, albendazole and maduramiun ammonium were approved for use in food animals along with formalin solution for control of protozoa, trematodes and fungi in fish.

Dr. G. A. Mitchell, Director of CVM's Division of Compliance and Surveillance, reviewed several items of interest. The agency is taking a rigid stance relative to compliance. State/Federal agreements are in place in 20 states representing 60% of all swine production. Letters to producers of violative residues are intended to reduce repeat offenders. The status of prosecution and sentencing of numerous bulk drug violators was reviewed.

Dr. Mitchell indicated that the agency will monitor pioneer and generic drug suppliers equally with emphasis on drug listing and establishment registration, compliance reporting and good manufacturing practices. Monitoring inspection of bioequivalence drug testing and pivotal studies for agency approval evaluations will also be emphasized. The number of FDA inspectors assigned to these tasks is expected to increase.
SALMONELLA ENTERITIDIS CONTROL:  
REGULATORY CONSIDERATIONS  

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State Veterinarian, California Department of Food and Agriculture  
Sacramento, California

I suspect my observations on problem-solving are far from original — but I am impressed by one reality:

Most problems they face may be solved quite simply if only they would take an obvious action.

The kinds of problems I face are all terribly complex and lack simple straightforward solutions.

The selection of proper governmental response to Salmonellosis and to Salmonella enteritidis (S. e.), in particular, is probably one of the latter.

In this presentation, I will be making observations involving the Proposed Voluntary Model State Program for Salmonella enteritidis Quality Assurance. I do not want my remarks to be interpreted as critical of that Model. I respect the developers of the Model and applaud their efforts and product. I am particularly impressed with the proposal in view of the fact it was created at a time of urgency and under extreme pressure.

I believe the Model Plan has its most appropriate use at the primary breeder and multiplier levels of the egg laying industry. However, I have concerns about the possibility of its widespread application at the production level. Another concern is, even though the proposal is voluntary, government and sectors of industry have a way of applying pressure through required or threatened marketing constraints which make volunteerism mandatory. Live animal and product movement constraints are a necessary function of disease control. Often, however, they may be ill-advised because the implementation is demanded through fear, perceived problems, and sometimes for marketing advantages.

Clearly, we cannot be sanguine about a problem such as Salmonellosis which touches half a million lives each year and costs society millions of dollars annually in direct and indirect losses.

It is unacceptably cold and unfeeling for us to categorize those who have experienced personal tragedy as an insignificant few whose misfortune really lies in other disease processes or immune impairments, or who are trapped in an institutional feeding environment.

Also, whether we could so choose or not, we are bound to respond to those who would imperil the nation’s animal industry and deprive its citizens access, through fear, to the world’s finest, least expensive, and by far its safest supplies of human food.

Dr. Duncan McMartin, University of California, Extension Service, recently emphasized: “Few animal products are so safe for human use as
those from poultry. Both nature and human design have contrived to protect the egg’s final production site from all exogenous disease agents through a most extraordinary filtration and detoxification process and then pre-wrapped it in a biologically safe container that, barring exceptional abuse, guards its contents almost as well as the fanciest plastic package.”

Poultry producing industries are highly sophisticated and efficient. Disease and quality control are vigorously pursued to insure maximum return with minimum losses.

Poultry meat has a relatively short production life with limited time for entry and amplification of dangerous agents. The environment of modern poultry processing operations is clean, decontaminated and disinfected regularly with environmentally safe procedures.

The consumer is the ultimate beneficiary with highly nutritious, properly safe, and extraordinarily economic supplies of human food.

Nevertheless, we must address the continuing consumer fear of disease and a deteriorating confidence in foods of animal origin. The same concerns, so often fueled by an incompletely informed or willfully disruptive press, spill over into allegations of governmental malfunction; or, at the very least, a failure of direction.

The economic pressures that follow inevitably drive attempts to avoid loss or to gain market advantage. The process of convincing frightened consumers they need to choose only a specific brand or a different source for their food to avoid the awful Salmonella is happily assumed by advertising and promotion people whose responsibility has always been the “selling” of perceptions unhindered by truth.

A recent Center for Disease Control’s (CDC) investigation made a hypothesis that disinfected table eggs were the source of human disease. However, repeated attempts to establish experimentally a model of nature that would confirm egg-borne transmission as a determinative feature have encountered minimal success.

The scientists whose business it is to understand the agent and its ecological niches continue to have difficulty establishing that anything really new or different is happening with Salmonella.

The most frightened epidemiologists seem to be those who ignore some pretty basic observations that serious disease among humans with S. e. is, and always has been most notable among the immune-impaired. The epidemics or clusters of infection always seem to be related to food abuse. The epidemics have been seasonally associated with warm weather that exacerbates food abuse problems.

At this time, permit me a couple of disclaimers. First, I am not a research microbiologist. I am possibly capable of spelling Salmonella generically, but have some real trouble with some of the species. There are a whole lot of things that a whole lot of you, the participants at this annual meeting,
could tell me about this microbe. Second, I am not in the livestock production industry. I seldom own more than a couple of frozen fryers at any one time, and only rarely a whole turkey.

But I am in the Regulatory Medicine business, and I am in a State with a major poultry industry contributing significantly to gross state production.

And, as many of you, I am proud and gratified to recall the long list of accomplishments of regulatory veterinary medicine — and the many difficult campaigns conducted by Veterinary Services and by the states in shielding America’s livestock and its citizens from dangerous or exotic disease. But, I can reflect as well upon the great costs borne — both directly and indirectly in the effort, by the production industries and by the public through forfeiture of other societal needs in order to muster the required resources.

California is home to thirty million people whose lives are enhanced by wholesome and economically plentiful products of our — and the nation’s — animal industry. There are many of those thirty million people in California who have no knowledge of their sometimes fragile and vulnerable food production miracle, but they have plenty of fear.

Fear is the consequence of sensationalism in the media, which is often nourished by agencies within government and inadequately informed local health and agricultural officers. Fear is also perpetuated by our own too often histrionic efforts to pursue some only partly-related agenda.

In my opinion, a national commodity promotional organization that describes terrible health hazards likely to be found in their own product simply to attempt to force a government to assign assets or impose sanctions is a classic example of effective marksmanship using their own big toe as the target.

I think too that those who are attracted to organized activities for organization’s sake — and perhaps too bemused over the efficacy of rules or regulations — trade far too often on the perception of risks regardless of the likelihood of success of their hypothetical solutions.

In response to governmental and public concerns about S. e. California, as have many states, has placed an emphasis upon surveillance for, and the epidemiology of, S. e. infections. In California, we have been typing Salmonella isolates from our livestock for several years and have a pretty fair estimate of the spectrum of paratyphoid that is likely to be found in our livestock, their feedstuffs and environment. We have identified S. e. twenty-odd times since the problem in the Northeast was announced. About half of those identifications were in mammals and the others in poultry, including chickens, turkeys, and ducks. The isolates involving poultry were almost entirely environmental. Twice, we have identified S. e. in white leghorn flocks.
The two white leghorn flocks did not fit the criteria on systemic infections as outlined in the model program. However, the diagnosticians involved were concerned about systemic infection in at least one case. These two cases did provide an opportunity for an in-depth investigation to test the basic hypothesis of the importance of egg-transmission, which is driving us toward a control program directed at this serotype. It is this hypothesis that has been extended to include internal contamination of table eggs, which has been so heavily emphasized by the CDC investigation and others that have imperiled the producer in the Northeast.

In the first of these outbreaks, about forty-thousand pullets approaching caging were quite uneven and suffering from a variety of problems, including severe coccidiosis. Sample mortality in the laboratory also revealed S. e.

An investigation included the testing of the breeder source of these birds. Every hen owned by the breeder firm was tested. One animal with S. heidelberg was revealed in this 100% test. There was absolutely no evidence implicating the breeder birds in the S. e. event.

The second outbreak was among an even larger placement of chicks consisting of both California and out-of-state hatched birds from a single genetics firm. Five-day mortality was slightly high and routine laboratory submissions revealed about 10 percent infection rate of S. e.

Once again, every single hen owned by this genetic firm contributing eggs to the hatch was serotested. A very large number of cloacal and environmental swabs were cultured. Absolutely no S. e. was revealed.

Now, I want to point out that these were not sample tests of three hundred or so animals, but every animal (100%) of the known breeder sources for the production flocks was serologically tested. Both these genetics firms had actually already confirmed, repeatedly, that there was no paratyphoid activity within their operations, simply because they cannot afford to be identified as the source of some serious Salmonellosis event in a production flock. This effort continues, and will continue, as these firms address their own responsibility without further governmental dictum. Nevertheless, it is unlikely that any amount of breeder flock testing would have interdicted these infections.

In the first of these events, S. e. was not found in environmental samples. Several paratyphoid organisms, not S.e., were found in the feed at the time of investigation. This may or may not have been the source of infection, since we were one complete feed-delivery behind the event. In the second case, feed was almost certainly the decisive factor, with a garden of Salmonella being found in almost all feed samples. One environmental sample from one of the hatcheries providing the chicks revealed S. e.

We believe the investigations have demonstrated that in these cases breeding stock were not determinative sources, and those stocks already have in place a highly effective surveillance and preventage program.
enhancing the National Poultry Improvement Plan. I doubt this effort can be improved upon, regulations or no. The outbreaks demonstrate that having S. e. free genetic source flocks did not prevent these two white leghorn flocks from becoming infected.

The proposed Model Plan for S. e. advocates initial emphasis on testing of primary breeder and multiplier flocks. This testing has been accomplished by many states, including California. However, if the Model Plan is extended to include all production flocks, its imposition would be an onerous burden and may actually jeopardize our ability to protect animal and human health.

In California we maintain an average of thirty million birds in lay. They are managed within about 90 ownerships on 430 separate premises. If we consider only the fifty-odd major owners, they will have about 1,000 different age/house groups of birds. For these owners to comply with the draconian requirements already being attempted by one state; and to continue that surveillance for qualification, the resulting burden on California’s veterinary diagnostic laboratory system would preclude most other functions including prosecution of legislatively mandated programs in brucellosis, for example, let alone any opportunity to support endemic and exotic disease surveillance. Far more dangerous threats to economic production would have to remain unaddressed.

Nor would this effort really contribute to public protection. The marketplace would advertise a product with such terms as “Salmonella free”, only to inevitably be found wanting next summer as the potato salad and warm mayonnaise season comes again.

Government then has a real opportunity to “do something” out of fear. It is important that we not seize that opportunity and embarrass ourselves and our industries. It is urgent that we not contribute to further degradation of public confidence when a sample plan with very limited sensitivity is used to support a “certification” program that contributes little or nothing to disease control.

But, let us suppose we do choose to mount a truly determined campaign to eradicate S. e. Full implementation of the Model Plan will require an extensive eradication campaign, resulting in high societal costs and extensive industry disruption. We need only look at our struggling counterparts in England for a microcosm of such a campaign in the United States.

A precedent is being set by agricultural agencies to resolve a public health problem with a program directed toward an agent causing little economic impact to poultry. There are no measurements of program effectiveness other than reported human cases, which could be environmental in origin, food items other than eggs or due to food abuse. The brucellosis and tuberculosis eradication programs addressed major public health issues in addition to eradicating two diseases which are economically catastrophic to the livestock industry. Yet with these programs,
restrictions on food products were incremental, not abrupt, as proposed for S. e. infection. Herds were handled on a test and slaughter basis, with the loss of individual reactor animals only. Only in the last few years has herd depopulation become a program element. S. e. proposals allow only depopulation of an affected laying flock because egg marketing is completely disrupted.

After we have eliminated S. e., where will we be? We will be facing new allegations that some other paratyphoid has sickened or killed someone who must have been infected through an egg. In other words, what Salmonella will be the next target of a Model Plan?

To me, it makes absolutely no sense at all to mount an eradication effort against a single serotype. It might be observed that successful programs have been directed against single serotypes of Salmonella, such as S. pullorum and S. gallinarum. These organisms are highly adapted to the avian species that allowed eradication procedures to be successful. However, the other 200 some paratyphoid serotypes that have been isolated from domestic poultry are ubiquitous, lending to a strategy of control rather than eradication.

What do we know about the source of Salmonella in poultry? Many studies over the past several decades have demonstrated that paratyphoids occur in poultry from one or more of three sources: egg transmission, environmental contamination, or feed contamination.

The determination of the source in the two events previously described was not definitely established. However, large scale epidemiological investigations conducted in California (Dillon Beach and California/Minnesota Salmonella projects) have established the most common source of Salmonella infection is environmental or contaminated feed.

The Model Plan does address feed and environmental management, but it does so by admonition alone, simply because that's about all you can do. I question if a national program is required to make that admonishment any more effective.

What should and what should not we be doing? I believe we certainly should not be restricting interstate commerce by ill-considered restraints. By this, I mean not instituting requirements for the purpose of "window dressing" protection. In California, we are quite dependent upon the free entry of poultry products from many other states, including several millions of started leghorn pullets from Missouri and tens of millions of broiler hatching eggs from Arkansas, Colorado and others. We have no evidence that our poultry or poultry products in shipments are putting us at risk; therefore, we do not plan on instituting changes in interstate shipping requirements.

I also believe we should not be strongly advocating the implementation of the Model Plan on the egg production level, because there are more cost effective measures for controlling Salmonella.
SALMONELLA ENTERITIDIS CONTROL

Clearly, there are things we should be about — to address this troublesome and costly source of human and animal disease. In my opinion, we need to do a lot more of the things we already know how to do and we know will work. We do need to support disease-free foundation primary breeder and multiplier flocks to place specific disease-free chicks on production farms. The production farms need further instruction in providing the means to reduce environmental and feed delivery system contamination to a minimum. We then need further exploration of means to utilize the bird's own immune system or other physiological or microbiological strategies to reduce the need for drug therapy.

The Salmonella public health problem is appropriately of major concern. S. e., however, represents only a part of this problem. The Model Program and societal pressures are addressing the resolution of only the S. e. segment. The main thrust of the approach is to stop spread in humans via egg transmission. True vertical egg transmission plays an insignificant role in disease spread. A more success-oriented program would be to place emphasis on hygienic food delivery systems and reducing Salmonella contamination of poultry products by addressing management factors involving environmental contamination at the production, slaughtering and processing levels, and most importantly at the feed production, delivery and storage levels.

Let us avoid further exacerbation in the press and in the minds of critics that our food-animal industries are in some way mindlessly risking the public's health and happiness. The food-animal industry has been, and is, providing a safe and economical food supply to this country. There are adjustments that are continually being put into the system to make it more successful. Let us — government, scientists, and industry — be certain that the prescribed adjustments are necessary and effective.
Microbiological Contamination of Raw Poultry Meat and Eggs

Salmonella Enteritidis Seminar Co-sponsored with the Committee on Salmonella.

In 1988, USAHA President Phil Bradshaw appointed an ad hoc Committee on *Salmonella enteritidis* to recommend how USAHA might involve itself effectively in the national discussion and resolution of the issue of increased prevalence of *Salmonella enteritidis* infection in humans associated with consumption of Grade A shell table eggs. This committee recommended a joint seminar meeting of the Committee on Salmonella and this committee.

The rationale for the joint meeting was that the membership of these two committees represented much of USAHA's and the nation's expertise on the subject of salmonella infection in poultry and, through the joint meeting, resolutions could be generated which would guide USAHA and the nation towards effective solutions.

The joint meeting/seminar was held on Tuesday, October 31, 1989, and
the seminar proceedings are reported elsewhere in these USAHA proceed-
ing.s.

Two resolutions, regarding *Salmonella enteritidis*, were adopted unani-
mously by the two committees and were submitted to the Committee on Resolutions for consideration. One pertained to the need for the develop-
ment of a contingency plan for the eradication of *Salmonella enteritidis* Phage Type IV if it is introduced into U.S. poultry flocks. The second addressed the need for formation of a task force involving USAHA, USDA, U.S. Department of Health and Human Services, state agencies and the poultry industry to develop a control program for *Salmonella enteritidis* infection in the commercial table egg industry.

**CONTINUED DISCUSSIONS ON SALMONELLA ENTERITIDIS**

The committee continued discussion on *Salmonella enteritidis* on No-
vember 1, 1989. Most of the discussion was about the announcement by Dr. Joe Madden, Food and Drug Administration, of that agency's intent to propose a national, mandatory *Salmonella enteritidis* testing program for egg type chicken breeds and commercial laying flocks.

The committee was unanimous in its strong opposition to the Food and Drug Administration intent. It is felt by the Committee that the proposed testing program was premature and that there was not a scientific base of knowledge necessary to construct a dependable program at this time.

A third resolution on *Salmonella enteritidis* was passed by the commit-
tee in response to this discussion and was forwarded to the Resolutions Committee for consideration. The resolutions conveyed the sense of the committee opposition to the Food and Drug Administration's proposed testing program and the reasons for the feeling.

**DISEASES OF IMPORTANCE AND RELATED ISSUES**

*Chicken Anemia Agent (CAA)*

Dr. S. Harris, APHIS-NVSL, reported that chicken anemia agent (CAA) infection was identified in commercial broilers in the Delmarva area in May of 1988. The agent had previously been shown to be in the United Kingdom, Europe and Japan. Surveys prompted by this isolation indicated that it is widespread in the United States. Flocks supplying specific pathogen free (SPF) eggs have been shown to be sero-positive using an indirect immuno

munofluorescent antibody (IFA) assay and a virus neutralization (VN) test. There is a concern that poultry biologics manufactured using these eggs may be contaminated with extraneous CAA. In an effort to help the egg suppliers establish and maintain CAA-free egg sources, the National Veterinary Services Laboratories (NVSL) has been supplying the SPF egg producers with the reagents necessary to perform the IFA test. These reagents are also being supplied on request to the biologics manufacturers.
Several projects are underway at the NVSL to improve our CAA diagnostic capabilities and to further characterize the U.S. isolates. An enzyme linked immunosorbent assay (ELISA) for CAA antibody is presently being developed. The ELISA is being compared to the IFA test and the VN test with respect to sensitivity and specificity. A monoclonal antibody (MoAb) specific for CAA was produced and characterization studies are underway. The MoAb will be used as the capture antibody in an ELISA system to detect extraneous CAA in vaccine master seed and serials. A study to compare the pathogenicity of the United States, Japanese, and German isolates was delayed last year due to a lack of CAA antibody-free eggs, but this study has been re-scheduled for November 1989.

VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE (VVND)

Dr. M. Mixson, APHIS-VS, reported that during Fiscal Year 1989, there were 43 foreign animal disease investigations in which VVND was suspected. During this period, three outbreaks of exotic Newcastle disease were diagnosed and eliminated in pet birds. There was no involvement with or spread to poultry.

On June 14, 1989, the National Veterinary Services Laboratories (NVSL), Ames, Iowa, confirmed exotic Newcastle disease in 17 Amazon parrots and a sun cockatoo from a private bird collector located in Stratford, Connecticut. The owner claimed to have purchased seven young Amazon parrots from a dealer in Houston, Texas. All birds bought and sold by the collector were traced, quarantined, and tested for Newcastle disease. All attempts to trace the source of infection resulted in no further VVND isolations. Specimens collected from one of the parrots which was purchased from the collector and was in a pet shop were positive for VVND virus. All birds belonging to the collector and the pet store owner were depopulated and both premises were cleaned and disinfected. No domestic poultry were involved in the outbreak.

In July 1989, Fish and Wildlife agents of the U.S. Department of Interior seized approximately 115 allegedly smuggled birds on a premises in Spring Valley, California. The seized birds were placed in the U.S. Department of Agriculture’s (USDA) Quarantine Station at San Ysidro, California. Later, several of the birds appeared sick and samples were collected and submitted to NVSL, Ames, Iowa, where an exotic Newcastle disease virus was isolated. The exposed birds, some of which are on the endangered species list, were maintained in isolation at the quarantine station for 120 days and were tested negative for Newcastle disease, before they were released to Fish and Wildlife Service authorities.

On August 2, 1989, NVSL isolated a Newcastle disease paramyxovirus from specimens submitted from pigeons in Columbus, New Jersey. The case was reported after pigeons developed several central nervous system (CNS) symptoms and many died. Several guinea fowl on the same premises
TRANSMISSIBLE DISEASES OF POULTRY

subsequently developed similar CNS symptoms. This is believed to be the first case in the United States where the pigeon paramyxovirus spread from pigeons to other avian species. Fourteen isolations of pigeon paramyxovirus, all identified at NVSL, had mean death times within the lentogenic range and are being typed by monoclonal antibody to separate from other PMV-1 isolates from poultry.

On September 28, 1989, Emergency Programs was notified that NVSL had isolated exotic Newcastle disease virus from conures in the USDA Quarantine Facility in Mission, Texas. The virus was isolated from pet birds that had been seized by the Texas Border Patrol on a premises in Brownsville, Texas. The premises were placed under a State of Texas quarantine and, as a precautionary measure, game chickens on the premises were destroyed and submitted to NVSL for virus isolation. Intensive surveillance activities, including the swabbing of chickens on other premises in the area were conducted. All of these remained negative for virus isolation at NVSL.

On May 9, 1989, a report was received that Texas A&M University's Diagnostic Laboratory suspected exotic Newcastle in game chickens in Dublin, Texas. Chickens in flocks in the area had been to an exhibition in Oklahoma. Chicken flocks on premises in both Texas and Oklahoma were inspected and swabbed and specimens were submitted to NVSL. All specimens were negative for Newcastle disease virus.

During Fiscal Year 1989, the campaign to alert bird owners of the risk of dealing in smuggled birds was continued. Fact sheets and a press release were distributed to the media and sent to bird owners and dealers. Coverage was also given by trade publications. It is believed that these campaigns have reduced the incidence of the disease in pet birds. In Fiscal Year 1987, there were 15 outbreaks in 10 states, and in Fiscal Year 1988, there were six outbreaks in five states. As stated previously, there were only three outbreaks involving pet birds on three premises in Fiscal Year 1989.

TURKEY RHINOTRACHEITIS (TRT) AND SWOLLEN HEAD SYNDROME (SHS)

Dr. J. E. Pearson, APHIS-NVSL, reported there is still no evidence that the virus that causes this syndrome is in the United States. During the last year, 360 domestic turkey and 181 domestic chicken serum samples were submitted to the National Veterinary Services Laboratories (NVSL). All were negative for antibody. An additional 64 turkey and 480 chicken serum samples from birds outside the United States were also negative. These birds were being tested to meet United States import requirements. The awareness of this disease should have been increased as there were presentations by scientists from the United Kingdom at the American Association Avian Pathologists meeting in Orlando, Florida, The Respiratory Disease Symposium in St. Paul, Minnesota, and at the Poultry Health and Condemnation meeting in Ocean City, Maryland.
REPORT OF THE COMMITTEE

CHLAMYDIA

Dr. J. E. Pearson, APHIS-NVSL, reported that between October 1, 1988, and September 30, 1989, *Chlamydia psittaci* was isolated from 25 of 471 pet bird submissions to the NVSL. The last outbreak in turkeys was in 1988 in Missouri. There are still no licensed antigen capture or antibody detection kits available.

AVIAN PARAMYXOVIRUSES

Dr. J. E. Pearson, APHIS-NVSL, reported that PMV-2 was isolated from an Iowa turkey flock in 1989 with a respiratory disease. Also, PMV-2 infection, based on serology, was diagnosed in several turkey flocks in California that had signs of respiratory disease. There have been no reports of PMV-3 isolations. However, there has been serologic evidence of infection and the vaccine has been sold to the following states: California, Minnesota, Colorado, Missouri, Washington, Indiana, Ohio, Virginia, North Carolina, and Pennsylvania.

During this fiscal year (FY) 1989 (October 1, 1988-September 30, 1989), there have been 96 PMV-2 and 233 PMV-3 isolations from imported pet birds. This is a decrease from 255 PMV-2 isolations last year.

AVIAN IMPORT-EXPORT ACTIVITIES

Dr. W. Ritchie, APHIS-VS, reported that an interim rule dated April 12, 1989, and finalized July 17, 1989, was published requiring that all table eggs imported into the United States must be certified to be from flocks that were tested and found free of *S. enteritidis*.

There were 6,976,997 poultry including day old chicks, and 22,321,412 hatching eggs imported into the United States during FY 1989. Most of the eggs were from Canada except approximately 2,000,000 hatching eggs.

Approximately 250,000 turkey hatching eggs were imported from the United Kingdom, quarantined for 8 weeks post hatch with serological testing to determine their status for viral turkey rhinotracheitis prior to release. Quarantines were conducted in California and West Virginia.

Currently USDA is requiring serologic negative certification for adenovirus 127 (egg drop syndrome), *S. enteritidis*, and the causative agent of viral turkey rhinotracheitis (swollen head syndrome in chickens), in addition to the required health certification in CFR Part 92.5 for all chickens and turkey hatching eggs.

Importation of commercial birds continued at the FY 1988 level. There were 447 lots of 368,098 birds offered for importation with 4 lots of 3,202 birds being refused entry due to VVND. Those birds were from Honduras and Indonesia.

In response to a request by the Association of Avian Veterinarians, the USDA promulgated regulations to permit USDA license vaccines, except
vaccines for Newcastle disease, avian influenza or other hemagglutinating virus of poultry, be administered to birds while in quarantine. As a result, two killed virus products, Pacheco's disease and psittacine pox vaccines, have been given conditional license for use. A third vaccine, psittacine reovirus, is currently being used in field trials prior to conditional licensing. These products are available for use with birds quarantined in USDA approved stations, and for use within the domestic population with state official approval.

A proposed rule to change the regulations allowing for the offspring of birds that originate in the United States and placed in a closed breeding colony outside of the country to enter into the United States without quarantine was published on December 6, 1988, with a 90-day comment period. There were approximately 700 comments opposing the rule change, and three comments in favor of the proposal. No final action has been taken at this time on the proposal.

Pet birds are currently being imported and quarantined at New York, Miami, Los Angeles, and Honolulu. There were 2,811 pet birds imported and quarantined during FY-89. All pet birds were tested for VVND. VVND was isolated from one bird.

There were 1,095 smuggled birds quarantined and auctioned to the public as permitted by a Memorandum of Understanding between the Departments of Agriculture, Interior, Justice, and Treasury.

There were only two lots of smuggled birds positive for VVND during FY-89. One lot consisted of 115 birds, with numerous endangered species involved. These birds were part of a Fish and Wildlife Service undercover operation in Southern California in which 12 people have been charged with smuggling.

On June 22, 1989, the Deputy Administrator of the Animal and Plant Health Inspection Service placed a ban on the issuing of permits for the importation of ostriches. On August 21, 1989, an interim rule was published which bans the importation of all ratites (ostriches, rheas, emus, and cassowaries). This rule was published as a result of the exotic ticks that were found on imported ostriches which had been located in Texas and Ohio. It is not the intent of the USDA to permanently ban the importation of all ratites. Rather, USDA feels they should be prohibited until methods can be developed, and regulations published, to permit their safe entry into the United States without a risk to our domestic animal population. These exotic ticks are known to be vectors of heartwater and East coast fever of cattle.

A public meeting was held on August 17, 1989, with the Secretary's Foreign Disease Advisory Committee, to gather information on ostrich imports. When a method can be identified that would allow for the safe importation of ostriches, regulations will be promulgated.
REPORT OF THE COMMITTEE

Virus Isolation from Imported Birds
FY 1988 and 1989

<table>
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<tr>
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<td>191</td>
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</tr>
<tr>
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Dr. J. E. Pearson, APHIS-NVSL, reported that the number of lots of imported birds has remained about the same in spite of the pressure to decrease importation. The number of specimens submitted has decreased, which may suggest that the lots are smaller. VVND was only isolated from four lots of commercial birds, the virus was also isolated from a privately owned pet bird that was in a USDA quarantine facility. VVND was isolated from two lots of confiscated birds, one of which contained endangered species. The endangered species will be released from quarantine after three negative virus isolations from swabs collected 2 to 4 weeks apart. The number of PMV-2 isolations decreased from 255 in 1988 to 91 in 1989. The lentogenic NDV and PMV-3 isolations remained about the same as previous years. There were two isolations of avian influenza (AI) from import birds. H4N3 was isolated from a swab collected from what was identified on the
laboratory submission form as a “zoo bird,” and H6N2 was isolated from a pygmy goose.

NATIONAL POULTRY IMPROVEMENT PLAN

Dr. I. Peterson, APHIS-VS, reported that in calendar year 1988, 36 isolations of Salmonella pullorum and 2 isolations of S. gallinarum were reported to the NPIP Staff.

During the present calendar year January to October 1, 1989, there were 83 isolations of S. pullorum and one isolation of S. gallinarum reported from 18 states. One outbreak in a state involving many interstate shipments was responsible for 73 of the isolations. Most of these shipments were from what is commonly referred to as backyard type operations.

The S. gallinarum isolation was from a small flock of 50 — 6 months old meat-type birds. They had experienced essentially no losses. The birds had been fed table scraps from at least two sources, some of which had been stored under questionable conditions. The hatchery is not believed to be the source of this infection. The flock was depopulated.

During the last year, West Virginia, New York, Wisconsin, and Michigan received the classification “U.S. Pullorum-Typhoid Clean States.” This brings the total number with this classification to 38 states. Louisiana has requested that the state be determined as qualified for this classification. When this occurs, only 10 states in the western United States remain to be classified. Staff’s goal is still to have all 48 contiguous states classified by the end of 1990. The Committee passed a resolution encouraging all states to qualify themselves for the “U.S. Pullorum-Typhoid Clean State” classification and forwarded it to the Resolutions Committee.

REVIEW OF ACTIVITIES OF THE USDA NVSL DIAGNOSTIC BACTERIOLOGY LABORATORY

Dr. W. Frerichs, APHIS-NVSL, reported that the General Bacteriology Section, Diagnostic Bacteriology Laboratory (DBL) provides support to the National Poultry Improvement Plan. This support most recently has been in the form of nationally distributed chicken and turkey serum check tests to determine the uniformity of serological test results for avian mycoplasmosis. The results of the check test survey will be published in Avian Diseases. The production of mycoplasma reference antigens, both plate and hemagglutination inhibition (HI), and reference sera has been another form of on-going support. Concerns have been expressed about certain problem areas in avian mycoplasma serology involving plate and HI test antigens from various sources. Long range goals at the NVSL involve the examination of antigen production and problem areas in avian mycoplasmosis serology. These areas include antigenic cross-reactivity and specificity. An additional area of interest is the development of a diagnostic capability to differentiate between field and vaccine strains of M. gallisepticum.
REPORT OF THE COMMITTEE

During the period from July 1, 1988, to June 30, 1989, the Bacterial Typing Section of DBL received 17,302 salmonella cultures for serotyping. Of these, 6,135 (35%) were from chickens and 4,117 (24%) were from turkeys. S. heidelberg was the most frequently identified serotype from both chickens (22% of isolates) and turkeys (17% of isolates). Other common serotypes from chickens in decreasing order of frequency were S. typhimurium, S. hadar, S. kentucky, S. infantis, S. thompson, S. enteritidis, S. agona, S. mbanda, and S. johannesburg. Other common serotypes from turkeys were S. saint paul, S. reading, S. senftenberg, S. anatum, S. kentucky, S. hadar, S. agona, S. montevideo, and Arizona 18: Z4,Z32. An annual summary of serotyping data will be published in the Proceedings of the U.S. Animal Health Association.

In August, the Bacterial Typing Section of DBL received bacteriophages for identification of Salmonella enteritidis serotype enteritidis (SE) isolates. The phages were received from Dr. Bernard Rowe of the Central Public Health Laboratory in London, England. Dr. Rowe's phage typing system is recognized by the World Health Organization as the definitive method for identification of SE strains. A NVSL microbiologist received training in SE phage typing in Dr. Rowe's laboratory earlier this year. Preliminary testing of reference cultures is now under way, and we plan to begin routine phage typing of SE isolates by November 1.

AVIAN INFLUENZA SUBCOMMITTEE REPORT

Avian Influenza in FY 88

A detailed report on Avian Influenza during the past year was made by the Chairman of this subcommittee, Dr. Ben Pomeroy, with contributions from other committee members.

Dr. M. Mixsom, APHIS-VS, reported that in July 1989, H7N3 antibodies were detected at the National Veterinary Services Laboratories (NVSL), Ames, IA, on a serological test in two broiler-breeder flocks in the Shenandoah Valley, Virginia. One flock was located in Page and the other in Rockingham County. Both flocks were related and were serviced by the same company. Morbidity and mortality in both flocks remained normal with little or no change in egg production, feed, or water consumption, and no significant changes were found on post-mortem examination.

Sentinel chickens were placed in both flocks with no sero-conversion or virus isolation. Both AI sero-positive flocks were depopulated by the owner and the premises were cleaned and disinfected. Extensive testing and surveillance of all production and backyard flocks within a 4-mile radius of each flock were conducted. In addition, all broiler production and breeder flocks in the Shenandoah Valley were tested with no further sero-positive titers found.

On May 11, 1989, it was reported that NVSL had identified H5 antibodies in serums submitted from a chicken flock in Pennsylvania. Later H5N2
antibodies were identified in serums from other birds in the flock. Extensive sampling was conducted and no virus was isolated. There were no clinical signs or abnormal mortality associated with this flock.

AI surveys were conducted at 73 live bird markets in New York City and in New Jersey during March and April of 1989. Swabs were collected from poultry on the premises and specimens were sent to NVSL, Ames, IA. All specimens were negative for the AI virus. As reported last year in March 1988, a H5N2 AI virus was isolated from swabs submitted from chickens on two premises in Fort Lauderdale, Florida. The virus did not kill chick embryos and was non-pathogenic to laboratory chickens. AI was last isolated from this Florida location in 1987. Virus “fingerprinting” at the St. Jude Research Center, in Memphis, Tennessee, revealed this virus to be similar to the AI virus which was identified in 1986 and similar to the Pennsylvania AI virus. In October of 1989, as a part of a continuing surveillance program, a H5N2 AI virus was isolated from sentinel chickens placed in a pet store in Miami, Florida. The isolate is being sent to the St. Jude Research Center, Memphis, Tennessee, for “fingerprinting” and susceptible chickens are being inoculated at the National Veterinary Services Laboratories, Ames, IA.

There has been a report from the Republic of Ireland, of a H7N7 avian influenza isolation from a chicken flock that was experiencing a higher mortality than normal. The details of this case are not known at this time.


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<tr>
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**REPORT OF THE COMMITTEE**

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</tbody>
</table>

*Live Poultry Market*

**INDIVIDUAL STATES**

A questionnaire was sent to State Veterinarians in 44 states and members of the committee. Replies were received from 35 states. Some of the states that did not reply had positive samples which were identified by NVSL during the period October 1988–September 30, 1989.

**Turkeys**

**Arkansas**

NVSL reported H1N1 positive serology from turkeys, but report from the state indicated no incidence of AI.

**Colorado**

NVSL reported H9N2 positive serology and isolation from turkeys, but no report was received from the state.

**Indiana**

H1N1 was identified in one turkey flock and confirmed by serology. One turkey flock from Indiana, that was slaughtered in Pennsylvania, was identified serologically with H2N2. This was not on the NVSL report.

**Iowa**

NVSL reported H1N1 and H2N2 positive serology from turkeys, but no report was received from the state.

**Michigan**

Two flocks involving 40,000 birds was found infected with H1N1 by serology.

**Minnesota**

The most extensive outbreak of AI occurred in the State in 1988.
TRANSMISSIBLE DISEASES OF POULTRY

Previously, extensive outbreaks were encountered in 1978-79 and 1972-73. A total of 256 flocks on 192 farms were involved with estimated loss of $3,328,000. The outbreak was first identified in July, 1988 and continued until January 1989.

The serotypes and number of flocks and farms involved were as follows:

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Flocks</th>
<th>Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7N9</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>H2N2</td>
<td>81</td>
<td>54</td>
</tr>
<tr>
<td>H9N2</td>
<td>102</td>
<td>77</td>
</tr>
<tr>
<td>Comb H2 &amp; H 9</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>H5N6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>H4N6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>H1N1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Multiple serotypes</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>256</td>
<td>192</td>
</tr>
</tbody>
</table>

This mortality ranged from 0-49% and condemnations from 0-14%. A total of 2 million doses of vaccine was used in the period of July 1, 1988 to June 30, 1989.

Serotypes used were H1, H2, H4, H6, H7, and H9.

In 1989, the first flock was identified in May and next reported flock was in August. A total of 6 flocks on 4 farms have been identified up to October 1. Only one serotype was isolated, H1N1. Other serotypes identified serologically were H9N2, H10N7, and H4N8.

North Carolina

NVSL reported the identification of H1N1 and H6N6 positive serology — 148 flocks involving 1,059,143 birds were vaccinated with H1N1 vaccine.

Ohio

No outbreaks were reported in Ohio, but 41 breeder flocks were vaccinated with H1N1, involving 488,000 birds.

Pennsylvania

One turkey flock (3,900) was identified with H1N1 based on serology as a result of surveillance program.

Utah

There were 4 turkey flocks vaccinated with combination H6 and H10 vaccines. NVSL reported positive serology for H4.

Virginia

NVSL reported positive serology for H2N9 and H4N6 in turkey flocks.
REPORT OF THE COMMITTEE

Wisconsin
NVSL reported positive serology in turkey flocks for serotypes H1N1, H2N2, and H9N2. No report was received from the state.

Other States
No clinical outbreaks and serological evidence of AI were reported from California, Missouri, Texas.

USE OF AVIAN INFLUENZA VACCINES FY 89
Influenza killed vaccine was used extensively in Minnesota (H1, H2, H4, H6, H7, and H9). In Ohio and North Carolina H1 vaccine was used primarily in breeder flocks. No reports were received on the use of vaccines in Iowa, Missouri, and Kansas.

Chickens
Minnesota reported one broiler breeder flock (13,000) with serological positive H9N2. No spread occurred in broiler and breeding flocks. Extensive monitoring of broiler flocks since this episode in October 1988 has been negative.

Pennsylvania reported one egg type chicken flock (1,000) with serological positive H5N2. No virus isolations were made. Flock was identified on monitoring program.

Virginia reported two broiler breeder flocks (42,000) with serological positive H7N3. No virus isolations were made and sentinel birds remained negative.

Other Fowl
Pennsylvania reported one guinea fowl flock (2,150) with serological positive H11N4 with no isolations. Flock was identified on monitoring program.

SUMMARY
AI outbreaks were identified in the following species and states in FY 1989. No highly pathogenic serotypes were identified by NVSL.

Turkeys
Arkansas H1N1
Colorado H9N2
Indiana H1N1, H2N2
Iowa H1N1, H2N2
Michigan H1N1
Minnesota H1N1, H2N2, H4N6, H5N6, H9N2, H10N7
New Jersey H9N2 Live Poultry Market
TRANSMISSIBLE DISEASES OF POULTRY

North Carolina  H1N1, H4N6
Pennsylvania   H1N1
Utah           H4
Virginia        H2N9, H4N6
Wisconsin      H1N1, H2N2, H9N2

**Chickens**
- Florida       H5N2 Live Poultry Market
- Minnesota    H9N2
- Pennsylvania H5N2
- Virginia     H7N3

**Guinea Fowl**
- Pennsylvania H11N4

**Vaccine Use**
No AI vaccine was reported used in chickens. AI vaccines were used in turkeys in Minnesota, Ohio, and North Carolina. No reports were received on use of vaccine in Iowa, Missouri and Kansas.

**AVIAN INFLUENZA SINCE 1964**

The following table represents the avian influenza serotype isolated from turkeys, chickens and other domestic fowl in the U.S. since 1964 or based on serology.

**AVIAN INFLUENZA SEROTYPES ISOLATED FROM TURKEYS, CHICKENS AND OTHER DOMESTIC FOWL IN THE U.S. (1964-1989) OR BASED ON SEROLOGY**

<table>
<thead>
<tr>
<th>YEAR FIRST IDENTIFIED</th>
<th>STATE</th>
<th>HEMAGGLUTININ ANTIGENS IDENTIFIED</th>
</tr>
</thead>
<tbody>
<tr>
<td>California  1964</td>
<td>H5, H6, H9</td>
<td></td>
</tr>
<tr>
<td>Massachusetts 1965</td>
<td>H6</td>
<td></td>
</tr>
<tr>
<td>Wisconsin  1965</td>
<td>H1, H2, H5, H6, H9; 1989: H1, H2, H9</td>
<td></td>
</tr>
<tr>
<td>Minnesota  1966</td>
<td>H1, H2, H3, H4, H5, H6, H7, H8, H9, H10; 1989: H1, H2, H4, H5, H9, H10</td>
<td></td>
</tr>
<tr>
<td>Washington  1967</td>
<td>H6</td>
<td></td>
</tr>
<tr>
<td>Oregon      1970</td>
<td>H6, H7</td>
<td></td>
</tr>
<tr>
<td>Iowa        1971</td>
<td>H1, H4, H5, H6; 1989: H1, H2</td>
<td></td>
</tr>
<tr>
<td>Colorado    1972</td>
<td>H1, H5, H9; 1989: H9</td>
<td></td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE

<table>
<thead>
<tr>
<th>State</th>
<th>Year</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohio</td>
<td>1975</td>
<td>H1</td>
</tr>
<tr>
<td>South Dakota</td>
<td>1978</td>
<td>H1</td>
</tr>
<tr>
<td>Texas</td>
<td>1979</td>
<td>H5, H7, H9</td>
</tr>
<tr>
<td>Indiana</td>
<td>1980</td>
<td>H1, H10; 1989: H1, H2</td>
</tr>
<tr>
<td>Missouri</td>
<td>1980</td>
<td>H1</td>
</tr>
<tr>
<td>Kansas</td>
<td>1980</td>
<td>H1</td>
</tr>
<tr>
<td>North Dakota</td>
<td>1981</td>
<td>H5</td>
</tr>
<tr>
<td>Arkansas</td>
<td>1981</td>
<td>H1; 1989: H1</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1982</td>
<td>H1; 1989: H1, H4</td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H1, H2, H5, H10; 1989: H2, H4</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>H1, H5; 1989: H1</td>
</tr>
<tr>
<td>Michigan</td>
<td>1985</td>
<td>H1, H9; 1989: H1</td>
</tr>
<tr>
<td>Utah</td>
<td>1985</td>
<td>H6, H10; 1989: H4</td>
</tr>
<tr>
<td>Nebraska</td>
<td>1988</td>
<td>H1</td>
</tr>
<tr>
<td>New York</td>
<td>1988</td>
<td>H9</td>
</tr>
</tbody>
</table>

Chickens

<table>
<thead>
<tr>
<th>State</th>
<th>Year</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>1975</td>
<td>H4</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1978</td>
<td>H6; 1989: H9</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983, 86</td>
<td>H1, H5; 1989: H5</td>
</tr>
<tr>
<td>Maryland</td>
<td>1983, 84</td>
<td>H5, H9</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1983, 86</td>
<td>H5</td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H5, H7; 1989: H7</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>New York</td>
<td>1986</td>
<td>H5</td>
</tr>
</tbody>
</table>

Chickens — Live Market

<table>
<thead>
<tr>
<th>District of</th>
<th>Year</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columbia</td>
<td>1980</td>
<td>H1; 1984: H5</td>
</tr>
<tr>
<td>Connecticut</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>Florida</td>
<td>1986</td>
<td>H5; 1989: H5</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>&quot;”</td>
<td>1989 turkey</td>
<td>H9</td>
</tr>
<tr>
<td>New York</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>1986</td>
<td>H5</td>
</tr>
</tbody>
</table>

Chickens — Dealer

<table>
<thead>
<tr>
<th>State</th>
<th>Year</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohio</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>Georiga</td>
<td>1987</td>
<td>H5</td>
</tr>
</tbody>
</table>

Other Species

<table>
<thead>
<tr>
<th>State</th>
<th>Year</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania</td>
<td>1969</td>
<td>Ducks NA, H3</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1974</td>
<td>Geese NA</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>Guinea Fowl NA</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>Pheasants H3, H7;</td>
</tr>
</tbody>
</table>
TRANSMISSIBLE DISEASES OF POULTRY

Pennsylvania 1983 Ducks H3, H4, H5, H6, H11
Maryland 1984 Guinea Fowl, Quail H5
1984 Ducks, Guinea Fowl H3
1985 Chukar H5
1985 Ducks H4
Washington 1985 Pheasant H9
Virginia 1985 Ducks, Swans,
1986 Geese H7
Oregon 1986
Pennsylvania 1986 Guinea Fowl H1, H6;
1989: H11
1986 Guinea Fowl,
1986 Chukar H5
Georgia (Dealer) 1987 Guinea Fowl H5
Maryland 1987 Ducks, Geese H9
Wisconsin 1988 Pheasant H9
Pennsylvania 1988 Geese H1
North Carolina 1988 Ducks H6
NA = Not Available

PROPOSAL FOR THIRD INTERNATIONAL SYMPOSIUM

The Committee passed a resolution proposing that USAHA consider sponsoring a Third International Symposium on Avian Influenza in 1991 or 1992 and forwarded it to the Resolutions Committee for consideration.

DISEASE ACTIVITY WITH MIGRATORY BIRDS

Dr. T. Roffe, USDI-National Wildlife Health Research Center (NWHRC) reported that these data are limited to those incidents reported to the NWHRC by a variety of state and federal agencies and private individuals. The data is confined to reports with confirmed diagnoses. During fiscal year 1989 (October 1, 1988, through October 1, 1989) approximately 200 epizootics were reported to the Center.

MAJOR EPIZOOTICS — FY 1989

The Atlantic Flyway continues to have outbreaks of duck plague. The Mississippi Flyway's major outbreaks were primarily cholera and lead poisoning. Cholera was observed for the first time at Lacassive NWR, LA. The Central Flyway is dominated by botulism and cholera outbreaks. In addition, necrotic enteritis in snow geese is now become more apparent in South Dakota and southern Canada. Only very incomplete information is received from Canada. The Pacific Flyway, particularly California, continues to be a major botulism and cholera area. In addition I have indicated the major oil spill mortality in Alaska and Washington. The latest report from
REPORT OF THE COMMITTEE

the Exxon Valdez oil spill was that in excess of 33,000 birds had been picked up. This obviously grossly underestimates total mortality.

AVIAN BOTULISM TYPE C

Botulism continues to be one of the primary problems and is widely distributed across the U.S. The greatest number of outbreaks occur in the Central Plains and western states. Of particular note is the major outbreak in southern Manitoba (at 3 closely associated sites) where an excess of 20,000 mallards are estimated to have died. This outbreak occurred in August. In California, the Tule-Klamath area, botulism mortality was considerably less severe than in previous years with just over 2,000 reported dead. North Dakota outbreaks were also reduced this year with only a couple of epizootics having mortality in the one to two thousand range.

AVIAN CHOLERA

Avian cholera occurred previously in the central U.S. and California. Part of the visibility of California is due to the frequent reporting by the California Department of Fish and Game. Many of the California outbreaks exceed thousands in mortality. As examples, Klamath Basin mortality in excess of 3,200 (snow WF goose, widgen, mallard); Delevan/Sacramento mortality exceed 1,400 (variety of geese, lesser numbers of various duck species); and Gray Lodge with mortality over 1,500 (primarily widgen, snowgoose, pintail).

In the central U.S., major outbreaks occurred in Southeast Texas where over 7,000 geese died in an epizootic around Attwater Prairie Chicken NWR. Illinois has seen increased numbers with Canada goose mortality over 1,500 in a couple of different outbreaks. We now have a confirmed report in Minnesota where over 5,000 Canada geese died at Lacqui Park in the last few weeks.

In general, cholera is a winter disease; however, in Maine we have regular mortality in nesting eider ducks in the spring. These birds nest on coastal islands.

SALMONELLA

Salmonellosis, as a major epizootic, is a relatively uncommon report. In one case, *S. typhimurium* caused mortality in an estimated 4,500 cattle egrets in southern California (Salton Sea Area). This is very much an approximation because habitat type at the egret rookery precluded ground counts over most of the area. The other *S. typhimurium* outbreak was reported by the Southeast Cooperative Wildlife Disease Study and involved 15-20 English sparrows.

DUCK VIRUS ENTERITIS (DVE)

DVE, or duck plague, was not reported in California this year. Thus far, with the exception of Lake Andes in 1973, and spillover into wild ducks in
TRANSMISSIBLE DISEASES OF POULTRY

the first Long Island outbreak in the 60's, we continue to see duck plague in game farm or urban duck (particularly muscovy) situation. The New York episode occurred at a rearing facility for Black Duck. Unfortunately this facility was near a major Black Duck refuge and had a history of releasing birds to the wild. However no wild bird mortality due to duck plague has been reported in that area. In Baltimore, muscovy (free-ranging) were the species affected. Here 7 muscovy died but they were in association with 100-150 mallards. These birds were depopulated. The Michigan incident involved muscovy, mallard and domestic geese but all birds were captives.

LEAD POISONING

Although the U.S. made the commitment to the use of non-toxic shot on a nationwide basis, lead poisoning due to ingestion of lead shot continues and probably will continue in certain areas for some time. Mortality is generally low level and outbreaks are often difficult to recognize. Many outbreaks are "discovered" because mortality of other causes brings attention to the site. This is the case in many of the California epizootics where cholera mortality leads to the finding of lead poisoned birds. The largest, strictly lead poisoning outbreak, occurred in Wisconsin where an estimated 500 Canada geese died at Rush Lake.

REPORT OF THE MODEL STATE PROGRAM FOR PET BIRDS SUBCOMMITTEE

Dr. Henry Virts gave the following report on the Maryland Cage Bird Improvement Plan (M-BIP).

M-BIP has been operational for one and one-half years, with 27 retailers and 7 breeders having achieved the official classification as an "M-BIP Participant." These 34 businesses have been professionally inspected for compliance with M-BIP rules, and are entitled to use the distinctive M-BIP logos in their sales and business dealings.

Numerous written inquiries have been received about the M-BIP from many states and three foreign countries. Sister cage bird improvement plans have been established in Florida and Virginia, with California and other states reported to be considering implementation.

The support of the M-BIP supplied by Dr. Archibald Park, Maryland Department of Agriculture, and Dr. Irwin Peterson, National Poultry Improvement Plan, USDA, APHIS-VS, has been the key to the success of this program to date.

Although required to be annually inspected for continued participation, all participants in the program for over one year have elected to remain in the program and have passed reinspection.
REPORT OF THE COMMITTEE

MYCOPLASMOsis SUBCOMMITTEE REPORT

Dr. D. McMartin, Chairman, reported that mycoplasmosis is a continuing disease threat to the poultry industries in the USA. Regional reports mention new *M. gallisepticum* (MG) infections in egg operations which are believed to have been free for years; MG in several meat turkey units; *M. synoviae* in turkeys as a threat to broilers and broiler breeders; and widespread *M. meleagrisidis* in turkeys in California, often with sinusitis, but usually in combination with other significant organisms.

For serological tests, plate, hemagglutination inhibition (HI) and ELISA are being used, either singly or in combination. Diagnostic laboratories continue to have reservations about sensitivity and specificity of all three tests, but it is not known to what extent infections might be missed or whether these perceived inadequacies contribute to spread of mycoplasmal infections. The National Veterinary Services Laboratories (NVSL) has maintained activity in this area to support laboratories in making correct diagnoses. Chicken and turkey serum "check tests" have recently been distributed nationally to determine uniformity of serological results. Findings of this survey will be published in Avian Diseases. The NVSL has also provided reference antigens, both serum plate and HI, and reference sera, to requesting laboratories. Long-term goals of the Laboratory include examination of problem areas in antigen production, particularly cross-reactivity and specificity, and development of a diagnostic capability to differentiate between field and vaccine strains of MG.

A resolution citing the need for better diagnostic procedures and reagents was passed by the committee and forwarded to the Resolutions Committee for consideration.

There is no report to date of F-strain MG having been found in a turkey flock. All isolates identified have been "wild" type.

No specific factor or factors have been incriminated in the continued appearance of MG in commercial flocks. Several different views are held.

- The commercial industry is letting its guard down.
- Infection is coming from small commercial and backyard flocks.
- Increasingly resistant MG strains are surviving longer off the host.
- A still unidentified method of spread exists.

No new information has been reported on methods to eradicate Mycoplasmas from endemically infected sites. The subcommittee encourages further laboratory studies on temperature sensitive mutants of MG (MGts), for example, duration of the organism in vaccinated flocks, whether reversal to virulence occurs after repeated bird passage, and whether MGts prevent establishment of field MG in the turbinate and sinus mucosa (the turbinates probably being the primary site of airborne MG infection).
Dr. M. Cover, Chairman of the SEPEA Research Committee, reported that this association has always relied on and supported research. At the present time there are three phases to the project: (a) general research including nutrition, management, waste disposal, disease, etc., (b) Microbiological including Salmonella, Campyobacter and Listeria and (c) rendering, now included only research to improve feather meal. These three programs include research projects requiring slightly more than two million dollars. The general research program includes projects in the following areas. Disease - 12, management - 5, further processing - 4, cholesterol - 1 and handling waste - 2. A total of 24 projects. The microbiological section has projects in the following areas. Salmonella - 10, Salmonella enteritidis - 2, Listeria - 4 and Campylobacter - 2. A total of 20 projects.

Information on completed research is available to any interested party and may be obtained by calling the Southeastern office, (404) 377-6465. This office is in Decatur, Georgia.

REPORT OF THE SUBCOMMITTEE ON INFECTIOUS LARYNGOTRACHEITIS (ILT) ERADICATION

Infectious laryngotracheitis is a highly contagious disease of chickens that is responsible for egg production and death losses. The eradication of ILT is considered feasible and would be of great benefit to the poultry industry. An ILT-free poultry industry would enhance the exportation of poultry and poultry products.

An ILT national eradication program encompassing the broiler industry, exhibition and backyard poultry, that can be implemented on a state or defined area basis is presented with enough flexibility to encompass all segments of the poultry industry. The subcommittee solicits comments on this preliminary program proposal.

Phase I
A. Reportable disease — Mandatory
B. Diagnostic capability should be developed — Histopathology, virus isolation, FAT, serologic tests, and any others that may be developed and approved.
C. Vaccine — Unrestricted use should be discouraged. State restrictions should limit use to birds kept to maturity (leghorns, broiler breeders) and in endemic areas only.
D. Surveillance — A surveillance monitoring program should be developed.
REPORT OF THE COMMITTEE

E. Biosecurity — Educate and practice including:
   1) service people
   2) flock caretakers
   3) hatcheries and related activities
   4) live haul operations
   5) litter removal, hauling, and mortality disposal
   6) egg transportation
   7) feed transportation
   8) poultry exhibitors
   9) game fowl growers

Phase II
A. Reportable disease — Mandatory
B. Diagnostic capability readily available
C. Surveillance monitoring program in place
D. Vaccine
   1) Restrict vaccine sales and use to holders of a valid permit issued by
      the State Animal Health Official for specific premises within a de-
      fined endemic area.
   2) Vaccinated flocks must be identified by type, number of birds, be
      quarantined, and moved on a permit issued by the State Animal
      Health Official.
E. Biosecurity should be promoted and practiced — Continue biosecu-
   rity education programs as in Phase I-E.
F. Quarantine infected flocks:
   1) Move on permit issued by State Animal Health Official
   2) Vacant infected houses:
      (a) shall have the litter wet down, closed up, and heated for 3 days
         to a 100°F. temperature and remain empty for up to 3 weeks min-
         imum
      or
      (b) remove litter to a compost pile, clean and disinfect house and
         equipment; then leave vacant for one week.
G. For exhibit, game fowl, and other backyard flocks, establish “ILT
Tested Flock” status. At least 25% of adult birds in flock will be blood
tested negative at an approved laboratory every 6 months by proce-
dures acceptable to the Official State Agency.

Phase III
A. Continue parts A, B, C, & E of Phase II.
B. Vaccine — Prohibit the sale and use of ILT vaccine.
C. Quarantine infected flocks:
   1) Move commercial poultry flocks to processing on a permit issued
      by State Animal Health Official after production cycle in selected
      cases where adequate biosecurity measures can be maintained.
TRANSMISSIBLE DISEASES OF POULTRY

2) Depopulate and dispose of all others by an approved method with indemnity.
D. Continue "ILT Tested Flock" status as in Phase II, G.

Phase IV — ILT Free
A. No known ILT infection for 24 months.
B. Depopulate and dispose of all infected poultry (commercial and non-commercial) by an approved method with indemnity.
C. Continue parts A, B, C, and E of Phase II.
D. Vaccine — Prohibit the sale and use of ILT vaccine.

THE FOLLOWING SUBCOMMITTEES WERE FORMED:

Avian Influenza: R. A. Bankowski; C. Beard; F. Craig; D. King; D. Halvorson; J. E. Pearson; I. Peterson; and B. S. Pomeroy, Chairperson.

Mycoplasmosis: D. Johnson; E. T. Mallinson; H. O. Opitz; B. S. Pomeroy; I. Peterson; W. Towers; R. Yamamoto; and D. McMartin, Chairperson.

Model State Program for Pet Birds: S. Clubb; D. J. Ligda; E. T. Mallinson; M. Meyers; L. Phillips; T. Tramel; and H. Kahan, Chairperson.

Paramyxovirus Evaluation: C. Beard; I. H. Kahan; D. King; C. Weston; R. A. Bankowski; and J. E. Pearson, Chairman.

Definition of Avian Influenza: B. E. Easterday; C. Beard; F. Craig; J. E. Pearson; B. S. Pomeroy, R. Webster; and R. A. Bankowski, Chairperson.

Infectious Bronchitis: C. Beard, R. Eckroade; H. Lasher, M. Opitz; and F. Craig, Chairperson.

Infectious Laryngotracheitis Eradication: F. Hoer; H. Lasher; D. McMartin; C. Weston, and D. Johnson, Chairperson.
National Poultry Improvement Plan
U.S. Pullorum-Typhoid Clean States

0/0 Qualifying Date
- Classified for Turkeys Only
- Classified for All Poultry

August 1989
FERAL SWINE AND DISEASE
George W. Beran
Department of Veterinary Microbiology
Iowa State University
Ames, IA 50011

INTRODUCTION

On his second voyage across the Atlantic Ocean in 1493, Columbus brought eight swine to the West Indies. In 1539, DeSoto brought 13 sows, descendants of these, to Florida from Cuba. Eleven months later, it was estimated that there were 300 in Florida, but 27 progeny from each of DeSoto's hogs in less than a year seems unlikely. There were many more introductions of European swine into North America; much of their husbandry permitted these swine to propagate on their own; and these became the first ancestry of feral swine of the United States. In Florida, hog claims by ear slits registered with the city clerk were recognized until 1949. Feral swine are a significant resource in southeastern U.S. With a feral swine population of over one-half million, 1-1/4 times the domestic swine population, feral swine earn 8.3-10 million dollars per year to Florida. Hunters pay $10.00 or more per pound of feral swine pork; in the market, these swine are worth about 10 cents per pound. Feral swine populations are endemic in at least 18 states with major populations in Florida, Texas and California, over one-half of the U.S. total being in Florida.

Pseudorabies in feral swine

Pseudorabies (PR) has been diagnosed serologically in at least ten states, Alabama, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, South Carolina and Texas, with isolates of PR virus in California and Florida. In several serologic studies, positive findings were reported in Florida in 34.7% of 190 feral swine tested by serum neutralization test in 1977-1978, in 41.5% of 578 (19% of them tested by serum neutralization and the rest by ELISA) in 1983, and 11.4% of 35 tested by latex agglutination test in 1985; in Texas in 36% of 124 in 1986; and in California in 3% of 135.

Brucellosis in feral swine

Brucellosis has been diagnosed serologically in at least eight states, Arizona, California, Florida, Georgia, Hawaii, Louisiana, South Carolina and Texas with 13 isolates of Brucella suis biotype 1 in Florida and Texas and one of biotype 3 in California. In several serologic studies, positive findings were reported in California in 15% of 136 feral swine, in Texas in 3.2% of 124, and in 6-53% of feral swine tested in Arkansas, Florida, Georgia, Louisiana and South Carolina. Human infections have followed exposure during field dressing and preparation of feral swine in Alabama and Florida.
Vesicular stomatitis in feral swine

Vesicular stomatitis (VS) has been diagnosed serologically in 7.3% of 1,054 feral swine in Arkansas, California, Florida, Georgia and Louisiana. On Ossabaw Island, Georgia, the New Jersey type has been isolated in 1983, 1987 and 1988. Feral swine are among many species of domestic and wild animals infected with VS virus but do not appear to serve as reservoir hosts, primary infections appearing each year transmitted from other species.4,10

Leptospirosis in feral swine

Leptospirosis has not been reported as a clinical disease in feral swine but serologic studies reported on 418 sera yielded positive results against pomona in 4.8%, hardjo in 14.8%, grippotyphosa in 2.4%, icterohemorrhagiae in 4.8% and canicola in 2.6% in one study,4 and in another on 124 sera against bratislava in 17%, icterohemorrhagiae in 21%, pomona in 12% and tarassovi in 8%.6 Isolates of autumnalis, pomona, canicola and grippotyphosa have been reported.4

Other infections recorded in feral swine

Serologic evidence of the following viral infections has been reported in feral swine: parvovirus infections (7/7 in Florida, 1988),5 Venezuelan equine encephalitis (31/51 in Texas, 1971-1973),11 reoviruses,4 influenza,4 and hemagglutinating encephalomyelitis.4 Prior to 1960, hog cholera was reported in feral swine in Tennessee, Florida, Georgia and California but surveys on 1,218 feral swine in 15 states between 1979 and 1987 were all negative.4 Serologic evidence of Q fever, a rickettsial infection, has been reported in 50% of 135 feral swine in California between 1981 and 1983. Among bacterial infections, Bordetella spp.4 and plague infections7 have been reported. At least 25 spp. of endoparasites including trichina,4 toxoplasma (18/135 in California, 1981-1983; 7/7 in Florida, 1988),5 lung worms, kidney worms, thorny headed worms, stomach worms, hookworms, nodular worms, thread worms and sarcocystis.4,12 At least seven spp. of ectoparasites have been reported on feral swine.4,12

Recommendations for disease control in feral swine

The U.S. Animal Health Association Subcommittee on Feral Swine has recommended additions to the Program Standards for Pseudorabies Eradication and to the Uniform Methods and Rules for Brucellosis Eradication to focus on prevention of transmission of these diseases between feral and domestic swine and to develop control in feral swine. The following definitions are proposed:

Feral swine. Swine which have lived any part of their lives free roaming. Feral swine may be reclassified as domestic swine by a negative official pseudorabies/brucellosis test conducted after at least 30 days confinement separate and apart from any infected or free roaming swine.
FERAL SWINE AND DISEASE

**Monitored negative feral swine population.** Feral swine which have originated from areas which have been geographically defined and under continuous surveillance yielding no evidence of infection may be classified by the pseudorabies/brucellosis epidemiologist as a monitored negative feral swine population.

**Recommended controls on movement of feral swine**

Movements from non-qualified/non-validated feral swine populations may be on negative serologic tests within the past 30 days. Feral swine may be moved from monitored negative feral swine populations without individual animal testing. Pseudorabies qualified/brucellosis validated feral swine herds may be created. They may be kept as confined herds or they may be permitted to roam in a geographically defined area devoid of any other swine. The stocking population must be found infection-free in complete herd tests and then one-fourth of the adult swine must be captured quarterly and demonstrated to be serologically negative. Feral swine may be moved from these populations without further serologic tests. Feral swine reclassified as domestic swine must meet all movement regulations for domestic swine before transfer into farm herds, to hunting preserves or game farms, or to zoos or animal parks.

Movement of feral swine to hunting preserves or game farms is not classified as shipment to slaughter. States are encouraged to approve and regulate hunting preserves, ensure that they are located away from farms raising domestic swine and require that they be adequately fenced to contain feral swine.

**Recommended epidemiologic research on feral swine**

Research on the role of feral swine in the epidemiology of diseases of swine and other species of animals transmitted through direct or indirect contact with feral swine must be a priority. Certain data are available at this time. The serologic prevalence of infectious and parasitic diseases in feral swine is largely known in areas of high feral swine population density but not at the peripheries of their habitats. Hog cholera occurred in feral swine when it was endemic in domestic swine but when it was eliminated from domestic swine, it disappeared from the feral population. Bovine tuberculosis was transmitted from cattle to feral swine on Molokai Island, Hawaii, but disappeared following elimination of the infected cattle herd. On Ossabaw Island, Georgia, it was found that vesicular stomatitis virus must be reintroduced into the feral swine population each year from reservoir hosts which maintain it.

Extensive evidence has been provided that feral swine maintain pseudorabies in the absence of domestic swine, as on Ossabaw Island, Georgia. They have become latently infected when experimentally exposed to strains originating from feral swine populations. Shedding of virus has been reactivated but such experimentally infected feral swine have shed
less virus and for a shorter time than have domestic swine. Epidemiologic investigations of pseudorabies outbreaks in domestic animals have traced them to feral swine in an outbreak in a farrow-to-finish swine operation in Tennessee, and in an outbreak in heifers exposed when a drought in Texas caused feral swine to seek feed and water in a feedlot.

Feral swine have been shown to maintain swine brucellosis and are capable of transmitting it. In 1988, transmission to cattle in Florida was epidemiologically traced to feral swine. Human infections have followed field dressing and preparation of feral swine pork in Alabama and Florida.

Feral swine have been shown in some geographic areas to maintain leptospirosis, toxoplasmosis, parvovirus infections, Q fever, a wide variety of internal parasites and several ectoparasites. Studies are needed on the extent to which other microbial and parasitic agents which infect feral swine are dependent on other maintaining animals as sources of reinfec-
tion, and which are maintained in feral swine and thus are sources of infection to domestic swine or other animals. Knowledge is quite fragment-
tary about the ecologic conditions which foster transmission. Concern over entry of exotic swine diseases includes both domestic and feral swine in foot and mouth disease, hog cholera, African swine fever, vesicular exanthema-
San Miguel Sea Lion virus and others; perhaps Venezuelan equine en-
cephalitis is already in feral swine.

Recommended research on control of feral swine diseases

Studies are needed on practical measures to segregate feral from domes-
tic swine, or more probably to segregate domestic from feral swine. Feral swine population control, including managed hunting, selective removal of adult swine, and depopulation-repopulation approaches need to be as-
sessed.

Two preliminary studies have been carried out on oral vaccination of swine with modified live pseudorabies virus, one of them using a genetically engineered gene deletion virus in feral swine. Both oral vaccines stimulated measurable antibody responses but much more investigation is needed, including effects on non-target species. Trials are needed on the effectiveness of the Chinese S-2 strain oral vaccine against swine brucellosis in feral swine.

Studies on peccaries (javelinas)

Peccaries range from Texas, New Mexico and Arizona south to Argentina. Experimental studies have shown them to be susceptible to pseudorabies, vesicular stomatitis, foot and mouth disease, vesicular exanthema, rinder-
pest and hog cholera but not to African swine fever. In a serologic survey on 218 peccaries in Arizona, one showed a 1:16 titer against pseudorabies virus, none were positive against brucella, 8% were positive against New Jersey but not Indiana type vesicular stomatitis and 23% were positive
FERAL SWINE AND DISEASE

against 11 serovars of leptospirosis. Further studies on the entry of peccaries into transmission cycles of diseases of swine are needed.

CONCLUSIONS

No state can be classified free of pseudorabies or brucellosis if these infections persist in feral swine in that state. Feral swine must be seriously considered in the programs for eradication of these two diseases, as well as in the control of other diseases intertransmitted between domestic and feral swine.

REFERENCES

FERAL SWINE AND DISEASE


I appreciate the opportunity to provide the USDA-APHIS report to this distinguished committee of pseudorabies eradication supporters.

APHIS would like to acknowledge the excellent agenda put together by USAHA Pseudorabies Committee Chairman Dr. Ed Slauter. Many important issues are being discussed which are vital to the efficient eradication of pseudorabies.

The past year has seen substantial accomplishment in developing the basic program policies and procedures which will carry the States rapidly through the eradication stages to a Pseudorabies Free National Swine Herd:

1. A nine member USDA/APHIS Swine Brucellosis/Pseudorabies Surveillance task force reviewed the APHIS Swine Brucellosis and Pseudorabies Surveillance Program and reported their findings to Dr. Lonnie King, Deputy Administrator of Veterinary Services, in January 1989. Recommendations identified the need to combine pseudorabies and swine brucellosis surveillance programs and to stop testing non-herd-of-origin identified swine.

2. The Program Standards for Pseudorabies Eradication approved by the Executive Committee of the USAHA at Little Rock last year, were distributed by APHIS in March of 1989. This important document is the State-Federal-Industry “blue print” for eradication.

3. A series of four Veterinary Services Memoranda was prepared and distributed to State and Federal Animal Health Officials. These Memoranda establish policy for implementing provisions of the program standards and recommendations contained in the Swine Brucellosis-Pseudorabies Surveillance Task Force Report.

4. The Alabama Pseudorabies Eradication Program was reviewed by Staff along with a representative of the NPPC. This was the first State program review to utilize the expertise of a swine producer and is a procedure which will be continued during FY 1990.

5. On October 12 and 13, 1989, an ad hoc committee composed of practicing veterinarians, producers, NPPC and LCI leadership, and State and Federal regulators met in Des Moines, Iowa, to develop a plan for implementing large herd cleanup studies in seven states. The details of the plan will be available within a few weeks. APHIS-VS will contribute 50% of the costs of the study and has allocated approximately $150,000 to the study for FY 1990.
(6) Staff developed a proposed regulation which would, if promulgated, permit the establishment of qualified PRV/Marker vaccinated/gpX negative herds. This docket is presently in the approval process and will be published in the Federal Register for public comment on October 31, 1989.

The subject test, if made official, would permit vaccination of breeding swine prior to movement and contribute to herd cleanup by enhancing the resistance of replacement breeding swine before their introduction into infective environments.

(7) A federal swine symposium held in Orlando, Florida, contributed substantially to our knowledge of the feral swine problem and led to two basic conclusions:

(a) Feral swine are here to stay.

(b) Feral swine infected with pseudorabies and swine brucellosis are here to stay for the foreseeable future. Therefore, our efforts should address procedures for maintaining effective biosecurity between the domestic and feral swine populations as well as maintaining appropriate surveillance in these two populations.

(8) In April, a four day Pseudorabies Training Seminar for 35 State and Federal veterinarians was held at NVSL. Participants were exposed to a variety of attitudes regarding Pseudorabies control and eradication by the speakers, two of which were practicing veterinarians.

(9) A National quarterly data report was developed utilizing State quarterly report data. Copies of this report are provided to all interested parties, including State, Federal and Industry program leadership. During January, the VS Swine Diseases Staff with the assistance of Neal Black and Dr. Frank Mulhern will produce the first of 10 annual APHIS-NPPC Reports To The Industry covering the first 12 months of program activity. This report will be directed to the swine producer and will be made available to members of the agriculture press.

(10) The Swine Diseases Staff, working cooperatively with the National Pseudorabies Control Board, has assigned program status to seven States as follows: Stage I — Arizona, Missouri, and Texas. Stage II — Georgia and Illinois. Stage III — Arkansas and Wisconsin.

Applications for program status have been received from Indiana, West Virginia, Kansas, Alabama, Florida, and Oklahoma, and are in the review process. We have been advised that several additional States will be submitting program status applications within the next few months. By October 1, 1990, we expect that all States will have been assigned Pseudorabies Eradication Program Status. The procedure for applying for status is addressed in Veterinary Services Memorandum 566.2.

(11) Cooperative agreements were developed with LCI for distribution of the monthly Pseudorabies Eradication Progress Report by Neal Black and with Dr. George Beran for his quarterly Epidemiologic Evaluation of
STATE/FEDERAL PSEUDORABIES ERADICATION PROGRAM

*National Data.* Additionally, APHIS has contributed to the cost of producing the *Pseudorabies Epidemiology Report* which is edited by Dr. Robert B. Morrison, University of Minnesota. Anyone interested in the epidemiology of pseudorabies should contact Dr. Morrison and ask to be put on his mailing list.

(12) Program Aid No. 1301 (Pseudorabies in Swine) was amended in February. Forty-thousand copies have been distributed to field locations.

(13) Individual States are given an option of receiving limited APHIS surveillance funds for either major packer surveillance or for other surveillance within their state.

States are encouraged to limit MST surveillance activities until such time as they are actively investigating, quarantining, and testing MST reactor animals. We believe limited surveillance funds can better be used to circle test and to test purchases and sales from known infected herds, in States which do not have mandatory test authority.

As a part of our effort to support National Pseudorabies Surveillance, a survey of slaughter establishments was conducted. The survey provided detailed information regarding where and how sows and boars are killed in each State. As additional surveillance funding becomes available, this information will be very valuable to program managers.

(14) Pseudorabies Eradication funds are allocated to the regions in accordance with the breeding swine population and number of infected herds within the States that make up the four APHIS Regions. The actual assignment of funds to State program activity is made by the Regional Director and may be based on several factors in addition to swine populations including State funding, State program quality and industry attitudes.

During FY 1990, APHIS will be involved in a number of activities, including:

(1) A 4th National Pseudorabies Training Seminar for State and Federal veterinarians at the National Veterinary Services Laboratory.

(2) Three regional pseudorabies — swine brucellosis training courses, the first of which is scheduled for December 5, 6, and 7 in Atlanta, Georgia for Southeastern Region State and Federal personnel.

(3) Implementation of the seven State Herd Cleanup study.

(4) A second graduate student will be enrolled in swine diseases training at North Carolina State University in January 1990.

(5) Refinement of the Quarterly State PR Eradication Activities Report (VS Form 7-1) and an intensification of effort to ensure only quality information goes into our data base.

(6) Dr. Joe Annelli will complete his studies at the University of Minnesota in June and will then be assigned to program activity.
There are several areas which we believe represent opportunities for significant enhancement of the program efficiency:

1. The identification of slaughter sows and boars remains a serious obstacle to efficient slaughter surveillance, which is potentially our cheapest and best surveillance procedure.

   We believe that enlightened swine producer leadership offers the quickest and best way out of our present dilemma.

2. Unfortunately, the degree of intensive epidemiologic study of individual herds and state programs has not met expectations. It is important that we carefully evaluate the most efficient procedures for herd cleanup and then publish and distribute this information.

3. Case finding activities need to be randomized within each State to ensure that all herds, small and large, are subject to surveillance. We commend those States which are working in this direction and urge all States to carefully evaluate their case finding procedures. In some instances, State officials and their Pseudorabies Committees are actually determining that an unnecessary number of sows and boars are being surveyed and are cutting back to save scarce resources.

To close, I would like to say that we are extremely pleased with the enthusiasm for eradication that we detect among State and Federal regulatory officials.

They report strong support from their State Pseudorabies Committees, and field personnel, who seem to enjoy the challenge of pseudorabies eradication and the feeling that they are contributing to the economic well being of swine producers. Thank you very much.

Slide 1.

The National Center for Animal Health Information Systems, Fort Collins, Colorado, has developed the *Pseudorabies Monitoring System* which automatically produces:

- Letters for owners of Qualified Negative and feeder Pig Monitored Herds.
- The Quarterly State PR Activities Reports (VS-Form 7-1)
- Slaughter Traceback Investigation Form
- Cleanup Plans
- Epidemiology Reports
- Herd Status Records
- Other Reports
Slide 2.
PRMS allows entry and Tracking of Different Types of Records
- Traceback of animals from slaughter
- Test Records
- Vaccination Records
- Cleanup Plans
- Epidemiology Reports
- Herd Status Records

Slide 3.
(PrC) PRMS Implementations
States Trained in FY 1989
Alabama
Arkansas
Michigan
Minnesota
North Dakota
South Dakota
Tennessee
Virginia

States to be trained in FY 1990
California
Florida
Georgia
Michigan
Missouri
New Jersey
North Carolina
Ohio
Oklahoma
Pennsylvania
South Carolina
Texas
West Virginia
Wisconsin
The National Pseudorabies Eradication Program began January 1, 1989, and will terminate no later than 10 years from its beginning on that date. One State, Arizona, gained Stage I program status during the reporting period. All States are expected to have gained program status by the end of FY 1990.

**Slide 1.**

Pseudorabies Eradication Program — Non-reporting States

Thirty-four States were participating in program activity by the end of June 1989.

**Slide 2.**

Herd Status — Participating States

At the end of June, 2.2% of the herds in the 34 participating States were considered infected, 1% were Qualified Pseudorabies Negative, 18.2% were monitored for feeder pig sales and 0.6% were under a written herd cleanup plan.

**Slide 3.**

Percentage of Swine Herds Infected by Region

At the end of June, 1.87% of the Northern Region participating herds were considered infected, 0.66% of the Southeastern Region herds, 3.68% of the Central Region herds, and 0.08% of the Western Region herds.

**Slide 4.**

Herds Under a Cleanup Plan

At the end of June, 34% of the known infected herds in the Northern Region were under a cleanup plan, 9% in the southeastern region, 20% in the Central Region, and 38% in the Western Region.

**Slide 5.**

MST Program — Breeding Swine Surveyed in Participating States

During the 6 month reporting period, 5.14% of the breeding swine were surveyed in the Northern Region, 3.54% in the Southeastern Region, 1.14% in the Central Region, and 17.74% in the Western Region.

**Slide 6.**

Summary of Qualified Negative Herds Infected

During the 6 month reporting period, 8 QN herds in the Northern Region were determined infected, 4 in the Southeastern Region, 20 in the Central Region, and none in the Western Region.
Slide 7.

Source of New Herd Infections

The second quarter report showed 35 new herd infections resulting from the purchase of infected feeder pigs, 41 from purchased breeding stock, 8 from feral swine, 77 from area spread, 4 from infected carcasses, 39 from herd division, 122 were of unknown etiology and 2 reported to be caused by other factors.

Slide 8.

Circle Testing — Infection Found

During the second quarter, 9.4% of the herds tested as a part of circle testing in the Northern Region were found infected, 40% in the Central Region, and 50% in the Western Region.

Slide 9.

Qualified Negative Herd Tests in Participating States

During the second quarter all 4 Regions reported testing fewer than 100% of their qualified negative herds.

Slide 10.

Case Finding January 1–June 30, 1989

During the 6 month reporting period, 2,143 epidemiologic herd tests were conducted which identified 660 new herd infections. (30.8%)
**Pseudorabies**

**Sources of New Herd Infections**

January 1, 1989 to June 30, 1989

Cooperating States

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<thead>
<tr>
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<th>Q1</th>
<th>Q2</th>
<th>Total</th>
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## Pseudorabies

### Selected State Herd Clean Up Activity

(January 1, 1989 to June 30, 1989)

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<th>State</th>
<th># Herds</th>
<th>% Infected Herds (June 1988)</th>
<th># Herd Retests</th>
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## Aphis - Veterinary Services
### Swine Diseases Section

**Summary A - Herd Status Data**

For the Period 4/1/89 to 6/30/89

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<th>State</th>
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<th>Number Infected Swine</th>
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<th>Feeder Pig Monitored Herds</th>
<th>Controlled Vaccinated Herds</th>
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</table>
Pseudorabies

Herd Status
In All Participating States

1st Quarter

2nd Quarter

- Known Infected: 2.1% (1st), 2.2% (2nd)
- Qualified Negative: 1.0% (1st), 1.0% (2nd)
- Feeder Pig Monitored: 17.3% (1st), 18.2% (2nd)
- Under Herd Cleanup: 0.7% (1st), 0.5% (2nd)
Pseudorabies

Percentage of Swine Herds Infected

- **Northern Region**: 1.86% 1.87%
- **Southeast Region**: .52% .66%
- **Central Region**: 3.63% 3.68%
- **Western Region**: .06% .08%
- **National**: 2.13% 2.18%

- **March 1989**
- **June 1989**
Pseudorabies

Herds Under a Cleanup Plan

<table>
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<tr>
<th>Region</th>
<th>First Quarter</th>
<th>Second Quarter</th>
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<td>79%</td>
<td>34%</td>
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<td>17%</td>
<td>9%</td>
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<tr>
<td>Central Region</td>
<td>17%</td>
<td>20%</td>
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<tr>
<td>Western Region</td>
<td>40%</td>
<td>38%</td>
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<tr>
<td>National</td>
<td>36%</td>
<td>23%</td>
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</table>
Pseudorabies

MST Program Breeding Swine Surveyed in Participating States
March and June 1989

- Northern Region: 5.14%
- Southeast Region: 3.54%
- Central Region: 1.14%
- Western Region: 17.74%
Pseudorabies

Summary of Qualified Negative Herds Infected
January 1 - June 1989

Northern Region: 8
Southeast Region: 4
Central Region: 20
Western Region: 0
Pseudorabies

Source of New Herd Infections

- Purchased Feeders: 19 March 1989, 36 June 1989
- Purchased Breeders: 10 March 1989, 41 June 1989
- Feral Swine: 1 March 1989, 8 June 1989
- Fomites (Bedding...): 1 March 1989, 0 June 1989
- Area Spread: 77 March 1989, 77 June 1989
- Infected Carcasses: 0 March 1989, 4 June 1989
- Herd Division: 6 March 1989, 39 June 1989
- Other: 2 March 1989, 0 June 1989
Pseudorabies

Circle Testing
Infection Found

March 1989

June 1989

14.8% 9.4%

18.2% 0%

40%

50%

20.9% 10.6%

Northern Region

Southeast Region

Central Region

Western Region

Nationally

* MN - No report - March, 89
Pseudorabies

Qualified Negative Herd Tests in Participating States
April 1 - June 30, 1989

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<tr>
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<th>Number of total herds</th>
<th>Number of herds tested</th>
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<td>1,548</td>
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<td>Southeast</td>
<td>275</td>
<td>166</td>
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<td>Central</td>
<td>1,236</td>
<td>1,008</td>
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<td>Western</td>
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## Pseudorabies

### Case Finding

**January 1 - June 30, 1989**

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<th># Found Infected</th>
<th>Reason for Test</th>
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<td>89</td>
<td>Slaughter Traceback</td>
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<td>61</td>
<td>22</td>
<td>Market Traceback</td>
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<td>Tracing Sales</td>
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<td>Other</td>
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REPORT OF THE COMMITTEE ON PSEUDORABIES

Chairman: Dr. J. E. Slauter, Jefferson City, MO
Vice Chairman: Mr. D. D. Gingerich, Parnell, IA

W. L. Adams, GA; F. J. Alderink, MD; J. M. Alumbaugh, IA; M. J. Bartkoski, Jr., MO; G. W. Beran, IA; C. C. Black, GA; N. Black, MN; P. E. Bradshaw, IL; D. R. Bridgewater, CO; G. Brown, NC; G. C. Edwards, NC; W. D. Felker, IA; T. W. Freas, IN; D. Galbreath, MD; A. M. Gallina, WA; T. J. Hagerty, MN; H. T. Hill, IA; L. W. Hinchman, IN; D. Hoogenstraat, SD; I. H. Huff, ND; D. D. Hupe, KS; C. L. Kanitz, IN; J. P. Kluge, IA; J. H. Lang, WI; W. J. Mackey, MN; A. D. Moles, MO; R. B. Morrison, MN; F. J. Mulhern, MD; J. Nehay, CA; R. R. Ormiston, MD; R. Polen, NJ; J. R. Ragan, TN; L. W. Schnurrenberger, AZ; R. L. Sharpee, NE; M. L. Snyder, ME; T. E. Socha, NE; P. L. Spencer, MO; C. E. Starkey, AR; W. C. Stewart, MD; A. C. Taft, IL; D. G. Thawley, MN; J. B. Thomas, SC; D. L. Thompson, CA; D. E. Thompson, WI; E. Thurber, NE; W. H. Waldo, NE; D. L. Weiss, IA; L. Williams, NE.

The committee met Oct. 31 and Nov. 1, 1989 with 37 members answering the roll call and more than 100 guests present.

Dr. Robert Ormiston of APHIS reported on the status of the control/eradication program (general session presentation). He listed accomplishments in a number of areas in the program. Program status has been assigned to 7 states: Stage I, Arizona, Missouri and Texas; Stage II, Georgia and Illinois; Stage III, Arkansas and Wisconsin. Applications for program status have been received from Indiana, West Virginia, Kansas, Alabama, Florida and Oklahoma and several additional states are preparing applications. By Oct. 1, 1990 he said all states are expected to have achieved status in the program.

Don Gingerich of National Pork Producers Council reviewed activities by that organization in the following areas:

1. Organization of state committees — all but four states have formed advisory committees (Washington, Oregon, Idaho and Utah).

2. Gathering information of progress from state committees — Dr. Merle Lang has worked with state advisory groups, has attended a number of state meetings and continues to be available.

3. Model state regulations and herd records have been distributed to state committees.

4. Funding — NPPC has secured increases in federal funding through its contacts in Congress for the past two years. The NPPC oversight committee has met on several occasions with APHIS regional directors to discuss state allocations of federal funds.

5. Information — NPPC cooperates with LCI in production of the monthly PRV Progress Report which is mailed to more than 4,500 leaders
PSEUDORABIES

in the effort nationally. A listing of information aids (appendix 1) has been distributed to advisory committees. NPPC has produced two videotapes, one on the disease and the second on the program, that are available for use by anyone through state pork producer organizations. An exhibit on the program is available on loan for use at meetings.

In the area of funding producers support development of a formula that would allocate federal funds to areas based on the density of pig population and the prevalence of the disease.

Gingerich said he has a major concern regarding the administrative load placed on funds appropriated by Congress for the program. A major portion of additional funds secured by producers for finding the disease and cleaning up herds is being skimmed off for administrative purposes. The increase in program funds did not increase administrative costs and we feel should not be used for that purpose, he said.

Neal Black presented a set of goals approved by Livestock Conservation Institute and referred to other organizations involved in the program, as follows:

1992 — All states in Stage II or higher
   22 states Stage IV or Free
1995 — All except Iowa in Stage III or higher
   40 states Stage IV or Free
1996 — All states Stage III or higher
1998 — All except Iowa Stage IV or Free
2000 — All states Free

On motion and vote by the committee the goals were approved.

While these goals may seem optimistic to some, Black said, they are achievable, especially when compared with previous programs at a similar stage, such as the hog cholera eradication effort. “PRV eradication is much farther along than the cholera effort when it was begun. We have a much more united industry, much stronger producer and swine practitioner organizations and tools such as the computer we didn’t even dream of then.” We have better vaccines and diagnostics. When the industry started talking about cholera eradication, he reminded the committee, “there wasn’t even a test for determining whether hogs were infected. Sure we’ll have some problems and some opposition, but we’ve never been better prepared to overcome them.”

Dr. Frank Mulhern discussed major obstacles to the program and their solutions. His presentation is attached as Appendix 2.

Dr. Robert Morrison’s paper on elimination of PRV from large swine herds is attached as Appendix 3.

Dr. Paul Doby of Illinois discussed the advisability of coordinating
REPORT OF THE COMMITTEE

requirements in the swine brucellosis and pseudorabies eradication programs and advised state regulatory officials to review their regulations frequently and rescind those no longer needed to reduce burdens on the industry. He indicated that Illinois officials are planning to require a retest of breeding stock from qualified negative herds from other states. He suggested that the degree of infection in the vicinity of such qualified herds be considered in evaluating the need for retesting breeding stock.

Dr. George Beran of Iowa State University presented a report of the USAHA feral pig sub-committee which was also presented to the general session.

Dr. Richard Ford of the Australian embassy discussed feral swine in Australia, noting they are very adaptable to the different habitats and climates in that country and are fairly widely distributed over the eastern portion of the nation. They are considered an agricultural nuisance, chiefly as a result of the predation of lambs, up to 50% of the lamb crop in some areas. Wild swine have been implicated in two swine brucellosis cases. Control of populations has been successful by trapping and shooting from helicopters. Recovery of the population in areas where it has been reduced depends on climatic conditions, he said. Poisoning has been used but its acceptance is declining.

A panel of producers offered the following comments on their experience with the disease.

Gerald Gehlbach of Illinois reported the successful elimination of the virus from his herd which annually produces about 12,000 hogs. Major factors in the success were continued vaccination and mostly one litter system used in the herd. About 80% of litters are from first-litter gilts and no sows are kept for more than two litters. The gilts were vaccinated at 7 months and again at 12 months of age. Another herd in the area of a similar size cleaned up at the same time, he said.

Jim Lewis of Minnesota, has two 280-sow herds on separate locations in total confinement in a highly endemic area of the state. One of his herds broke with the disease in May of 1989, with the probable source a herd a mile away which became positive at the same time. He said his home herd, the one he thought had the best security, was the first to become infected. A problem in his state is the lack of sufficient quarantined feedlots to handle feeder pigs from infected herds, a situation which has compelled him to build finishing units for the pigs he formerly sold as feeders. His herd had been vaccinated for a year and he is continuing to vaccinate sows before farrowing and weaned pigs intranasally. Other problems in his state are (1) false positives with the differential test, which now may have been solved, (2) the need for obtaining funds to match $150,000 provided by the state legislature but only if matching funds are forthcoming (this is in addition to other state PRV appropriations, and (3) failure to obtain the state's share of federal funds.
Bob Ivey of North Carolina, owner of four 250-sow herds, two purebred and two crossing herds. One of the nucleus herds was found to be positive on a regularly monthly 10% test. Because he was on a monthly testing program, he found the infection when titers were low and none of the herds which had received breeding stock became infected, nor did his crossing farms where animals from the nucleus herd were removed and the rest of the herds tested and found to be negative. It was necessary to obtain samples from 4,400 hogs — every pig in the herds — in a two-day period to lift the quarantine on herds, which he considers a waste of money and manpower, suggesting a random statistical test would have been as good.

Eric Dee, chairman of the Iowa PRV advisory committee, noted that Iowa has an estimated 25% prevalence. He asked that hogs vaccinated with differential vaccines per permitted to move, and asked for more cooperation between states, pointing out that an estimated 60% of Iowa herds are vaccinated. He suggested the biggest problem in Iowa will be funding — counties are waiting to become part of the down-the-road testing program which is being expanded from county to county as fast as funds and manpower permit.

Ivey credited success in obtaining state funds for the program to the political contacts established by the producers, while Lewis credited the success in his state to employment of an experience lobbyist by the state producer association.

A panel of veterinarians offered the following comments:

Dr. Bernard Curran of Iowa, a member of the state advisory committee — considers PRV a management disease. Through use of vaccination, all-in/all-out production and rolling over the sow herd rapidly the virus can be eliminated from herds. Funds are needed in Iowa for finding infected herds. He said federal field people have been instructed to reduce the time spent on PRV and asked for reconsideration of that decision. He also asked for an increase in the fee paid for collecting blood samples.

Dr. Wayne Johnson of Illinois — Everyone is in this together and it takes a neighborhood approach to solve problems. Remove the political restrictions on vaccination and get more pigs vaccinated, especially in dense pig areas, to reduce the amount of virus moving around neighborhoods. Vaccination is one wheel on the cart, the others are management practices.

Dr. Tim Loula of Minnesota emphasized the all-in/all-out concept for reducing the amount of virus circulating on an infected farm, a major factor in reducing transmission within herds. Producers in his area of southern Minnesota have been successful in stopping virus cycling in their finishing herds and are willing to keep their sow herds vaccinated, “but that’s as far as they are willing to go without help from the program.”

Dr. John Bush for Indiana indicated his first experience with the disease was in 1956 and producers in his area have learned to live with it. As a result of the hog cholera depopulation over a major portion of the county in 1972,
REPORT OF THE COMMITTEE

producers aren't anxious to see a mandatory herd cleanup program in that area.

Among other comments by the panel were:

To stop cycling in finishers, sows are vaccinated before breeding and again before farrowing, the time when antibody wanes in the pigs is determined and a decision is then made on when to vaccinate the finishers (Loula). Rolling over the sow herd in a two or three-year period, along with good management, can eliminate the virus from a sow herd (Curran). Circle vaccination is needed as well as circle testing around infected herds to reduce virus production (Johnson). In an endemic area unless all herds clean up at the same time, the effort is useless (Bush). The opportunity to move pigs off the farm is needed to facilitate clean-up of herds, emphasizing the need for contract finishers (Loula). Common mistakes are acting too fast in a herd without time for the outbreak to settle down, inadequate sow separation (Curran). Compromises in herd cleanup plans add risks (Loula) and handling cull sows is a major factor in spreading the virus, since weaning is probably the greatest stress on a sow.

Dr. Joe Annelli of APHIS reported that Minnesota data indicates the prevalence of seropositives in infected herds may not be as high as levels on which statistical testing protocols are based and states with low herd prevalence might consider testing more than 30 animals in a herd to detect infection. More data should be studied to determine if these preliminary conclusions are justified, he said.

Representatives of companies producing vaccines and diagnostic tests reported as follows:

Bioceutics — A new killed gp1-deleted vaccine has been licensed but is not yet on the market. It is chemically inactivated and contains a dual adjuvant. Recommendations are a single dose of 2 ml. administered semiannually. Trials indicate the product reduces time and amount of virus shedding after challenge.

Tech-America — A new gpIII-deleted vaccine has been licensed, along with a screening kit. Data has been submitted for licensing of a differential diagnostic kit. Studies on the diagnostic indicated 100% specificity and sensitivity on known field samples, as well as 100% correlation with the standard ELISA. No serum dilution is required for the test.

Norden — A differential test effective for all Norden vaccines has been licensed and went into use in diagnostics labs the week before the meeting. Field trials indicate a sensitivity of 97.9% compared with the SN and 98.3% compared the standard ELISA. The test includes a suspect range for low titers a few days after exposure which could be retested two weeks later. Results from six diagnostic labs indicate 100% agreement on negatives and agreement between labs on all but two of 166 positives, with two being in the suspect category in some labs.
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SyntroVet — The first differential test licensed for PRV has been proposed for recognition as an official test in a Federal Register publication Oct. 31, 1989. The vaccine which is paired with that test has performed better than expectations, with three million doses having been sold in 18 months. The vaccine will be available soon in combinations for more convenient use before breeding.

IDEXX — In herd studies using the Herdchek differential test indications are that the vaccine stops virus shedding and that the test is effective in differentiating between field and vaccine titers. Over-vaccinating in a herd rapidly shuts down virus shedding.

Dr. John Cobb of Georgia discussed feral pigs in his area, pointing out that most wild pigs in the south should be considered exposed and one test of such pigs for movement may be deceptive. The disadvantage of requiring testing of feral pigs being moved, such as to shooting preserves, he said, is that the requirement will drive the movements underground. States with lots of wild pigs need heavy hunting to keep populations down and reduce exposure of domestic swine. He urged allowing movement of wild pigs as though they are going to slaughter, identified with backtags for movement into preserves with escape-proof fencing and requirements that wild pigs released for shooting must be killed within a day or two.

Dr. Arnold Taft of Illinois discussed use of vaccines in cleanup programs in that state (appendix 4).

Dr. Walter Felker of Iowa said the present level of funding of the Iowa eradication program, both federal and state, is inadequate. He reminded that while the number of infected herds in the state is large, there are still about 35,000 herds which are not infected. The PRV law which passed in the state legislature unanimously provides that on Dec. 1, 1989 all swine sold except to slaughter or to an approved premise must be vaccinated with a differentiable vaccine. The new gp1-deletion test licensed by Norden has been approved for program use in the state. He said the new APHIS strategic plan seems to abandon the former emphasis of the agency. (We must spend funds to eradicate disease, not just measure it," he said.

Dr. Daniel Cohen of the University of Pennsylvania reported on studies of PRV titers in wildlife (appendix 5).

Dr. Quentin Tonelli of IDEXX discussed the PCFIA test, which permits a very large volume of tests with minimum labor requirements. The company is satisfied with performance of the test and is proceeding with validation of the assay. The test permits simultaneous tests for multiple diseases, with swine brucellosis and PRV being studied developed.

Actions by the committee included:

1. Approval of the report of the feral pig sub-committee as it applies to the pseudorabies eradication effort.
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2. Approval of a report of a sub-committee on program standards as follows:

Slaughter surveillance not be implemented in a state until herd prevalence as reported in Section A of the National Pseudorabies Report has declined to 5% or less. States using first-point sampling as a surveillance method exclude from sampling animals from known infected herds.

Revision of program standards to provide that when the owner of a herd to which a positive test result has been traced chooses not to test within 30 days of the positive trace, for whatever reason, the herd must be considered infected and quarantined. In Stage III or higher, even if such a herd is sold out, circle testing around the herd is required.

3. Approval of a recommendation that the chairman of the PRV committee appoint a sub-committee on information with the objective of coordinating information activities for the program. Membership should include USAHA, APHIS legislative/public affairs staff, Livestock Conservation Institute, National Pork Producers Council, Extension Service, American Association of Swine Practitioners, American Farm Bureau Federation, at least one representative from one of the state advisory committees and others as appropriate. The committee should meet annually in advance of the PRV committee session at the annual meeting and at other times as necessary.

4. Approval of a motion thanking the retiring chairman of the committee, Dr. J. E. Slauter for his service to the committee and urging the president of USAHA to appoint the committee’s vice-chairman, Don Gingerich, to replace him.

APPENDIX I

PRV INFORMATION TOOLS
(11/01/89)

The following information materials for PRV Eradication are available from the source listed and at the prices listed.

PAMPHLETS

Swine Pseudorabies Eradication Guidelines (Second Edition), from Livestock Conservation Institute, 6414 Copps Ave., Suite 204, Madison, WI 53716, Phone 608-221-4848; 12-page pamphlet covering the plans for elimination of the virus from a herd; used as basic reference material for use in developing plans for herd clean-up by all states with eradication programs; $20 per 100 copies.

PRV Fact Sheet from Pork Industry Handbook; Cooperative Extension Service, Purdue University, West Lafayette, IN 47906; Phone calls to Jim Foster, 317-494-4837; also available through extension service at most
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land-grant colleges; call Foster for price and to order copies from Purdue; also available for $20 negatives suitable for printing with space for logo of source on front page; available in quantity with NPPC logo from National Pork Producers Council, phone 515-223-2600, ask for Dave Meeker: 2 pages, front and back of one sheet; covers history, clinical signs, immunity, spread of infection, diagnosis and control of infection.

_Pseudorabies in Swine_; USDA program aid #1301; revised edition available after May 1, 1989; available through all USDA Veterinary Service offices; no charge; 8 page pamphlet intended for producer information programs includes information on history of PRV, prevalence, signs, testing, means of spread and controlling spread, and vaccination.

_Pseudorabies Cleanup Programs_, By Wayne Johnson, D.V.M., veterinary practitioner working in herd cleanup in Illinois; 1-page review of field recommendations for herd cleanup, stressing the use of vaccination; copy available for copying for distribution from Dr. W. C. Stewart, USDA-APHIS-VS, 6505 Belcrest Rd., Hyattsville, MD 20782; phone 301-463-7767.

PUBLICATIONS

_PRV Progress Report_, published by Livestock Conservation Institute; monthly newsletter on progress in eradication program; mailed to leaders of eradication effort throughout U.S.; Editor, Neal Black, 2825 Vilas Lane, Eagan, MN 55121, Phone 612-454-5928; covers developments nationally and in states contributing to eradication; to submit names or suggest editorial material, contact Neal Black at address above; contents may be copied in part or entirely for any use to further eradication, including mailing to all quarantine herd owners, for example.

_PRV Progress Report, Introductory Issue_, published by Livestock Conservation Institute, provides background on industry deliberations which resulted in decision to eradicate; suitable as initial informational material for those unfamiliar with program; suggested for duplication by committees for use in their state either by photo copying or printing; to obtain copy for duplication, contact Livestock Conservation Institute or call Neal Black at 612-454-5928.

_Pseudorabies Eradication - State-Federal-Industry Program Standards_, published by Veterinary Services, Animal and Plant Health Inspection Service, USDA; contains all the details on the eradication program, definitions, stages, administrative procedures, standards for participation in herd plans and release of quarantines; available from federal offices of Veterinary Services in each state or Dr. W. C. Stewart, USDA-APHIS-VS, 6505 Belcrest Rd., Hyattsville, MD 20782; phone 301-463-7767.

VIDEOTAPES

_The Last Years of Pseudorabies_; 18 minute videotape featuring Dr. George Beran of Iowa State University discussing the disease, symptoms,
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losses, testing and cleanup plans; ideal for informational meetings as kickoff to presentations and discussions by state and local officials; copies available through state pork producer associations or on loan from National Pork Producers Council, Box 10388, Des Moines, IA 50306; Phone 515-223-2600, ask for Laurie Dvorak.

Pseudorabies Eradication: A Producers Program; 20 minute videotape featuring pork producers Don Gingerich of Iowa; Hilman Schroeder of Wisconsin and Willard Korsmeyer of Illinois discussing their experiences with the disease. Also includes other leaders discussing the eradication program and how it evolved; available through pork producer associations or from National Pork Producers Council, Box 10388, Des Moines, IA 50306; Phone 515-223-2600, ask for Laurie Dvorak.

EXHIBITS

Tabletop display for use as an exhibit at trade shows, conventions, meetings; contains state status map, program goals and information on program stages; self contained in shipping container; intended for use with state pamphlets and program handouts; no charge for use; to reserve call Laurie Dvorak, National Pork Producers Council, Box 10388, Des Moines, IA 50306, Phone 515-223-2600.

APPENDIX 2

MAJOR OBSTACLES AND SOLUTIONS IN PRV ERADICATION

Dr. Frank Mulhern

When Ed Slauter asked if I would talk to you on the subject of major obstacles and solutions in PRV eradication, I reflected on whether or not I was capable of covering that subject. I recalled that over a 40-year span I have been directly, or indirectly involved in the successful eradication of FMD in Mexico and Canada, vesicular exanthema of swine, hog cholera, sheep scabies, virulent newcastle disease of poultry, Venezuelan equine encephalomyelitis, screwworm, African swine fever in Haiti and the Dominican Republic, the Mediterranean fruit fly and citrus canker in Florida, brucellosis and boll weevils. Even a humble Irishman should learn something from that experience.

Industry participation and active support — It’s always been my contention that none of these types of programs can or could be successful without the industry’s participation and active support. So that has to be the number one obstacle to the eradication of pseudorabies. This cannot be glossed over or misread, especially in the eradication of PRV. This is the first program I know of that is being touted as a producer’s program, which is interpreted as their having more control over the implementation of it. It’s really a new role for producers that needs to be fully understood by the
membership, because it carries a lot of responsibility. In the past APHIS assumed more of the leadership role than they plan to fulfill in the PRV eradication program, so producers and state officials must fill the gap, if it is to be successful. Also, previously APHIS worked with the national leadership of the industry involved and tried to get the commitment of as many producers as possible. In reality, this varied state by state. The program implementation schedule was made by APHIS and the state regulatory officials in consultation with the leaders of the industry.

APHIS took the position to eradicate the disease in the shortest time possible, because it was the least costly. Industry leaders, in some cases, sought to modify the program or prolong the schedule. Also, certain states would move into more advanced stages of their program than other states and this caused marketing problems. There were times when this created hardship upon certain segments of the industry involved, and this caused resentment on the part of some producers.

The pilot studies provided a means in states like Iowa and Illinois, where different approaches were used and producers had a strong influence on how the studies were implemented. I have been told that is also true of the programs carried out in Wisconsin and other states. Producers are exercising their influence through the state advisory committees. This is especially true where there are enough producers on the committee so they can’t be out-voted by the other members of the committee.

In the past APHIS provided follow-up to meetings such as this through contacts and participation with state regulatory officials. This helped keep the program moving. In the future producer organizations within the states must do more of this. In other words, instead of reacting to actions by APHIS, they will be expected to lead, and APHIS will support their efforts. I would assume the state regulatory officials will take a stronger leadership role in this program. Perhaps USAHA, through this committee, will also fill some of the roles formerly carried out by APHIS. It will be quite an accomplishment when the goal is finally reached and producers will be proud to say it was their program and that they got it done with the support of the state and federal regulatory officials.

APHIS participation — Formerly APHIS took the bit in its mouth and went marching down the road to eradication. Now they are telling us their role is one of support consisting primarily of providing epidemiological evaluation and surveillance analysis. They are there to provide help upon request. This is going to take a considerable amount of adjustment not only on their part, but also on the part of the state and industry participants. All will be confronting traditions, which is always a real challenge to overcome.

They have always controlled federal funding with a minimum of scrutiny by the industry and state officials. Now procedures are being set up for industry representatives to work with them beginning at the planning stages of their budget cycles and at periodic intervals throughout the
process to the time funds are allocated and spent at the state level. The industry and the states are working on the process, so there are open channels of communication between all parties.

Funding — Over time I’m confident that some of the current problems over federal funding will be understood, if not readily accepted. Congress doesn’t appropriate money for salaries and administration for personnel and operations above the people like Stewart and Ormiston, so they take it out of each program that APHIS has. This may vary each year depending on the status of the program and funds available. We can challenge that as an industry and we should, but don’t be disappointed if you don’t resolve it.

If federal funding is going to be involved, it must come through APHIS. USDA and Congress are going to demand that APHIS be responsible for the allocation of funds. So we can challenge the method of allocation, but we can’t ignore APHIS responsibility. We must strive to have frank, open communication between APHIS, NPPC and the state regulatory officials.

Discontent has been expressed on how little of current funding is distributed to the states and how much is taken off the top. This has generally been accepted or not understood in the past. It’s going to get much more scrutiny in the future and my contacts in APHIS indicate the agency is eager and willing to participate with industry and the states in this regard.

Formerly APHIS presented and fought for funding within the department, OMB and Congress. The industry only got involved when the budget went to Congress and APHIS sought some of the industry leaders to intervene on behalf of the program. In 1989, during the annual NPPC legislative seminar meeting in Washington, 250 producers met with their members of the Senate and House concerning budget priorities of the organization. Pseudorabies was one of those priorities. This type of action produced funding for the pilot studies, even in a year when it was included in the APHIS budget. This year we obtained an increase in the Senate and lost in the House. So the conference split the difference. If we get our act together I’m confident we will get it funded adequately in the future.

When an industry participates to this degree to seek funding, naturally it wants to have greater input into how the money is being spent. This is going to cause some friction until we reach a better understanding and get accustomed to the new process and interrelationships.

State participation — In the past states developed their programs and APHIS coordinated the total effort to minimize conflicts as states moved through the different stages. It seems to me that this burden is going to be more on the shoulders of the industry and the states. Since this coordination is so important to all those involved, the responsibilities of each group need to be clearly identified. Eradication takes place in each state, so there has to be maximum cooperation between the swine industry and the state regulatory officials, with the leadership coming from the advisory committee.
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Epidemiology — Technical Basis for the Program

Pseudorabies, like other eradication programs must have sound epidemiological data to support the program. We never have had all the answers but we need the essential factors before we launch the program and as it is being implemented. The Technical Advisory Committee members have been providing that. In any national program there isn't any reason why the best qualified persons in the country shouldn't be called upon to participate. The industry relies upon the federal and state governments to provide that support. They are expected to call upon the experts whether they are at universities, diagnostic, research establishments or state or federal governments, etc.

It's my understanding that APHIS plans to have regional epidemiologists to implement recommendations of experts on the epidemiology of this disease, just as they have done on the brucellosis program. Experience with the disease is not the basis of determining expertise. We had these types of experts, galore, during the eradication of many diseases who gave us more problems than those who hadn't had any experience with the disease at all. Neither do we need a large group of experts that usually keep us confused. We need those experts who have had years working with the virus, who are actively trying to know more about it, and who can pinpoint from the findings of the regional epidemiologists, the adjustments that must be made in the programs and the short and long term research needs. However, as to long term goals, they must bear in mind we are in a ten year eradication program. That may not be in concrete, but at this stage, we should plan like it is.

Vaccination

When we began to eradicate FMD in Mexico we killed over a million animals trying to eradicate it and were still unsuccessful. Then we vaccinated the 16 million susceptible animals in the infected area 3 times, at 4 month intervals to keep the total population as immune as possible. The data from past experience showed that the virus did not live outside the live animal after a year. During the twelve months we had the total population vaccinated, we killed a little over 12,000 animals that broke through the vaccine. Thus we prevented new strains from becoming established. That's how we eradicated the disease. Needless to say, we, and others, had a bad experience with FMD vaccines but it answered our problem in Mexico. Had we continued with the slaughter program, we may have eradicated those engaged in the program! However, most of the Mexican producers were in favor of the vaccination program and very little attention was paid to the depopulation of the 12,000 head. Today there would have been less apprehension because it seems a large part of the population are using needles to inject drugs.

Most of us who were involved with hog cholera eradication realized the obstacles that use of various vaccines presented. It was unbelievable the
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number of papers presented year after year on the mysterious findings reported which was undoubtedly linked to the various types of vaccines used and the native viruses which underwent periodic mutations. Many people with both technical and producer backgrounds became anti-vaccine critics. Similar problems were encountered with the Brucellosis program.

However, technology has come a long way since those types of vaccines. Also I feel that we need to get maximum benefits from the use of vaccines as a tool towards eradication. In large infected herds we need to repeat the vaccination process used in Mexico and try to build up immunity in those herds to minimize spread and eventually reduce and, if possible, eliminate the disease. I know experience tells us that it will only prolong eradication, but it may shorten it by getting cooperation from owners that are not willing at this time to eliminate the disease from their herds. So, I feel where it is needed, especially in areas of high infection, the vaccines should be an essential component of the eradication program. Their use should not be belittled. They should be encouraged.

Tailor-made Eradication Programs

Some of the seedstock producers have been critical of the program because they say, to them, the solution is worse than the disease. We need to address their specific needs just like we are doing with the large herd problem. Some have said they are unwilling to modify their production and marketing practices to eliminate the disease, but have we seriously sought solutions that would work for them or are we adamant against deviations because it is easier for regulatory officials to implement standard procedures.

If we are willing to develop trials for large herds, it seems we should do the same for seedstock producers. In fact, this program is developing a prototype for future programs. Thus, we must find ways to get maximum cooperation of all segments of the industry involved. We must find a way that producers can cooperate in the eradication of the disease — a workable plan. A way to get maximum disease eradication with minimum interference of their normal production and marketing practices.

Feral Swine

The known presence of pseudorabies in feral swine may or may not present an obstacle to the eradication of pseudorabies in our domestic swine. A positive step to evaluate and seek solutions to this situation was taken after the meeting this spring in Florida. At least priorities for studies were established and hopefully funding can be provided to implement them. This must be given a high priority and not left hanging. All interested groups must keep this on the front burner.

One important conclusion reached at the meeting was that we are not going to be able to eliminate the feral or wild pig population. So we must determine if they are a real source of pseudorabies to our domestic swine.
and if so, what measures need to be taken to prevent spread from them to our domestic swine that will allow eradication of pseudorabies in our domestic swine to be a realistic goal.

Information — Education

Believe me, no animal disease eradication programs can be effective without an active, well coordinated information/education program. In fact, this is an area that can’t be overdone. Even though it took us over 15 years to eradicate hog cholera, some people in the final years were still complaining that we needed a better information program. When the leaders of the beef cattle industry protested during 1976 against the eradication of cattle brucellosis and demanded a thorough evaluation, an important finding was that even though the program began in the late 1930s one important flaw was the deficiencies in the information/education phase of the program. It was emphasized that some of the people who needed it the most were veterinary practitioners and some state and federal veterinarians who had worked on the program for years. This information phase must continue throughout the life of the program. It is just as important in the final stages as it is in the developing stage.

Communication

One point that bothers me about the current pseudorabies program is the lack of outward concern by the majority of producers. At times, I get the feeling that it may be indifference to the program on their part. Others have told me there is strong support among producers, otherwise the program wouldn’t be progressing in some states like it has. Some have said that a lot of the controversy was reduced by the pilot studies. I do recall at a convention meeting in Iowa back in the late 70s, before I retired from APHIS, that there was standing room only and different segments were quite outspoken about their viewpoints. In that environment, even though there was a lot of tension, key points got identified and eventually resolved. Conflict needs to come to the top and be recognized and resolved, otherwise it is like fighting ghosts. Besides, programs without controversy get boring and dull. At times, critics can be a real pain but they play an important part in these programs unless they are ignored. They will identify the weaknesses in the approach from their viewpoint that needs to be evaluated as to its validity.

All groups involved need to have good, effective communication among them. It can’t be assumed. The program leaders need to always recognize its importance and work on it. I still remember some of those shouting matches during the hog cholera and brucellosis programs. There were times when I thought the differences would never get resolved. Some say the critics caused unnecessary delays and increase the cost of the program. However, there were times when they were right and if the program wasn’t modified, they would have failed. In cases where they were wrong, that is the cost one must pay in our system of democracy.
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Costs / Benefits

Some producers have questioned the cost/benefits of eradicating pseudorabies from their herds or the country. I don't know of any other animal disease eradication program that has had more extensive cost/benefit analysis done before the program began than this one. Nevertheless, I firmly believe that these studies need to continue to evaluate the economic conclusion mainly reached during the pilot studies.

It is true that sometimes the virulence of the virus varies as the dynamics in its perpetuation and spread occurs and this can be deceiving. We probably need more data as to its economic effects during the chronic stage as well as during the acute stage.

Some producers have told me that even though they feel they can live with it through the use of vaccines, they would like to get rid of it, if they could. Others have told me the losses or cost of living with the disease doesn't justify the costs of an eradication program. It is interesting that the producers whose swine are not infected tend to receive the greatest benefits from an eradication program both from not experiencing losses due to the disease and the cost of the use of the vaccine. However, most are passive. Do you know that the sale of vaccine before the hog cholera eradication program began was $25,000,000 during one year? This was the sale of the vaccine and didn't include administration costs.

Sound cost/benefit analysis needs to be the basic foundation for any eradication program from a producer's standpoint unless a public health issue is involved. Again if this program is to be the prototype for any future eradication program the cost/benefit issue must be satisfactorily addressed.

In conclusion, I have identified what I consider major obstacles facing the program namely: 1. Industry, state & federal roles, epidemiology, vaccination, tailor made programs, large herd, seed stock feral swine information/education, communication cost/benefits.

I have tried to give a realistic analysis, I have proposed some resolutions. However, I see the major challenge being the recognition and the need to clearly understand the new roles between the industry, APHIS and the state officials in what is being called “The Producers Program of the Future.” Since I spent close to 38 years with the government and the last 3 years with the swine industry I hope I can help resolve the problem associated with this transition.
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APPENDIX 3

ELIMINATION OF PRV FROM LARGE SWINE HERDS

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University of Minnesota

Introduction — Pseudorabies virus (PRV) was successfully eliminated from 116/119 (97.5%) swine herds that were involved with the PRV pilot project for at least 18 months. However, the mean number of sows in these herds was only 117, and no large herds (>400 sows) were included. The swine industry and scientific community are concerned about the special difficulties that owners of large herds face as they attempt to eliminate PRV. Therefore, a resolution was passed unanimously at the 1987 LCI annual meeting calling for increased research efforts to “investigate cost-effective methods for PRV clean-up in large swine herds.” The purpose of this report is to review current knowledge and the approach that our research team is taking to address the problem.

Current Status of Herd — Before contemplating a herd plan, one should know the PRV status of the area. Risk of re-infection is higher for herds in a high prevalence area. Therefore, one should plan less aggressive (costly) plans for these herds.

In most large herds, PRV must be eliminated from the growing/finishing and the breeding sections of the herd. An initial step is to determine the seroprevalence and the approximate age of infected individuals. In the growing section, 10 representative pigs that are older than 4 months of age, from each barn will indicate with 95% confidence whether more than 30% of the pigs have been infected. The age and location of each pig should be noted on the test chart. In the breeding herd, an estimate of the seroprevalence is needed. One is more confident that the seroprevalence in the sample of adults accurately reflects the prevalence in the breeding herd as the number in the sample increases. Thirty representatives gives a reasonable estimate of the seroprevalence, but if greater accuracy is desired, more sows need to be tested. Rather than 30 randomly selected sows, one alternative is to breed representative sows by parity. This may be more informative as to the status of the breeding herd.

When selecting a herd-plan, one should attempt to have more than one plan underway to reduce the chances of complete failure. The three basic programs include test and removal (T & R), offspring segregation, and depopulation-repopulation and the basic methods of these were recently reviewed.

Depopulation/repopulation — To reduce the cost of downtime in a depopulation plan, try to arrange offsite finishing, and breeding of the new...
replacement stock. If whole herd downtime is being contemplated, consider shortening the downtime to <30 days. The increased risk of doing so is impossible to quantify; however, the reduction in cost of the program can be substantial. This option may necessitate bleeding the breeding herd (or at least a representative sample) to remove quarantine. Check with the official state PRV coordinator beforehand. According to federal program standards, the minimum whole herd downtime (generally regarded as 30 days) appears to be negotiable.

An even more risky innovation of depop/repop involves a rollover of the breeding herd with no whole herd downtime. That is, the “old” herd is gradually marketed as the “new” herd is introduced. If PRV is spreading in the herd, contact between the infected and new stock must be minimized, if not eliminated. The “old” herd should be vaccinated to decrease the amount of virus being shed by recently infected swine. Additionally, vaccinating the “new” herd should be considered, since it may decrease the chance of infection. The state PRV regulations will determine whether the “new” herd can be vaccinated prior to the release of quarantine. This program is being attempted in a 500 sow farrow to finish unit and is progressing well to date.

**Offspring Segregation** — The major disadvantage of the traditional PRV offspring segregation program is the major disruption it causes to the herd management with the only benefit being the elimination of PRV. Recent innovations of the program have permitted the elimination of other diseases simultaneously. The goal is to wean the piglets at 5–10 days of age before they become infected with infectious agents such as *Actinobacillus (Haemophilus) pleuropneumoniae*, *Haemophilus parasuis*, *Streptococcus suis*, *Treponema hyodysenteriae*, *Leptospira spp.*, and PRV. One can anticipate this modified offspring segregation to be even more successful than the traditional version, since the chance of having infection in the pigs prior to segregation will be less. However, we do not recommend this procedure for producing breeding stock for sale, since circumstantial evidence suggests that piglets (or fetuses) may be latently infected and yet seronegative by conventional serologic tests.

**Test and Removal** — In this program, noninfected breeding stock are introduced into the existing infected breeding herd and gradually replace all infected sows and boars (figure 1). There are three basic steps for T & R to work. Firstly, spread of PRV must be not occurring in the grow/finish section. Secondly, spread must be not occurring or be very minimal in the breeding herd. Thirdly, all infected sows and boars must be replaced by noninfected stock and the herd needs to be released from quarantine.

Figure 1. Seroprevalence of PRV in an example breeding herd that was infected with PRV but spread has halted (the herd has a 50% annual culling rate and no culling for age).
Step 1 — PRV spread in grow/finish — Pseudorabies virus is usually spreading continually among growing pigs in large herds. It has been proposed that spread within this section of the herd can be halted by moving pigs in an all-out flow (1–2 weeks age spread optimum; 4 weeks maximum), clean rooms between groups, avoid mixing within groups, control other diseases such as A. pleuropneumoniae, minimize other stresses, and strive for an effective vaccination program. It is not known how separated these all out units need to be from infected pigs to eliminate “contamination.”

A dilemma exists for vaccination in growers vs. breeders. Pre-existing anti-PRV antibody does not prevent infection and titer has little correlation with severity of clinical signs upon challenge. Additionally, there is a negative association between the concentration of passively acquired anti-PRV antibody and the pig’s response to vaccination. Therefore, a program which stimulates maximal immunity in the breeding herd will also evoke maximal passive antibody in the pigs and consequently, there will be more inhibition of the growing pig’s response to vaccination.

If PRV is spreading within the grow-finish and breeding herd, there are three approaches to maximizing the effect of the vaccination program in the growing pigs. The most common method is to delay vaccination until approximately 8–10 weeks of age when passively acquired antibodies are thought to have decayed sufficiently. The major problem with this is that PRV infection may have already occurred. A second approach is to bypass the systemic immune system by administering the vaccine intranasally. By stimulating local immunity, decreased clinical severity and less viral shedding have been reported. The intranasal route is the method of choice in several European countries and two products have been licensed recently in the US for intranasal administration (Boehringer-Ingelheim, Bio-Ceutic; Grand Labs, Pseudo Cell). A third approach is to concentrate on the grow-finish vaccination program first and consider no or minimal vaccination in the breeding herd (If negative gilts are being introduced from an outside source, they should be vaccinated). As soon as PRV has stopped spreading within the grow-finish section, an aggressive vaccination program can be started in the breeding herd. This approach appears more
risky at first glance; however, in herds where gilt replacements are supplied from the growing/finishing section, close to 100% of the breeding herd will already be seropositive and vaccination may have no value.

What is the potential of vaccine? Can it reduce shedding after infection to a noninfectious amount? One researcher reported that in pigs that were infected with PRV and then re-infected, PRV shedding could not be detected after the re-infection. This indicates that if pigs are sufficiently immunized, shedding may not occur after infection. Several researchers have reported that pigs given certain vaccines (not available in the US) did not shed virus after challenge. While these experiments need to be repeated, they indicate that vaccine efficacy may continue to improve as technological advances are made.

**Step 2 — PRV spread in the breeding herd** — Preliminary results from a study of 20 quarantined herds in Minnesota indicate that spread of PRV in the breeding herd is a very slow and irregular event, rather than continuous. This occurs even in the absence of any intervention, other than biannual vaccination. Whether new infections in the breeding herd occur by contacting PRV shed from a sow that recrudesced virus, or from PRV being shed in the growing section of the herd is unknown. These observations give reason for cautious optimism for developing programs to eliminate PRV from the breeding herd.

Once the grow/finish is negative, the vaccination program in the breeding herd becomes critical since it is the main method of control that exists at present. The goal is to inhibit recrudescence of latent PRV and eliminate new infections if a sow does recrudesce virus. The frequency of administration has an effect on the immune response but no data exist on the optimum interval. Currently, most veterinarians recommend bi-annual or pre-farrowing vaccination and some are recommending quarterly administration.

Figure 2. Hypothetical effect of vaccination frequency in infected breeding stock on probability of recrudescence and on cost per sow.
Other than vaccination, there are few other recommendations for controlling spread in the breeding herd at this time. One generally accepted guideline is to reduce stress whenever possible. Stressed sows have lower productivity, but more importantly in this case, is that their immune response can be suppressed. Known stressors include fighting, extreme environmental temperatures, housing changes, and rough handling. Although it will be exceedingly difficult to document, the manager’s attitude towards the pigs and his/her husbandry skills probably have a critical effect on a sow’s immune system, and consequently, may be the most important determinant in a herd’s chance of eliminating PRV.

**Step 3 — Eliminating all infected and release of quarantine** — The new PRV Program Standards have details on PRV quarantine removal methods. The standards are less restrictive for farrow to finish herds and representative sampling is considered adequate for early in the program. However, the owner should not be led to believe that the herd is not infected unless a whole breeding herd test is done. The main question is when should this whole herd test be performed. The risk of a second outbreak increases as the seroprevalence initially decreases in the breeding herd, but may actually reach a point where it decreases (figure 3). Although we have no evidence to confirm this theory at present, the implication is that a whole herd test MAY be unnecessary. The advantages of releasing quarantines by representative sampling are that the cost is less than a whole herd test and removing quarantines stimulates confidence in the program. The disadvantage is that there may be a large percentage of herds with <10% prevalence in the breeding herd and representative sampling, which is a less confident method, will not detect these herds.

Figure 3. Hypothetical relationship between prevalence of infected breeding stock and probability of second outbreak occurring within breeding herd.
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Herds removed from quarantine by representative sampling should remain in higher risk category and have frequent follow-up. This also applies to herds that were released by using new diagnostic tests until their sensitivity on field samples is known. Herds released by offspring segregation or T & R should not be permitted to sell breeding stock until all first generation offspring from infected sows are all out of breeding herd.

The use of serologic testing in the field is essential to relieve pressure on diagnostic labs, but more importantly, to give results rapidly (hours) to the producer. This will be more likely to be permitted when the there is a known status on all herds in a state.

Questions to Ponder:

1) After grow-finish is negative, what is source of infection for breeding females? Is reactivation of latent PRV a nonpredictable event or are there stress inducing factors associated with reactivation? farrowing? breeding? disease? more common in those with low antibody titer?

2) In crated gestation barns what is the importance of continuous water troughs vs nose to nose contact between crates? Consider as high a chlorine concentration as sows will tolerate and maintain consumption.

3) What is the “best” vaccine available in the US? There are no independent data available on comparative efficacy. Therefore compare cost of vaccine, availability and need of an accompanying diagnostic test, and license limitations. The effect of the particular vaccine on shedding after infection occurs is more important than the effect on weight gain after infection when you have a regulatory program and must eliminate PRV from infected herds.

4) Should the licensure of vaccines be limited to those having accompanying diagnostic tests? Should the number of compatible diagnostic tests be limited (which would eliminate some vaccines)? In much of Europe, only vaccines having a gp1 deletion are permitted. The alternative for the US is that the market will determine which products will survive.

Preliminary observations indicate that the diagnostic tests will not be compatible between vaccines having a gpX deletion (Tolvid, PRV-Marker), whereas they will be for those having a gp1 deletion (Bio-Ceutic, PR-Vac). This will have repercussions for diagnostic laboratories.
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Table 1. PRV vaccines that are currently available in the U.S.

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<thead>
<tr>
<th>Company</th>
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<td>KV</td>
<td>IM</td>
<td>gpI, gp63</td>
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</tr>
<tr>
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<td>Porci-Rab</td>
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<td>IM</td>
<td>gpI</td>
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<tr>
<td>Oxford</td>
<td>PRV Mune</td>
<td>KV</td>
<td>IM</td>
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<tr>
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<td>MLV</td>
<td>IM/IN</td>
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REFERENCES


USE OF PSEUDORABIES VACCINES IN A CLEANUP PROGRAM

By Arnold C. Taft, D.V.M.
Chief Veterinarian, Bureau of Animal Health
Division of Animal Industries
Illinois Department of Agriculture

Pseudorabies vaccines are used in the Illinois Control and Eradication Program for three different reasons:

(1) To reduce the losses during an acute outbreak
(2) To protect the neighboring herds against clinical pseudorabies. I recommend that all breeding animals within two miles be vaccinated.
(3) To aid the cleanup phase by protection given to negative offspring or purchased additions and to suppress virus shedding in the infected animals.

Permits authorizing the use of vaccine are issued to each herd owner, usually at the request of the practicing veterinarian.

Once a herd is known to be infected with pseudorabies, the first priority is to minimize the loss due to the disease. Modified live vaccines (MLV) have been effective in doing this in many herds. Herd owners are receptive to a cleanup program that employs the use of a vaccine. In addition to the protection from the clinical disease, a good vaccination program will greatly reduce the amount of circulating virus which is essential to the cleanup program. Most herd owners willingly accept a vaccination program for the breeding herd but are more reluctant to vaccinate the “finishing” animals. The vaccination of the “finishing herd” is essential if seroconversion is occurring. If allowed to continue seroconverting every time new pigs move into the finishing buildings, a “virus factory” is maintained which will perpetuate the disease. Our goal is to suppress the viral shedding while we are planning other management strategies to reduce the overall exposure to negative animals that enter the herd through birth or by purchase.

The management strategies to reduce exposure are really the most important part of the whole plan but they take time. Vaccination can be started immediately and will serve a very useful purpose while the management strategies are being implemented. Vaccination alone will not eradicate the disease. All in-all out management from the nursery through the finisher is our goal. Pen density, sanitation, and comfort of the pigs must be carefully monitored to reduce stress during the growing and finishing period. Flow patterns of the infected breeding herd are planned so as to reduce or eliminate exposure to the “finishing herd” and to negative replacement breeding animals. Elimination of common watering or feeding areas is another management factor that reduces exposure.
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The vaccination program is as follows:

• Use a modified live vaccine (MLV) that has a differential test.

• At initial outbreak:
  — Vaccinate all breeding animals and all pigs up to 12 weeks of age—optional on older pigs.
  — Continue to vaccinate newborn pigs until sows have had time to develop maternal antibodies to protect the pigs.

• After initial outbreak:
  — Booster sows pre-farrowing. This should continue so that all breeding animals receive at least one booster per farrowing.
  — Vaccinate all boars at least twice a year.
  — Booster replacement gilts at 5–6 months of age and again pre-farrowing.
  — Vaccinate non-vaccinated purchased replacements on arrival; booster in one month, and again pre-farrowing.
  — Vaccinate all pigs at 8-12 weeks of age.

Our cleanup programs for the large herds are based on:

(1) the protection that a vaccine can offer
(2) the reduction of virus circulation in the herd that is provided by a complete vaccination program
(3) the reduction of exposure time that can be provided by improved management
(4) the reduction of stress throughout the system by improved management
(5) the further reduction of exposure by rapidly replacing all older parity sows with known negative vaccinated replacements.

This program has been in place for varying times up to two years in twenty herds of five hundred (500) sows or more. To date, two of the herds have been released from quarantine—a 700-sow herd and a 500-sow herd. At the beginning, all herds were selling positive market hogs. Today, over one-half of these herds are producing negative market hogs. Comments from the herd owners are: “The pigs perform better.”; “My pigs go to market two weeks sooner.”; “The pigs have less pneumonia.”, and “My herd seems to be healthier.”

November 3, 1989
ABSTRACT

The persistence of pseudorabies (PRV) in a small focal area of Pennsylvania swine farms of Lancaster County, attracted epidemiologic attention. The role of wild animals in maintaining the infection was sought. Serologic evidence was uncovered for the possible role of two species, cats and raccoons, in maintaining the infectious virus on the premises after the disease has been discovered in swine. PRV antibodies at a 1:40 level or greater by the Latex Agglutination test were examined by immunoprecipitation and immunoblotting techniques. The sera from both suspect cats and raccoons were found to immunoprecipitate a number of PRV polypeptides. The potential presence in these sera of antibodies cross-reacting with PRV is being further defined.

INTRODUCTION

The persistence of pseudorabies in Pennsylvania swine has been a perplexing disease. Since it was recently uncovered some 10 years ago, it has defied attempts by regulatory agencies to eradicate it by the system of quarantine and depopulation. Despite heroic efforts, the disease has persisted in certain focal areas of Lancaster County. It was this geographic focus and its resistance to attempts to eradicate it that first drew our attention to the study of its epidemiology.

Pseudorabies in this area has persisted, giving rise to some 10–20 new outbreaks per year. The greater portion of the affected farms are to be found in an area surrounding the town of Effrata. Within this area there are 19 farms which have been known to have been recently affected with the disease. It is the purpose of this report to describe some of the efforts to determine the role played by wild animals as one mechanism by which virus has persisted in the community.

MATERIALS AND METHODS

Virus-Antibody Tests

For surveillance purposes the Pseudorabies Virus Antibody Test Kit produced by Viral Antigens Inc. of Memphis, Tennessee, was utilized. This
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kit, a latex agglutination test procedure, works effectively in identifying IgM or IgG antibodies in swine serum, or plasma. However, to ascertain the specificity of the test in wildlife, it was suggested by the manufacturer that the serum from non-swine sources, positive at a 1:4 dilution, be retested at a 1:40 dilution. If it is positive at 1:40 dilution it may be considered as specifically positive. If the serum sample is positive at 1:4 and negative at 1:40, the serum should be rechecked at 1:4 following heat inactivation. If it is still positive, then some other test must be utilized to see whether or not the antibody detected is specific for pseudorabies.

For this purpose, some of our positives were sent to the Pennsylvania State Diagnostic Laboratory at Sommerdale, PA, where they were subjected to a standardized PRV Serum Neutralization Test procedure. This test was performed in Bovine Kidney Cell Line culture (BK), utilizing 100-1000 TCID/50 amounts of the virus per test. The serum tested was not heat inactivated.

Immunoprecipitation and Immunoblotting Techniques

Additional specimens were sent to Dr. Roger Maes of the Michigan State University Veterinary School for confirmation utilizing the following methods:

a. Immunoprecipitation

Labeling of PRV antigens was done by infecting a monolayer of CRFK cells at a multiplicity of infection (MOI) of 10 pfu per cell. After the virions had absorbed for 1 hour at room temperature, the cells were washed and refed with EMEM and incubated for 3 hours at 37°C. The media was removed and the cells were overlaid with methionine-free EMEM (Gibco Laboratories) containing 1% FBS and 20 uCi/ml of 35S-methionine (Amersham Corporation). The cells were harvested 12-15 hours postinfection. They were first washed 3 times in ice cold sterile saline containing 0.1 mM PMSF. The monolayer was then overlaid with lysis buffer (10mM Tris pH 7.5; 0.15M NaCl; 0.5% NP-40; 0.5% Na deoxycholate) and incubated for 5 minutes at 4°C. The lysed cells were scraped from the bottles and centrifuged at 600g for 10 minutes to remove nuclei. The supernatants were aliquoted and frozen at -70°C.

Formalin-fixed Staphylococcus Aureus (Cowan strain I) was prepared by the procedure of Kessler. Immunoprecipitation was performed by first preclearing labeled antigens with normal serum at 4°C, overnight and then incubating the labeled antigens with hyperimmune swine serum or suspect cat serum at 4°C for 12–18 hours. The immune complexes were precipitated by the addition of protein A-producing Staph A, pelleted and washed four times with ice cold phosphosythesis buffer (0.1M NaH2PO4 – Na2HPO4, pH 7.5, 0.1M NaCl, 1.0% Triton X-100, 0.5% Sodium Deoxycholate, 0.1% SDS). The immunoprecipitates were analyzed by SDS-PAGE on 5% to 10% gradient gels. After separation of the polypeptides, the gels were fixed and...
fluorography was performed by soaking the gel in 1M sodium salicylate (pH 8.0) for 30 minutes. The gels were then dried and exposed to Kodak X-Omat X-ray film.

b. Western blotting

Purified PRV virions were prepared by infecting a monolayer of CRFK cells at a MOI of 0.01. When the cytopathic effect was advanced, the cell culture medium was collected and the virions were purified by centrifugation through a cushion of 30% potassium tartrate in 10mM EDTA (pH 7.5) solution. The purified virions were then separated on gradient SDS-PAGE and immunoblotted.

Trapping Procedures

For this study Safeguard whole body traps were utilized. Animals were trapped, tranquilized with Ketamine and bled utilizing a 20 gauge needle and a 3ml plastic disposable syringe. Traps were placed on farms which were identified by the State Department of Agriculture as having pseudorabies virus present. During the year these sites yielded a variety of feral animals, including: raccoons, opossums, ground hogs, squirrels, an occasional skunk, cats, rats and mice.

Additional samples of raccoon sera were obtained from sites around the State by requesting sera originally from animals trapped for a rabies study by Dr. Charles E. Rupprecht of the Wistar Institute. This included sera trapped from foxes and raccoons in Lacawac Preserve in 1984. At a later date, our trapper re-trapped this area which in 1984 yielded a completely negative series of samples.

Following bleeding, all animals except for raccoons were tagged and released. The raccoons were given 0.5ml. of T-61 Euthanasia Solution and put to sleep. The bodies were then taken to the laboratory at Summerdale of the Pennsylvania State Department of Agriculture for examination for rabies.

RESULTS

Table 1 shows the results from our overall survey. A total of 1380 animals were examined during a 13 month period. Of these 977 or 70.8% were from Lancaster County. A total of at least 23 different species were tested. Chief amongst them were raccoons (353), pigeons (213), opossums (195), mice (153) and cats (159). The results for all species are shown in Table 2. Only 3 of the 23 species tested resulted in positive samples. In Lancaster County, cats were 16.5% positive of the 139 tested, raccoons were 13.7% positive of 73 tested and pigeons were 2.9% of 207 tested. Eleven percent of 281 raccoon sera received from outside of Lancaster County including 3 of 21 trapped in the Lacawac Preserve were also positive.

Initially, some of the positive sera were sent to the Summerdale laboratory for confirmation by the serum neutralization test. Without heat
treatment of sera, the results are shown in Table 3. A close correlation between the two tests was shown. However, these cats all had low titers and when heat inactivated the samples tested negative.

Following these results, the manufacturers of the Latex Agglutination test were contacted to discuss test results and they presented a new protocol for interpreting positive test results in non-swine species. Results using this new protocol are shown in Table 4. It will be seen that only 3 of 10 of the raccoon sera, still available for retesting, tested positive at the 1:40 dilution compared to 0 out of 31 specimens tested from outside of Lancaster County. Only 5 of 26 cat sera available from Lancaster County retested similarly positive at the 1:40 dilution compared to 1 out of 20 cats derived from outside Lancaster County. The one positive animal was located about 1 mile from an infected farm. None of the pigeons from either within or outside of Lancaster County tested positive at the 1:40 dilution level.

Some of the positive (at the 1:40 dilution) cat and raccoon specimens were sent to Dr. Maes at Michigan State University for identification utilizing immunoprecipitation and immunoblotting techniques. Figures 1, 2, and 3 show the results.

Sizes of similar polypeptides from Immunoprecipitation (chart I) and Immunoblotting (chart II) of PRV antigens with suspect cat sera, suspect raccoon sera and hyperimmune swine sera, are:

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<th>Kd</th>
<th>II</th>
<th>Kd</th>
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<td>*</td>
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<td>B</td>
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<td>F</td>
<td></td>
<td>73</td>
<td>*</td>
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* Polypeptides of these sizes also precipitate with hyperimmune goat serum against FHV-1.

As a further check, Feline Herpes Virus 1 hyperimmune serum (Dr. Maes) and Feline Cytomegalo Virus hyperimmune serum (Dr. Kruger) were tested by the Latex Agglutination procedure and both gave a negative response.

DISCUSSION

The Latex Agglutination test, when employed at the 1:40 dilution, seemed a useful screening test for the selection of sera for further testing. Only raccoons and cats from infected farms in the Lancaster area and one cat collected from within a mile of a farm with pseudorabies in adjacent Berks County, tested positive to the Latex Agglutination test at the 1:40 dilution level. From these sera, samples were obtained for further testing by immunoprecipitation and immunoblotting procedures.
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The immunoprecipitation results indicate that sera from suspect cats show a polypeptide pattern that is very similar to the one obtained by reacting radiolabeled PRV antigens with high-titered antiserum to PRV. The immunoblotting data substantiate the presence of antibodies to polypeptides with sizes identical to those of PRV in sera both from suspect cats and raccoons.

The cross reactivity between serum antibodies against FHV-1 and PRV antigens should be further defined. Hyperimmune goat serum against FHV-1 precipitates PRV antigens very close in size to the ones that the suspect cat sera precipitate. Bands D and E from immunoprecipitation of PRV antigens with the suspect cat serum appear to be unique in size. Serum from an SPF cat previously shown to strongly precipitate FHV-1 antigens reacted very weakly with radiolabeled PRV, suggesting that the IPP patterns obtained with suspect cat sera and radiolabeled PRV are not strictly the result of homology between PRV and FHV-1. This would be consistent with the results found in an immunologic comparison of pseudorabies and bovine herpesvirus-1.9

This work suggests that cats are either infected with pseudorabies virus and survive, or are infected with another herpes virus that cross-reacts. If it is the former, then the cat could possibly play a reservoir role in maintaining pseudorabies on the farm.

The results in raccoons similarly seems to suggest that there are raccoons which seem to resist the pseudorabies virus from being considered as a 100% fatal infection. This observation has already been made by others6,7 but the role of the raccoon in perpetuating the disease on a given farm is still under debate.8 The raccoon may have its own herpes virus agents which might serologically cross-agglutinate with pseudorabies on the latex agglutination test. This would account for the 11% positives amongst those seen outside of Lancaster County at the 1:4 level.

The barn cat may represent a similar story. However, both the supportive results of the Serum Neutralization Test and the Immunoprecipitation Test and the negative results with FHV-1 and Feline Cytomegalovirus hyperimmune sera suggest that there may be a more specific response with the farm cat. This would fit with the repeated observation that some cats cohabitate with the swine in the barns on many of the farms. Some of these cats may survive challenge with pseudorabies from infected swine and stay on the farm even after the infected swine are removed. Under these conditions they may possibly serve as a source of infection for the next generation of clean swine that are brought to the affected farms. Further attention to the clarification of the role of the barn cat in the perpetuation of Pseudorabies Virus on affected farms is indicated.

ACKNOWLEDGEMENTS

A special note of appreciation is due to Mr. Dan Sullivan of Dr. Maes laboratory who ran the immunoprecipitation tests and took the photo-
PSEUDORABIES

graphs, Ms. Ann M. Rosenberg of Dr. Kim’s laboratory who performed the serum neutralization tests, Ms. Debbie Flad of Dr. Cohen’s laboratory who ran the Latex Agglutination tests and kept the records and to Mr. Ted Poper who trapped and bled the various species of animals tested.

REFERENCES

# REPORT OF THE COMMITTEE

## TABLE 1

**SPECIES SURVEYED FOR PSEUDORABIES VIRUS**  
**JULY 1, 1988 TO AUGUST 31, 1989**

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<thead>
<tr>
<th>SPECIES</th>
<th>LANCASTER</th>
<th>NON-LANCASTER</th>
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<td>Pigeons</td>
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TABLE 2
SEROLOGIC RESULTS WHEN TESTED BY LATEX AGGLUTINATION FOR PRV
JULY 1, 1988 TO AUGUST 31, 1989

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<tr>
<th>SPECIES</th>
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<tr>
<td>Cats</td>
<td>23/139</td>
<td>1/20</td>
<td>16.5</td>
</tr>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>Pigeons</td>
<td>6/207</td>
<td></td>
<td>2.9</td>
</tr>
</tbody>
</table>

All other species tested were negative.

TABLE 3
RESULTS OF TWO SEROLOGIC TESTS FOR PSEUDORABIES IN WILDLIFE

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LATEX AGGLUTINATION</th>
<th>SERUM NEUTRALIZATION*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raccoon</td>
<td>++</td>
<td>1:8</td>
</tr>
<tr>
<td>Raccoon</td>
<td>+</td>
<td>1:4</td>
</tr>
<tr>
<td>Raccoon</td>
<td>+</td>
<td>1:4</td>
</tr>
<tr>
<td>Cat</td>
<td>++</td>
<td>1:8</td>
</tr>
<tr>
<td>Cat</td>
<td>+</td>
<td>1:8</td>
</tr>
<tr>
<td>Opossum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Opossum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dog</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Performed at the Summerdale Laboratories of the Pennsylvania State Department of Agriculture. Serum not heated.
TABLE 4
RESULTS OF LATEX AGGLUTINATION RETEST OF CATS AND RACCOONS

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LOCATION</th>
<th>POS. 1: 40</th>
<th>TOTAL TESTED</th>
<th>% POS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RACCOONS</td>
<td>LANCASTER CY</td>
<td>3</td>
<td>10</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>NON-LANCASTER</td>
<td>0</td>
<td>31</td>
<td>0.0</td>
</tr>
<tr>
<td>CATS</td>
<td>LANCASTER CY</td>
<td>5</td>
<td>26</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>NON-LANCASTER</td>
<td>1*</td>
<td>20</td>
<td>5.0</td>
</tr>
<tr>
<td>PIGEONS</td>
<td>LANCASTER CY</td>
<td>0</td>
<td>4</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>NON-LANCASTER</td>
<td>0</td>
<td>2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*From site about 1 mile from an infected farm.

FIGURE LEGENDS

Figure 1. Title: Immunoprecipitation of 35S-methionine labeled cytoplasmic extracts of PRV with suspect cat sera.

Immunoprecipitation of uninfected, labeled cell extracts with suspect cat serum (Lane U1) and with hyperimmune swine serum (Lane U2). Lane I1: Immunoprecipitation of labeled PRV antigens with hyperimmune swine serum. Lanes 1-8: Immunoprecipitation of labeled PRV antigens with suspect cat serum.

Figure 2. Title: Presence of polypeptides with apparent homology to those of PRV in sera of suspect cats and raccoon as determined by immunoblotting.

Reaction pattern of polypeptides from purified PRV virions with: (Lanes A and B) hyperimmune swine serum; (Lanes C, D, and E) suspect cat serum; (Lanes F, G, and H) suspect raccoon serum.

Figure 3. Title: Crossreactivity between 35S labeled PRV antigens and antibodies against FHV-1.

Immunoprecipitation of PRV 35S labeled antigens with goat anti-FHV-1 serum (lane A), SPF cat anti-FHV-1 serum (lane B) and suspect cat serum (lane C).

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AFLATOXINS IN ANIMAL TISSUES DURING DROUGHT CONDITIONS

By J. Honstead, R. D. Stubblefield, O. L. Shotwell

aUSDA, Food Safety and Inspection Service, Science and Technology, Residue Evaluation and Planning Division
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I. Introduction

The purpose of this project was to determine the presence of aflatoxins in the U.S. meat supply in a drought year. The year in question was 1988, during which certain regions of the midwestern and eastern U.S. experienced a severe drought.

The Food Safety and Inspection Service (FSIS) has regulatory authority over the U.S. meat supply in federally inspected plants. The Residue Evaluation and Planning Division of FSIS is responsible for planning the testing of tissue samples for animal drugs, pesticides and chemicals or environmental contaminants. Compounds for which no tolerance has been established may be sampled for in exploratory studies in which information is collected and no regulatory action is taken. Aflatoxin testing in 1988 is an example of an exploratory program.

FSIS desired aflatoxin data on edible animal tissues in a worst case scenario based on the following:
1. Aflatoxins are highest in drought years (1988).
2. Aflatoxin occurrence varies among locales.
3. Swine concentrate aflatoxins more than do other animals.
4. Of the edible tissues, liver contains the highest concentration of aflatoxin.

II. Aspergillus flavus

Aspergillus flavus is a fungus that is found in soils in most parts of the United States. During drought conditions, two things happen that set the stage for aflatoxin contamination in corn. First, the corn is under stress from the lack of moisture and insect damage. Second, A. flavus is more easily carried to the corn plant via the dust.

Once A. flavus is carried to the corn plant, it contacts two critical areas: the silk and the insect-damaged skin of the corn seeds. A. flavus grows here and sends projections called mycelia into the endosperm of the corn kernel, where the aflatoxins are produced.

Fall harvest is often carried out during moist conditions. If the corn is
stored wet, the *A. flavus* will continue to grow and increase the aflatoxin concentration in the corn.

III. *Aflatoxins*

Many aflatoxins are produced but two are of toxicological interest in foods. Aflatoxins B1 and M1 have been shown to be hepatotoxic, carcinogenic and teratogenic in animals. These are both formed in corn and can be found in food products made from contaminated corn. If M1 is fed to dairy cows, it is concentrated in the milk.

When corn containing aflatoxin in high enough concentration is fed to animals, it can cause decreased weight gain, weight loss, liver damage or death, depending on the dose. Aflatoxins are metabolized rapidly upon absorption from the digestive tract and eliminated in urine and feces. If the animal goes to slaughter, there may be concentrations of aflatoxins in some of the edible tissues as metabolism detoxifies and eliminates it from the body. Ruminants tend to retain aflatoxins longer because of the slow transit time through the rumen. Table I illustrates the feed-to-tissue ratios for domestic animals indicating that, of the food-producing animals, swine has the lowest ratio and is therefore the species of choice in this sampling plan.

*Table 1.* Ratio of aflatoxin concentration in feed to residue concentration in edible tissues.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tissue</th>
<th>Aflatoxin</th>
<th>Feed/Tissue Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef cattle</td>
<td>Liver</td>
<td>B1</td>
<td>14,000/1</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>Milk</td>
<td>M1</td>
<td>75/1</td>
</tr>
<tr>
<td>Swine</td>
<td>Liver</td>
<td>B1</td>
<td>800/1</td>
</tr>
<tr>
<td>Broilers</td>
<td>Liver</td>
<td>B1</td>
<td>1200/1</td>
</tr>
<tr>
<td>Layers</td>
<td>Eggs</td>
<td>B1</td>
<td>2200/1</td>
</tr>
</tbody>
</table>

The Food and Drug Administration has set enforcement levels for aflatoxins in various foods and animal feeds. No tolerance has been set for meat and other tissues from food animals.

*Table 2.* Food and Drug Administration Enforcement Level for Aflatoxins

<table>
<thead>
<tr>
<th>Product</th>
<th>Enforcement Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>0.5 ppb</td>
</tr>
<tr>
<td>Corn (human consumption)</td>
<td>20 ppb</td>
</tr>
<tr>
<td>Corn (for animal feed):</td>
<td></td>
</tr>
<tr>
<td>—Dairy animals</td>
<td>20 ppb</td>
</tr>
<tr>
<td>—Immature animals</td>
<td>20 ppb</td>
</tr>
<tr>
<td>—Breeding beef cattle &amp; swine, mature poultry</td>
<td>100 ppb</td>
</tr>
<tr>
<td>—Finishing swine</td>
<td>200 ppb</td>
</tr>
<tr>
<td>—Finishing feedlot cattle</td>
<td>300 ppb</td>
</tr>
</tbody>
</table>

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IV. Sampling Plan — 1988

A worst case sampling plan of swine for aflatoxin was conducted from samples associated with the 1988 severe drought. Aflatoxins were determined to be present in corn in states which usually do not have any contamination. Six states were identified as having significant aflatoxins in their corn crop: Virginia, North Carolina, South Carolina, Texas, Iowa and Illinois. The region within each state having the highest aflatoxin in corn was described as follows:

- Virginia: S.E. corner of state, northeast and east of Richmond
- North Carolina: south and east of Raleigh
- South Carolina: east of Columbia parallel to the coast
- Texas: line from Wichita Falls to Waco to Arkansas border
- Iowa: Lyons County, Des Moines area, triangle: Cedar Rapids to Dubuque to Maquoketa
- Illinois: north of Interstate I-70

Medium-sized FSIS slaughter plants were selected for sampling in these areas because they can identify locally fed hogs at the plant. Instructions sent with the sample requests specified that hogs from local farms were to be collected when possible. This avoided auction hogs from farms out of the region.

The samples were collected in two time periods: January and April 1989. Due to the drought, much of the 1988 corn crop was fed to livestock soon after harvest which the January samples would have measured. The corn that was stored, started to be fed in the early Spring which the April samples would measure.

The tissues collected from analysis were swine liver and muscle. These were frozen and sent to the Agriculture Research Service (ARS) Laboratory in Peoria, Illinois. Aflatoxins were stable frozen at -20 C and can be stored for analysis.

V. Analytical Methods

The tissues were first screened by high pressure liquid chromatography. The detection limits for B1 is 0.04 ppb and for M1 is 0.1 ppb. If aflatoxin was found in the screen test, confirmation was carried out by two-dimensional thin layer chromatography.

VI. Discussion

The sampling plan accomplished the main goal of analyzing aflatoxins in worst case situations in federally inspected products. This was accomplished by testing 160 samples of liver from locally fed hogs in the regions with high aflatoxin corn from the 1988 crop. The expertise of the ARS lab in analyzing the tissues has been very useful in this cooperative effort between two federal agencies.
The results of the analysis indicate that these animals effectively metabolize aflatoxins present in feed under routine slaughter conditions. Eight of 160 (5%) liver samples had confirmed aflatoxin, and only 4 of those 8 exceeded 0.1 ppb. Only 1 of 160 liver samples was in excess of the milk tolerance of 0.5 ppb for total aflatoxin. Neither aflatoxin B1 nor M1 were detected in any of the 160 muscle samples tested.

In severe drought conditions, the presence of aflatoxins in animal feed does not result in a significant frequency in or magnitude of tissue residues in swine. Since swine appears to be the most sensitive species insofar as tissue concentration of aflatoxins, it would be logical to conclude that residues in other food producing species would be significantly lower.

ACKNOWLEDGEMENTS

The authors wished to thank J. I. Greer and G. M. Shanon for technical assistance and expert analyses.
THE APPLICATION OF HACCP TO POULTRY INSPECTION
STATUS REPORT OF A FEASIBILITY STUDY
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Ralph W. Johnston, B.S., MSc.
U.S. Department of Agriculture
Food Safety and Inspection Service
Washington, DC 20250

INTRODUCTION

In 1987, the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service, which is responsible for meat and poultry inspection in the United States, embarked on a study to determine if the Hazard Analysis and Critical Control Points (HACCP) concept can in practice minimize microbial contamination of poultry and if this approach can be effectively used to regulate the poultry industry. This paper is a report on the status of this feasibility study which is on-going.

USDA surveys have shown that approximately one-third of all chicken carcasses leaving poultry processing plants have some Salmonella cells on them.\(^1\) The actual number of Salmonella cells per bird appears to be extremely low.\(^2\) Nevertheless, low levels of Salmonella on fresh product can pose a health hazard if it is grossly mishandled and cross contaminates ready to eat foods. A large amount of poultry is consumed in the U.S., and the consumption rate continues to climb. In 1988, the average American consumed 80.6 pounds of chicken and turkey as compared to 63.1 pounds of pork and 72.7 pounds of beef. It is projected that chicken and turkey consumption for 1989 will increase to 83.5 pounds on the average.\(^3\) Public health data suggests the incidence of Salmonella food-borne disease in the U.S. is increasing.\(^4\)

The goal of USDA is to improve the safety of fresh meat and poultry. To accomplish this goal, USDA has begun to refine the way inspection interfaces with production. USDA believes it should set clear standards for the control of potentially hazardous points in the production process and for the safety of the finished product. These standards should be based on sound scientific principles. They should be demonstrated in the “real world” to work, that is, to cause the level of bacteria, including human bacterial enteropathogens, to be minimized. The plant should be held accountable for meeting these standards, while the inspection service should regularly monitor the operation of the plant to insure standards are met. The challenge facing USDA is how to accomplish these goals in a way most cost-effective for both the inspection service and the regulated industry.

In the Fall of 1987, USDA began a study to determine if a poultry company can apply the principles of HACCP and significantly reduce bacterial contamination on fresh product. The study is being conducted in
cooperation with an integrated poultry company in Puerto Rico. If the study proves successful, USDA would be in a position to mandate that all poultry processors operate using the HACCP model.

**METHODS**

For the purpose of this study, poultry production was divided into three zones: (1) the farm environment, (2) the feather removal operation, and (3) removal of the viscera. In the farm environment zone, four critical control points were sampled and hazard analyzed: (1) the eggs and the hatchery environment, (2) the feed, (3) the drinking water and (4) the chicken house environment. The eggs were sampled by swabbing the shell surface and collecting the egg shell fragments and residual fluff. Also, cloacal swabs were collected from culled chicks. In addition, transport tray liner paper was sampled. All the samples including the feed and water samples were collected in sterile plastic bags. Samples were taken of the farm environment using the drag swab method.

The feather removal zone was considered as a single major critical control point. This critical control point was hazard analyzed by sampling carcasses at the point where they were transferred from the feather removal room to the evisceration room. The carcasses were sampled using the whole bird carcass rinse method.

In the viscera removal zone, three critical control points were sampled and hazard analyzed: (1) the viscera removal operation, (2) the giblet harvest operation, and (3) the chilling operation. Carcasses and giblets were sampled using the standard carcass and tissue rinse method. Standard methods were used to analyze the samples for total number of aerobic bacteria, for the number of enterobacteria and *Escherichia coli*, and for the presence of *Salmonella*.

**RESULTS AND DISCUSSION**

Initially, 800 samples were collected and analyzed within the plant. Four drag swabs were collected and analyzed from each of 27 different farms. A total of 100 feed samples were collected and analyzed. Chicken drinking water samples were collected and analyzed from 10 different farms. Over 300 samples were taken of the eggs and the hatchery environment. These samples provide baseline data so that as various control measures are tested, their relative effectiveness can be determined.

Last year (1988) USDA concentrated its research effort in the zones where changes are generally the simplest and least expensive to implement. In the evisceration zone, 20 ppm chlorinated water hand rinse facilities were installed for all workers. Also, workers who used knives or scissors were provided two sets so that one could be soaked in a sanitizer between use. Workers were required to rinse their hands and change utensils whenever contamination occurred. In addition, shackles were marked to pass workers approximately every minute which prompted them
APPLICATION OF HACCP

to routinely rinse their hands or change utensils regardless of obvious contamination accidents.

In the feather removal zone, the scalder configuration was changed so that carcasses exited the scalder at the opposite end from where they entered. The water intake was moved to the exit end of the scalder so carcasses moved from the dirtiest to the cleanest water. Also, a hot water rinse cabinet through which carcasses passed after exiting the scalder was added.

These changes in toto appear to have had a significant impact on the bacterial quality of the finished product, but the results are not consistent for Salmonella contamination. This may be due to the variable levels of latent Salmonella infections in different flocks. Samples are continuing to be collected and analyzed at various critical control points. In addition, cecal samples have been added to the study design to try and determine the level of latent Salmonella contamination of various flocks being processed on the particular days sampling is being conducted.

Further studies this month (November, 1989) are planned for the feather removal zone including the effects of using very hot water (160 degrees F) on the carcasses as they exit the pickers.

In the evisceration zone, studies are under way on the effect of adding chlorine at the rate of 25 ppm. in the potable intake water to both the carcass and giblet chillers. This concentration of added chlorine leaves about 3–5 ppm. chlorine in the overflow water. Preliminary results suggest this chemical significantly decreases the presence of Salmonella on the finished product.

It appears however, that what the scientific literature\textsuperscript{11} has already suggested and what many working in this area believe, probably is true. That is, the best way, and perhaps the only way, to prevent or control Salmonella and other human enteropathogenic bacteria from contaminating the finished product is to prevent these organisms from entering the processing plant in the first place. With the current processing technology available, it appears that enteropathogenic bacteria hazard controls in the processing plant are effective only against rather minimal levels of latent Salmonella contamination and moderate to heavy flock contamination overwhelms the controls.

This supposition has led to shifting the principal focus of the study to the farm environment zone. A feed additive product made by Kemin Industries, Inc.,\textsuperscript{12} will next be tested. It is a mixture of organic acids that reportedly has already been used in Europe to some extent. In research studies done by the company, this additive has been shown to reduce Salmonella to a non-detectable level in the feed within three days. The additive also has been claimed to confer a high degree of resistance to subsequent re-contamination of the feed\textsuperscript{13}. A test of this product under field conditions should show if the levels of Salmonella can be reduced in birds before they arrive at the
PRUCHA, JAMES, JOHNSTON

plant and subsequently in the fresh product leaving the plant.

Within the next few days (early November, 1989) four farms will begin feeding this treated feed for the entire life of their chicken flocks. The study group of chickens will also be placed on fresh litter. The approximately 80,000 chickens making up the study group will be processed on a single day in late December or early January and both the pertinent critical control points and the finished product will be extensively sampled. A control group of chickens will be processed and slaughtered the following day.

CONCLUSION

USDA believes that systematically identifying hazards throughout the chain of poultry production, determining their impact on the finished product, developing control measures, and measuring their effect is a sound concept. Such a process is a large undertaking and will take time. Nevertheless, USDA is committed to testing all possible applications of HACCP to poultry inspection and determining which ones are both practical and effective in minimizing bacterial contamination of fresh poultry. USDA fully expects the industry will adopt any process controls found to improve the overall bacterial quality and safety of fresh poultry.

REFERENCES AND NOTES


6. For collecting the feed samples, a sterile plastic bag was held under the point where feed was augured into the feed troughs from the feed storage bins. When collecting the water samples, the water was first run for approximately 30 seconds, the faucet was then heated with a propane torch to sterilize it, then the water sample was collected in a sterile plastic bag.

7. The drag swab methodology was developed by Dr. E. T. Mallinson, Cooperative Extension Service, University of Maryland, College Park,
MD. The methodology is described in an article accepted for publication in Avian Diseases; 33: No. 4

8. The Whole Broiler Carcass Rinse Procedure is available from the Microbiology Division, Science and Technology, FSIS, USDA, Washington, D.C. 20250.

9. Bacterial analytical methods used are described in Microbiological Laboratory Guidebook. Available from the Microbiology Division, Science and Technology, FSIS, USDA, Washington, D.C. 20250.

10. The sanitizer used was Mikroklene DF manufactured by Klenzade Products. It is an iodine-based detergent sanitizer at a concentration of 25 ppm. titratable iodine.


12. The feed additive is called Prostat; it is produced by Kemin Industries, Inc., 2100 Maury Street, Des Moines, Iowa 50301.

INFECTIOUS WASTE LEGISLATION FOR MINNESOTA VETERINARIANS

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In May of 1989, an Infectious Waste Disposal Bill was passed in the Minnesota State legislature. This followed two years of study by the Attorney General's Office of real and potential generators of infectious wastes. The legislation will become effective January 1, 1990.

Action at the state level was precipitated by National reports of improper management of medical wastes, such as waste haulers being stuck by needles, and biomedical wastes washing ashore on beaches. In Minnesota, there were reports of sharps or needles being disposed of with regular garbage and medical wastes ending up on the tipping floors of solid wastes processing facilities. These incidents stimulated regulatory responses at the state and federal level.

Background

The 1976 Resource Conservation and Recovery Act (RCRA) of the U.S. Congress included infectious waste among the kinds of waste to be regulated by the Environmental Protection Agency (EPA). Proposed regulations published in December, 1978 contained broad definitions that would have treated practically all wastes from a veterinary hospital or laboratory as “hazardous.” The American Veterinary Medical Association (AVMA) extensively opposed the broad definition in that it would result in burdensome overregulation with no appreciable benefit to public health or the environment.

Following lack of implementation of regulations by the EPA, the President in 1984 signed a Congressional bill that contained a series of amendments to, and a four-year extension of the RCRA. This new law also directed the EPA to begin regulating “small-quantity” generators of hazardous waste (over 100 kg. and less than 1000 kg. per month).

Subsequently, the passage of the federal Medical Waste Tracking Act of 1988 resulted in development of rules for managing the medical waste stream. As a Great Lakes state, Minnesota had the option to participate in the federal program. Instead, Minnesota from the Attorney General's Office (AG) formed an Infectious Waste Task Force and opted out of the Medical Waste Tracking Act. In the Fall of 1988, the Attorney General’s Office in consultation with the Minnesota Pollution Control Agency (MPCA), the Minnesota Department of Health and interested parties drafted legislation that passed in 1989.

Veterinarians of the Minnesota Veterinary Medical Association (MVMA)
INFECTIOUS WASTE LEGISLATION

requested excluding Veterinary Hospitals and Clinics from many of the regulations that were being proposed for disposal of infectious wastes.

In the 1989 Legislation, Minnesota veterinarians were included in two areas: disposal of sharps and preparation of a management plan. Animal research was also included if agents infectious to humans were used.

The following definitions are selected from the legislative bill and are of importance to veterinarians:

**Infectious Agent**—means an organism that is capable of producing infection or infectious disease in humans.

**Infectious Waste**—means laboratory waste, blood, regulated body fluids, sharps and research animal waste that have not been decontaminated.

**Laboratory Waste**—means waste clusters and stocks of agents that are generated from a laboratory and are infectious to humans; discarded contaminated items used to inoculate, transfer, or otherwise manipulate cultures or stocks of agents that are infectious to humans; wastes from the production of biological agents that are infectious to humans; and discarded live or attenuated vaccines that are infectious to humans.

**Sharps**—are defined as (1) discarded items that can induce subdermal inoculation of infectious agents, including needles, scalpel blades, pipettes, and other items derived from human or animal patient care, blood banks, laboratories, mortuaries, research facilities and industrial operations; and (2) discarded glass or rigid plastic vials containing infectious agents.

Sharps, except those generated from a household or from a farm operation or agriculture business:

(1) must be placed in puncture-resistant containers;

(2) may not be compacted or mixed with other waste material whether or not the sharps are decontaminated; and

(3) may not be disposed of at refuse-derived fuel facilities or at other facilities where waste is hand sorted.

The second area for veterinarians is the preparation and submission of a management plan to the extent applicable to the facility, a person in charge of a facility that generates, stores, decontaminates, incinerates, or disposes of infectious or pathological waste must prepare a management plan for the infectious or pathological waste handled by the facility.

The management plan must describe, to the extent the information is applicable to the facility:

(1) the type of infectious waste and pathological waste that the person generates or handles;

(2) the segregation, packaging, labelling, collection, storage, and transportation procedures for the infectious waste or pathological waste that will be followed;
(3) the decontamination or disposal methods for the infectious or pathological waste will be used;

(4) the transporters and disposal facilities that will be used for the infectious waste;

(5) the steps that will be taken to minimize the exposure of employees to infectious agents throughout the process of disposing of infectious or pathological wastes; and

(6) the name of the individual responsible for the management of the infectious waste or pathological waste.

The management plan must be kept at the facility. To the extent applicable to the facility, management plans must be accompanied by a statement of the quantity of infectious and pathological waste generated, decontaminated, stored, incinerated, or disposed of at the facility during the previous two-year period. Quantities may be reported by weight, volume, or number and capacity of containers. The Commissioner of Health shall prepare a summary of the quantities of infectious and pathological waste generated, by facility type.

A management plan must be updated and resubmitted at least once every two years. Management plans prepared by facilities that generate infectious or pathological waste must be submitted to the Commissioner of Health with a fee of $225 for facilities with 25 or more employees, or a fee of $40 for facilities with less than 25 employees.

Research Animal Waste—means carcasses, body parts, and blood derived from animals knowingly and intentionally exposed to agents that are infectious to humans for the purpose of research, production of biologicals, or testing of pharmaceuticals.

Agency Rules—The agency (MPCA), in consultation with the Commissioner of Health, may adopt rules to implement. The agency has primary responsibility for rules relating to transportation of infectious waste and facilities, storing, transporting, decontaminating, incinerating, and disposing of infectious waste. The agency before adopting rules affecting animals or research animal waste must consult the Commissioner of Agriculture and the Board of Animal Health. The MPCA is currently establishing rules for the 1990 implementation.2

Health Rules—The Commissioner of Health after consulting with the MPCA may adopt rules to implement. The Commissioner of Health has primary responsibility for rules relating to facilities generating infectious waste. The Commissioner of Health before adopting rules affecting animals or research animal waste must consult the Commissioner of Agriculture and the Board of Animal Health.

Penalty—A person who knowingly, or with reason to know, disposes of or arranges for the disposal of infectious waste as defined, at a location or in a manner that is prohibited is guilty of a gross misdemeanor and may be....
sentenced to imprisonment for not more than one year, or to payment of a fine of not more than $10,000 or both. A person convicted a second or subsequent time under this subdivision is guilty of a felony and may be sentenced to imprisonment for not more than two years, or to payment of a fine of not more than $25,000 or both.

**Future**—The Pollution Control Agency, in consultation with the Commissioner of Agriculture and the Board of Animal Health, shall study the feasibility of establishing a collection system for sharps generated by farm operations or agricultural businesses.

**Guidelines for Veterinarians**—In the August 15, 1989 issue of the Journal of the American Veterinary Medical Association, an AVMA guide for veterinary medical waste management is published. This guide includes a model veterinary medical waste management program; however, it does not attempt to address all the variations in state and local regulations.\(^3\)

The veterinary profession must be strongly cognizant of its responsibilities on the handling and disposing of veterinary medical waste. Through the development of management plans, Minnesota veterinarians must look carefully at methods and procedures being used in their hospitals and clinics. We must make sure that we do not contribute to the pollution of the environment and thus necessitate costly regulations. Protection of animal/human health and prevention of environmental contamination must be the highest priorities of the veterinary profession.

**REFERENCES**


REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND
ENVIRONMENTAL QUALITY

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Miller, AL; J. C. New, TN; R. L. Parker, KS; M. E. Potter, GA; J. C. Prucha,
MD; S. K. Scott, IA; T. F. Siburt, VA; R. H. Singer, KY; C. D. Stumpff, KS;
J. Webb, DC; S. Williams, MD; L. D. Woodsor, KS.

The Committee on Public Health and Environmental Quality was called
to order by Dr. Stanley L. Diesch, Chairman, at 1:30 P.M., on Wednesday,
November 1, 1989. A total of eight (8) committee members and twenty-four
(24) guests were in attendance.

In the absence of Dr. D. F. Schwindaman, Dr. John New served as Acting
Vice Chairman.

Dr. John Prucha, Deputy Administrator, Science and Technology, FSIS,
USDA, gave a presentation on the application of HACCP to poultry
inspection. In 1985, the National Academy of Sciences issued a report on the
food inspection methods used by USDA. One recommendation was that
inspection should be based on risk assessment. HACCP (Hazard Analysis
of Critical Control Points) resulted from this report. Control of enteropatho-
gen contamination is a current focus using HACCP. A pilot project in a
poultry processing plant is currently underway. Critical Control Points in
poultry include eggs, feed, water and the poultry house. Samples are taken
and analysed at each point. CCPs in a processing plant include eviscera-
tion, giblet harvest and chilling. Interventions were designed based on
testing. Many interventions had an effect on reducing the level of contami-
nation including Salmonella species, but results were not sustainable.
Focus is now on the growing of poultry. The use of organic acids in feed is
being evaluated regarding its effect on contaminants, especially Salmo-
nella. A complete report of this paper will be published in the Proceedings.

Dr. Janice Webb, Veterinary Medical Officer, Science and Technology,
FSIS, USDA, Residue Evaluation and Planning Division, presented informa-
tion for Dr. John Honstead, on aflatoxins under drought conditions. Corn samples from five (5) states were collected, along with tissues from
market swine. Swine were considered a sensitive species so levels found in
them would probably represent maximum levels in livestock. This paper
will be published in the Proceedings.

Dr. Denise Sofranko, Staff Veterinarian, USDA, APHIS, VS, gave an
update on veterinary accreditation examination procedures. A 1984 task-
force recommended a new examination and self-study module materials.
Fourteen (14) modules (study units) were developed and distributed to veterinary colleges and Federal and State offices last summer. A new examination, which is computer based, is being developed. Between now and June, 1990, a pilot project will evaluate the units and the test.APHIS veterinarians have tested the process and provided comments. This material was designed as reference material and currently is not meant to be incorporated into curriculae of veterinary schools and colleges. An internal APHIS task-force has been establishd to review the entire accreditation process, including the examination. Comments should be sent to Dr. Sofranko.

Dr. Mike Pullen, Professor, University of Minnesota, College of Veterinary Medicine, gave a presentation on Lyme Disease in Minnesota. He reported that Lyme Disease (LD) was first identified in 1980 and became a reportable disease in Minnesota in August 1985. In 1987 there were 102 reported human cases in Minnesota, and 78 in 1988 according to the Minnesota Department of Health. Minnesota is among the top ten states in the U.S. relative to the number of human LD cases reported. The primary endemic area for LD in Minnesota encompasses nine east central counties. New cases of Lyme Disease are most prevalent in Minnesota during June and July, although cases have been reported from March to November. LD in dogs was briefly discussed. Preliminary results of surveys in horses were reported.

Dr. Stanley Diesch, Professor, University of Minnesota, College of Veterinary Medicine, presented a paper on infectious waste legislation for Minnesota veterinarians. He reported that Minnesota veterinarians under the 1989 legislation will be required to participate in State controlled disposal of sharps and are required to develop a management plan for their clinics and hospitals. Minnesota veterinarians opted for minimal regulations, similar to that emphasized by the American Veterinary Medical Association. Veterinarians of the Minnesota Board of Animal Health will have input in rulemaking for animals. This paper will be published in the Proceedings.

Discussion was held concerning E·coli, 0157:H7 and what could be done to better clarify its risk. Dr. Gary Cowman, Assoc. Director of Science and Technology, National Cattleman's Assoc., addressed the Committee. NCA is receiving inquiries regarding E·coli, and NCA has been encouraged to support research to determine the prevalence of this organism. How can field based research be designed to assess this problem? Dr. Larry Shipman, Regional Epidemiologist, USDA, APHIS, VS, indicated that farms can be identified where the organism is prevalent. The ability to find the organism is an important element in determining prevalence. The stigma of this organism's association with beef (dairy or beef breeds) is of concern. The Committee has submitted a resolution to the Resolutions Committee calling for the funding of epidemiologic research to establish the incidence and prevalence of E·coli 0157:H7 in livestock and poultry.
Dr. John New presented Resolution 19 from last year's Committee action. The Resolution and the USDA response was distributed. The Resolution was drafted to (1) commend USDA for its implementation of epidemiologic training, and (2) to express concern that non-degree training not replace graduate education for USDA veterinarians. The response received from USDA was ambiguous. The Committee realized that this Resolution was sent to USDA during a time of major reorganization. Discussion at the Committee meeting revealed that several USDA veterinarians have recently entered graduate programs in academic institutions. The use of NAHMS databases by graduate students was then discussed. Two concerns were voiced; such databases do not allow students involvement in (1) study design, and (2) data collection. Concern was expressed by participants on the validity of the data and lack of sub-sampling that exists in the data. These elements are critical elements of academic graduate epidemiologic training. The use of NAHMS databases will be a subject of discussion and policy development by the USDA in the near future. This was selected as an item for presentation and discussion at the 1990 Committee meeting.

The Committee subsequently met to consider resolutions. The Committee members present unanimously support the proposed resolution of the Salmonella Committee entitled “Foodborne Diseases and Biosecurity/Disease Prevention Programs.” The Committee then voted to develop a Resolution concerning establishing \textit{E. coli} 0157:H7 epidemiologic research to determine prevalence and incidence in livestock and poultry.

The Committee adjourned at 4:30 P.M.
SWINE AS A RESERVOIR OF SALMONELLA:
PERSISTENT INFECTION WITH
SALMONELLA TYPHIMURIUM AND SALMONELLA
NEWPORT TO MARKET AGE

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Salmonella typhimurium in cattle and poultry and S. newport in cattle
are well-known hazards in food safety and public health. Swine also
frequently carry salmonellae, especially S. typhimurium, which is an
important swine pathogen as well as a public health hazard. Less is
known about the ability of salmonellae that are important pathogens of
humans, but not of swine, to establish a carrier state in market pigs.
Salmonella newport, for example, is an important pathogen causing dis-
ease in humans, but seldom causes disease in swine, whereas S. ty-
phimurium is an important pathogen of both humans and swine. Studies
were conducted at the National Animal Disease Center to compare the
ability of S. typhimurium and S. newport to establish continual and
persistent infections in growing pigs, and to determine the organs in which
the bacteria tend to persist.

The studies with S. typhimurium and S. newport were conducted in 2
separate experiments, using a strain of S. typhimurium isolated from a case
of acute salmonellosis in swine, and an epidemic strain of S. newport that
had caused serious disease in humans. In each experiment, 6- to 7-week-
old Salmonella-free pigs were orally exposed to a single dose of 10^10 colony-
forming units of Salmonella culture.

The pigs were housed in isolation rooms with well-drained concrete
floors, which were washed down twice daily. Fecal samples were collected
weekly until 10 weeks after exposure, then biweekly until 28 weeks. They
were taken at random from the floors. The number of samples at each
collection equaled the number of pigs in the room.

In each experiment, pigs were necropsied at random at 2, 4, 8, 12, 16, 20,
24, and 28 weeks after exposure. Two to 4 pigs, usually 3, were necropsied
each time. Tissue specimens were taken for cultural examination from 11
segments of the gastrointestinal tract (stomach, duodenum, cranial, middle
and caudal jejunum, cranial and caudal ileum, cecum, ascending and
descending colon, rectum) and associated lymph nodes. Also specimens
were taken from 12 other lymph nodes of the head, neck, thorax, abdomen,
and pelvis, and from tonsils, liver, gallbladder, spleen, kidney, heart blood,
heart muscle, and lung — a total of 40 tissue specimens from each pig.

Specimens were homogenized with a small amount of phosphate-buff-
ered NaCl solution in a Stomacher blender and divided into flasks or tubes
of GN Hajna broth and tetrathionate broth (Difco) in a 1:10 ratio (w/v).
Duplicate rectal swabs were cultured in the same media. Incubated broth cultures were streaked onto brilliant green-sulfadiazine agar (BGS) (BBL), and also were subcultured into Rappaport-Vassiliadis broth, which was incubated and streaked onto BGS. Incubations were at 37°C for 24 hours, except tetrathionate broth was incubated 48 hours. Colonies having the characteristics of *Salmonella* were picked from BGS and examined to confirm that they belonged to the same serogroup as the respective exposure strain.

Duplicate samples of feces (10 gm) were suspended in 10 ml of tetrathionate broth, which was subcultured to BGS, and into peptone pre-enrichment, which was subcultured into Rappaport-Vassiliadis broth, and from there to BGS. Isolates were identified as to appropriate serogroup.

Exposure to *S. typhimurium* (29 pigs) induced a febrile response within 24 hours (mean temperature: 39.8°C). Temperatures returned to normal within 4 days. By day 2 after exposure, about 75% of the pigs had a watery, yellow diarrhea, and most showed mild depression and diminished appetite. The prevalence of diarrhea decreased to 20% or less by day 14. Four pigs died within 14 days after exposure (14% mortality), and were not used for bacteriologic data. Pigs that survived beyond 14 days after exposure (n = 25) returned to apparently normal health until scheduled necropsy.

Exposure to *S. newport* (22 pigs) induced a febrile response within 24 hours (mean temperature: 39.7°C) Temperatures returned to normal within 4 days. By day 2, about 30% of the pigs had mild diarrhea, characterized by unformed stools of normal color. Stools returned to normal by day 5 after exposure. Attitude and appetite of the pigs were normal throughout the experiment.

Rectal culture swabs taken daily for one week after exposure showed that at least 97% of the pigs in each experiment began shedding *Salmonella* within 24 hours.

In both experiments, fecal samples were 83 to 100% *Salmonella*-positive through postexposure week 22, then varied from 17 to 100% positive until week 28. Overall, fecal samples averaged 87% positive in *S. typhimurium*-infected pigs and 95% positive in *S. newport*-infected pigs.

*Salmonella typhimurium* and *S. newport* both persisted during 28 weeks after exposure in tonsils, cecum, colon, mandibular lymph nodes, ileum, rectum, caudal jejunum, and ileocolic lymph nodes (listed in decreasing order of frequency of *Salmonella* recoveries). These organs ranged from 89 to 41% positive during the 28-week period. Colic lymph nodes, cranial and middle jejunum, jejunal lymph nodes, stomach, and duodenal lymph nodes ranged from 33 to 8% positive, and the organisms persisted for a median of 12 weeks (range: 2–28 weeks). Duodenum, gastric lymph nodes, and superficial inguinal lymph nodes ranged from 7 to 5% positive, and the organisms persisted a median of 4 weeks (range: 2–20 weeks). The remaining organs examined (except medial iliac lymph nodes and heart blood, which were
negative in all pigs) ranged from 5 to 2% positive, and the organisms persisted a median of 2 weeks (range: 0–16 weeks). In this last group, nearly all the positive cultures (94%) were from S. newport-infected pigs.

Our findings indicate that the S. typhimurium and S. newport isolates utilized in these studies possessed a similar ability to establish a long-term infection in swine, in terms of persistence in the body as well as affinity for specific body organs. Salmonella newport was found in a wider variety of organs than S. typhimurium, especially during the first 2 to 12 weeks after exposure, in spite of the fact that S. newport induced a milder clinical response than S. typhimurium; therefore lower virulence for swine did not hinder the ability of S. newport to become widespread in the body and establish a persistent infection. From the results of these studies, we conclude that healthy growing market swine can readily become carriers of salmonellae following a single oral exposure, including a Salmonella serotype that is a significant hazard to human health, but not an important swine pathogen.

SUMMARY

Salmonella typhimurium in asymptomatic poultry, cattle, and swine and S. newport in cattle are important public health hazards. In addition, S. typhimurium is an important cause of swine salmonellosis; whereas S. newport seldom causes disease in swine. Studies were conducted to compare the ability of S. typhimurium and S. newport to establish a persistent carrier state in growing pigs, up to market age. Six- to 7-week-old pigs were exposed to S. typhimurium or S. newport, and body organs were examined at necropsy at time intervals until 28 weeks after exposure. Salmonella newport induced a milder clinical response than S. typhimurium, but the 2 organisms were similar in their ability to establish a carrier state. They persisted in the pigs throughout the 28-week period, primarily in the tonsils, mandibular lymph nodes, cecum, colon, and ileum.

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5. Ogilvie, T. H. The persistent isolation of Salmonella typhimurium from
WOOD


HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP)

APPROACH — POULTRY SLAUGHTER

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The Hazard Analysis Critical Control Point (HACCP) system, first presented at the 1971 National Conference on Food Protection, provides a more specific and critical approach to the control of microbiological and chemical hazards in foods than that provided by traditional inspection and quality control approaches. HACCP consists of: 1) identification and assessment of hazards associated with growing, harvesting, processing — manufacturing, marketing, preparation, and/or use of a given raw material or food product; 2) determination of critical control points to control any identifiable hazards; and 3) establishment of procedures to monitor critical control points. When properly applied, HACCP separates the essential from the superfluous aspects of hazard control by focusing attention on those critical points that directly affect food safety and quality and by monitoring to determine whether or not these points are under control.

In the United States, the HACCP system has been successfully applied to microbiological control of low-acid canned foods, in which case it is mandated by federal law. Some food processors have employed HACCP in the control of food products other than low-acid canned foods. However, the acceptance of the HACCP system by the food industry is far from universal.

In a 1985 National Academy of Sciences (NAS) report, HACCP was identified as a comprehensive approach applicable to the range of operations from production of animals to slaughter, processing, and handling in retail outlets, food-service establishments, and homes. The NAS Committee further recognized that in order to achieve both operational efficiency and the protection of public health, critical control points must be identified, food inspectors trained in the HACCP approach, and procedures regularly monitored.

Generalized critical control points in slaughtering operations for all species of food-animals include the health of the live animals; sanitary conditions during transport, slaughtering and dressing; the rate of a carcass cooling; and time-temperature conditions of storage and distribution of the carcasses.

In poultry slaughtering the primary health concern is microbiological hazards. Poultry carcasses are contaminated by a variety of microorganisms, including low levels of some pathogens. *Salmonella*, *Campylobacter* spp., *Clostridium* spp., and *Staphylococcus aureus* are frequently involved. Within the poultry slaughtering establishment there are a variety of procedures which contribute to the transfer of pathogenic organisms to the poultry carcass, neck and giblets (heart, gizzard, liver).
The initial point in poultry slaughter where cross-contamination can occur is in the holding area while the birds are still in their shipping crates. Because poultry may shed all types of bacteria; including pathogens, the longer the birds are held in the holding area before slaughter, the greater the likelihood of cross-contamination.

The next point in processing where cross-contamination can occur is when the birds are electrically stunned prior to bleeding. Involuntary expulsion of fecal matter from the cloacal opening may occur. The amount of fecal contamination depends on the amount of feces in the cloaca. For example, poultry recently fed prior to slaughter may contain considerable amounts of feces in the cloaca.

Scalding is usually accomplished by immersing the birds in a hot bath with the highest temperatures 60-63°C (140-145°F) for a few minutes. Even this high scald temperature is inadequate to kill all pathogens introduced from fecal material on the feet, skin or intestinal tract which is released into the scald water. A film formed on the surface of the scald tank water provides protection to the pathogens against the lethal effects of the hot water.

During the defeathering process the pickers become contaminated and consequently contaminate each bird as they pass through. The pressure of the picker fingers on the bird forces fecal material out of the bird's lower intestines which contaminates both the birds and the pickers. Pathogens can be forced into the skin or may enter the open feather follicles where they firmly attach and thus are protected from the washing procedure.

During the evisceration operation, birds may become contaminated when their intestines are accidentally severed by the equipment or by the person removing the viscera from the birds. If the intestinal contents spill out contamination of the bird, the equipment and other birds may occur.

Throughout the poultry slaughtering process the birds are handled many times by both plant employees, as well as federal inspectors. This multiple handling further serves as a mechanism of transferring pathogens. Although employees and inspectors are encouraged to rinse their hands frequently, this is not adequate to prevent the transfer of pathogens.

The removal of the giblets (heart, gizzard, liver) can result in the transfer of pathogenic bacteria. This microbial transfer is further enhanced with the subsequent water chilling of the giblets and necks in the chill tanks. The contaminated giblets and necks may be introduced into the dressed carcasses prior to shipment which results in further contamination.

In cases where the carcasses do not adequately drain following inside/outside washing, they enter the chill tank carrying pathogenic organisms into the chill tank. The chill tank provides an excellent environment for the transfer of pathogenic organisms from one poultry carcass to another.

It is imperative that the HACCP system be applied at one or more locations in order to effectively eliminate microbial pathogens. Certain
HAZARD ANALYSIS CRITICAL CONTROL POINT

measures, however, are critical in reducing contamination with pathogens during processing. Scalding in water at a temperature of 60°C (140°F) or higher significantly decreases microbial counts of many groups of organisms, including Salmonella, on poultry carcasses. Scald water temperature should be constantly monitored. The feature-picking operation is another major point of spread of Salmonella. Spray washing of carcasses after picking and at other stages during processing can partially remove the microbial flora from poultry surfaces. Further reduction of microbial flora can be enhanced by using efficient spray patterns and appropriate water volume and pressure.

Chilling is another critical control point. Chlorination of chill water kills detached Salmonella and therefore minimizes cross-contamination between carcasses. The chlorine concentration and water volume should be monitored. Continuous and liberal replenishment of cooling water aids in cleaning skin, and counter-flow immersion chilling decreases bacterial counts and minimizes cross-contamination. Periodic cleaning and disinfecting of equipment can prevent microbial build-up during shut-down time and reduce the transfer of bacteria at the start of operations, but transfer readily occurs following the processing of a few carcasses.

Dry chilling, temperature control, chemical use and improved sanitary dressing procedures all represent critical control points in the poultry slaughtering process. The use of approved anti-microbial compounds or processes in appropriate processing systems has been employed to some extent. The use of chlorination in both wash and chill waters contributes some increased effectiveness to the control program.

Reductions of Salmonella populations by as much as 25% have been reported through the use of 9ppm chlorine in chill water. Chlorine dioxide and certain acid compounds (sorbate, lactic, acetic) have been investigated as potential aids in controlling microbial contamination both in scalding and chilling. These compounds, however, are quickly neutralized by contact with organic matter other than bacterial. Other potential control measures, such as irradiation and chemical sprays or dips, are not currently utilized and generally have met consumer resistance to use in food products. Research indicates that greater than 90% of the bacterial contamination is associated with the poultry skin surface. Skin removal could eliminate a great deal of the bacterial contamination problem, although, this is not attractive to the processor due to loss of a saleable product.

In a preliminary report of the FSIS Puerto Rico study, adding 25 ppm of chlorine to the potable intake water to both the carcass and giblet chillers significantly decreases the presence of Salmonella on the finished product. This concentration of chlorine leaves about 3-5 ppm chlorine in the overflow water.

It appears from the preliminary report that the best way, and perhaps the only way, to prevent or control Salmonella and other human entero-
pathogenic bacteria from contaminating the finished poultry product is to prevent these organisms from entering the processing plant. Apparently enteropathogenic bacteria hazard controls in the poultry slaughtering plant are effective only against rather minimal levels of latent \textit{Salmonella} contamination and moderate to heavy flock contamination tends to overwhelm the controls.

**REFERENCES**


SALMONELLA SEROTYPES FROM ANIMALS AND RELATED SOURCES REPORTED DURING JULY 1988–JUNE 1989

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D. A. Miller, D.V.M., M.S.
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National Veterinary Services Laboratories
Science and Technology
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

SUMMARY
Serotyping results for 19,631 salmonella cultures from animal disease cases and epidemiologically related sources are reported for July 1, 1988, through June 30, 1989. A total of 17,302 cultures were serotyped by the National Veterinary Services Laboratories. The most frequently identified serotypes were *Salmonella typhimurium*, *S. heidelberg*, *S. cholerasuis* var. *kunzendorf*, *S. hadar*, and *S. kentucky*.

INTRODUCTION
Data for this report were accumulated at the National Veterinary Services Laboratories (NVSL), Science and Technology, Animal and Plant Health Inspection Services, U.S. Department of Agriculture, Ames, Iowa. The data, except for serotyping results, were provided by the many laboratories requesting serotyping services. Most of these laboratories appreciate the importance of accurate data and made a concerted effort to provide quality input. The reports were also screened for obvious errors. However, it was not possible to verify each entry, and the overall quality of the report is a reflection of the cooperative spirit of these laboratories.

This report also contains information submitted to the NVSL by several laboratories that serotype salmonellae. We are grateful to these laboratories for their cooperation in allowing their serotyping results to be included in this report. This will enable us to present a more complete summary of salmonella serotypes identified from animal sources in the United States.

The purpose of this report is to make the data available to epidemiologists and others who have a need for it. The data are presented in tables similar to those in previous reports in order that comparisons can be easily made. Isolates formerly identified as "Arizona," which are now reported on the basis of their corresponding salmonella antigens, are separately reported in Tables 4, 5, and 6.

DISCUSSION
This report includes serotype information on 19,631 isolates from animals and related sources from 49 states, the District of Columbia, and
Puerto Rico. The highest number of isolates identified in preceding years was 14,108 in 1988.

The tables listing distribution of Salmonella and Arizona serotypes by state have been divided into Eastern and Western states (Tables 1, 2, 4, & 5) in an effort to make them easier to read. The Mississippi River was arbitrarily chosen as the dividing line between Eastern and Western states.

A total of 224 serotypes were identified (Tables 1–6). The ten most common serotypes accounted for 58% of the total cultures identified (Table 12). Seventy-six serotypes were identified only once (Tables 3 & 6).

*S. typhimurium* and *typhimurium* var *copenhagen* have been combined for inclusion in Table 12 for the first time. Many laboratories are no longer distinguishing between these two serotypes, and *S. typhimurium* var *copenhagen* is no longer included in serotype lists from the WHO Collaborating Centre for Reference and Research on Salmonella\(^1\) or *Edwards and Ewing's Identification of Enterobacteriaceae*.\(^2\) The salmonella serotyping laboratory at NVSL continues to report both serotypes in order to provide additional information for epidemiologic studies.

*S. heidelberg* would have been the most frequently identified serotype if *S. typhimurium* and *S. typhimurium* var *copenhagen* had not been added together for Table 12. Seventy-eight percent of the isolates of *S. heidelberg* were from chickens and turkeys.

*S. hadar* isolations have continued to increase (Tables 3 & 12). The majority (62%) of isolates were from chickens, and 48% were from animals in Georgia and California.

Isolations of *S. kentucky* have also increased. Seventy-eight percent were isolated from chickens and turkeys (Table 3), and 52% were from sources in California.

*S. dublin* can no longer be considered limited to the Western states. Of 544 isolates, 209 (38%) were from 12 Eastern states (Table 1). Although the majority (94%) of *S. dublin* isolates were from cattle, 16 (3%) were from other species (Table 3).

A total of 488 *S. enteritidis* isolates were identified, compared to 376 reported in 1988.\(^3\) Two hundred fourteen of the isolates were from chickens, 110 from other species, and 164 from environmental and miscellaneous sources (Table 3). Isolates which were not identified as being from chickens or turkeys, but were submitted as “avian” are included in “miscellaneous sources.” The large number of *S. enteritidis* isolates from Pennsylvania (160) was due to ongoing research projects.

The percentage of salmonella isolates from chickens continued to increase (23% in 1988,\(^3\) and 31% this year), while the percentage of isolates from cattle and swine continued to decrease.

**REFERENCES**

1. WHO Collaborating Centre for Reference and Research on Salmonella.
SALMONELLA SEROTYPES


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Total: 355 cases
**SALMONELLA SEROTYPES**

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**TOTALS**


(A) Table 1. Lists the following isolates:
- From BM - 2 HANCO, 1 TYPHIMURIUM
- From CO - 1 YISOHIO, 1 TYPHIMURIUM (CROOKED)
- From ID - 1 VIRGINIA, 1 TYPHIMURIUM
- From IN - 1 VERMONT, 1 TYPHIMURIUM (CROOKED)
- From MD - 1 VERMONT, 1 MONTANA, 1 OHIO
- From PA - 1 MINNESOTA, 1 MONTANA, 1 OHIO

(B) Varieties:
- North Dakota
- Wisconsin

(C) Varieties:
- Copenhagen

525
Table 2. Distribution of Salmonella Serotypes by State from 07/88 through 06/89—Western States (A)

| SEROTYPE | AZ | CA | CO | HI | ID | IA | KS | ME | MT | NE | NH | NV | OR | SD | TX | UT | WA | WV |
|----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| ADELAIDE |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| AGADA | 20 | 1| 1| 20 | 1| 1| 1| 1| 1| 7 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ALICHA |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ALVY |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ALCINE |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ARKANSAS |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ATLANTIC |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| AVEO |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ATY |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| AZB | 30 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| AZED | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| BAK |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| BAVARIAN |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| BAY | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| BAY | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
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| BAY | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

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**FERRIS, MILLER**

**TABLE 3 DISTRIBUTION OF SALMONELLA SEROTYPES BY Source FROM 07/88 THROUGH 06/89**
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|-----------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|    |
| 1,13,23:G,Z51         | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 17:L,V-E,N,X,Z15      | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 18:L,V-Z              | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 18:Z4,Z32             | 0  | 3  | 0  | 0  | 8  | 0  | 2  | 31 | 0  | 2  | 15 | 0  | 4  | 0  | 73 |    |
| 35:K-Z53              | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 35:R-E,N,X,Z15        | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 3  | 0  |    |    |    |    |
| 38:K-Z35              | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 41:Z4,Z23             | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 1  | 2  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 44:Z4,Z23,Z32         | 0  | 0  | 2  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 44:Z4,Z24             | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 48:1-Z                | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 48:Z4,Z23             | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 48:Z4,Z24             | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 48:Z32-Z              | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 50:R-Z                | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 50:Z52-Z35            | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 51:Z4,Z23             | 0  | 0  | 0  | 0  | 0  | 0  | 3  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 61:1,5                | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 7  | 2  | 0  | 4  | 1  | 0  | 2  | 5  | 0  |    |
| 61:C-1,5              | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 61:C-Z35              | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 61:1-Z53             | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 62:G,Z51              | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |    |
| 65:L,V-Z              | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  |    |
| <strong>TOTALS</strong>            | 1  | 3  | 5  | 10 | 12 | 7  | 5  | 44 | 1  | 2  | 17 | 6  | 4  | 4  | 74 |    |</p>
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Table 12. Salmonella Serotypes Identified Most Frequently from July 1, 1988 through June 30, 1989 with Comparison Data for 5 Years (All Sources)

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<td>171</td>
<td>174</td>
<td>154</td>
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* Number of times serotype was identified
** Rank beginning with the most common
*** Includes *S. typhimurium* and *S. typhimurium* var copenhagen
REPORT ON THE COMMITTEE ON SALMONELLA

Chairman: Dr. B. S. Pomeroy, St. Paul, MN
Vice Chairman: Dr. G. H. Snoeyenbos, Amherst, MA

J. K. Ausburg, MD; C. W. Beard, GA; C. E. Benson, PA; D. F. Bisplinghoff, FL; B. O. Blackburn, IA; E. S. Bryant, CT; R. G. Burdett, OR; J. R. Cole, GA; D. Corrier, TX; M. S. Cover, MO; F. R. Craig, MD; J. R. DeLoach, TX; W. H. Dubbert, CD; R. J. Eckroade, PA; W. M. Frerichs, IA; G. Yan Ghazikhanian, CA; R. D. Glock, CO; Eric Gonder, NC; E. Grass, CA; R. W. Griffith, IA; T. J. Hagerty, MN; D. A. Halvorson, MN; D. D. Hancock, WA; D. W. Hird, CA; R. E. John, IL; D. C. Johnson, GA; D. D. King, MD; G. E. Kolb, WI; D. C. Kradel, PA; T. T. Kramer, IA; M. C. Libal, SD; E. T. Mallinson, MD; C. S. McCain, OK; R. H. McCapes, CA; P. L. McDonough, NY; L. McGovern, VA; E. L. Menning, DC; G. W. Meyerholz, DC; D. A. Miller, IA; K. V. Nagaraja, MN; H. J. Olander, CA; R. E. Pacer, FL; I. L. Peterson, MD; M. E. Potter, GA; W. H. Ritchie, MD; R. A. Robinson, MN; M. Rosenstein, AR; L. D. Schwartz, MI; W. G. Smith, MA; S. B. Suthern, NY; W. T. Tramel, MS; Keith VanSteenberg, MO; S. A. Vesey, GA; M. W. Vorhies, KS; D. U. Walker, VT; R. D. Welsh, OK; C. W. Weston, NH; W. O. Williams, Jr., DC; R. L. Woods, IA; R. L. Ziprin, TX.

Ex-officio — C. F. Langford, Canada

The Committee met at 1:30 pm Monday, October 30, 1989.

Thirty-two members and 27 guests were present.

Twelve reports were presented to the Committee.

A. Committee on Diagnostics, Data Collection and Epidemiology —

   Dr. D. A. Miller and K. E. Ferris
   NVSL-APHIS-USDA, Ames, Iowa
   The authors reported that 19,631 cultures were serotyped, increase from previous high of 14,100 in FY 1988. There were 224 serotypes identified which 76 were identified only once. Complete report will be published in the proceedings of this meeting.

2. Dr. Earl Grass, Chairman of the subcommittee proposed the following changes in the reporting system:
   a. The present quarterly report be changed to report only the 20 most frequently isolated serotypes from chickens and turkeys and the five most frequent serotypes from other species. The annual report should be continued except that sources of isolates should be designated as from either a) surveillance and research, b) environment or c) clinical cases. States not submitting salmonella serotyping reports done in their facilities...
REPORT OF THE COMMITTEE

would not receive the quarterly reports. These changes were recommended by a voice vote of the committee.

3. Recent Human Salmonella outbreaks associated with animal products were reviewed by Dr. Lisa Lee, CDC, Atlanta, Ga.

Dr. Lee reviewed the epidemiological investigation of the recent S. javiana and S. oranienburg outbreaks in humans in Minnesota, Wisconsin and Illinois related to contaminated cheese. She also related foodborne outbreaks in Nevada and the current outbreak of S. typhimurium in children in Ohio. Animal food products were implicated in these outbreaks.

B. Regulatory Programs

1. Dr. I. L. Peterson, NPIP coordinator, gave the following report:

In calendar year 1988, 36 isolations of Salmonella pullorum and 2 isolations of S. gallinarum were reported to the Poultry Improvement Staff.

During the present calendar year January to October 1, 1989, there were 83 isolations of S. pullorum and one isolation of S. gallinarum reported from 18 states. One outbreak in a state involving many interstate shipments was responsible for 73 of the isolations. Most of these shipments were what is commonly referred to as backyard-type operations.

The one S. gallinarum isolation was from a small flock of 50—6 months old meat-type birds. They had essentially no losses earlier. The birds had been fed table scraps from at least two sources, some of which had been stored under questionable conditions. The hatchery is not believed to be the source of this infection. The flock was depopulated.

During the last year, West Virginia, New York, Wisconsin, and Michigan received the classification “U.S. Pullorum-Typhoid Clean State.” This brings the total number with this classification to 38 states. Louisiana has requested that their state be determined as qualified for this classification. When this occurs only 10 states in the western United States, remain to be classified. Staff’s goal is still to have all 48 contiguous states classified as U.S. Pullorum-Typhoid Clean States by the end of 1990.

2. Processing — Dr. William James, FSIS-USDA reported on the progress of the Puerto Rico Project. The USDA has been convinced for some time that its traditional method of poultry inspection can be improved to focus on human pathogens and the microbiological safety of the product. Inspection resources must be used in the most effective ways. Safety related standards for both the finished product and the critical control points in poultry processing need to
be set. The poultry industry should apply controls to its processes to meet these standards. Inspectors should be used to monitor these controlled operations. A Bacterial Control project is underway to apply Hazard Analysis Critical Control Point (HACCP) principles to poultry processing technology, bacteria hazard controls in the processing plant are effective but are not completely effective. The study has now been expanded in scope to include the farm environment in an effort to reduce the level of live bird contamination.

C. Industry
1. Feeds and Feed Ingredients
   a. Dr. Fred Bisplinghoff, Animal Protein Producers Industry (APPI) reported the completion of the summer testing program and the incidence level of salmonella in rendered animal proteins is approximately the same as reported in the benchmark survey in 1985. The winter testing survey will be made in January, February, and March, 1990. APPI is encouraging cooperating members to do their own inhouse salmonella testing. Broader participation in the program is needed as many of the non-participants are the ones selling unground product to blenders. A number of plants are employing microbiologists to staff their quality control laboratories.

   Government assistance has been solicited to co-fund two research projects. The first is to contract with Silliker Laboratories to work with two renderers that have made a conscientious effort to reduce the incidence in their product but are still reporting above a 40% recontamination level. APPI plans to match the selected renderers expenditures to make necessary equipment modifications, dollar for dollar up to $5,000 per plant. The other project is to test various organic acid compounds as to their efficacy and safety in reducing salmonella in naturally recontaminated animal proteins.

   The committee commends the Animal Protein Producers Industry for its voluntary effort to reduce salmonella recontamination of animal protein products.

   b. Dr. George Mitchell, CVM, FDA, reported on a project completed this spring to sample rendered poultry products from plants receiving raw product from SE infected flocks to determine whether an SE contamination problem exists. Twenty-eight samples from 5 rendering plants were extensively examined which include raw and rendered products. Salmonellas were found in all raw and finished products, but no SE was identified. FDA will continue to sample similar types of rendering operations.
REPORT OF THE COMMITTEE

Another aspect of the project was to document hygienic practices in the plans and to document and initiate regulatory action against establishments in gross violation of the Federal Food, Drug and Cosmetic Act, thus posing a significant threat to public health.

As a result of this study FDA has proposed that the surveillance program of the rendering industry should be maintained. Also plant sanitation education workshops should be held. Selective sampling for *S. enteritidis* should be continued. Also research is needed to develop a more sensitive analytical method to assay rendered products for SE. Good manufacturing practices guidelines should be updated and published for processors of poultry products.

c. Dr. K. V. Nagaraja, University of Minnesota, reported on the cooperative salmonella surveillance program of animal protein products used by three large turkey production organizations. Animal protein products were examined from 12 suppliers. The results indicated that some suppliers have made significant progress in reducing salmonella contamination of their products. The three organizations over a period of years have eliminated suppliers with high salmonella contamination rates. Twenty-five salmonella serotypes were identified.

D. Research

1. Poultry

a. Dr. D. E. Corrier reported for the research group at USDA-ARS Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas — the research study presented involved the effect of dietary lactose on salmonella colonization of growing chickens.

Lactose provided continuously or intermittently in the water or feed ration of broiler chickens enhances natural mechanisms of host resistance and inhibits cecal colonization by *Salmonella typhimurium*. The number of *Salmonella* in the ceca of 10-and 20-day old broiler chicks, challenged with $10^6$ *S. typhimurium* at 3 days of age, was consistently decreased by $2.5 \log_{10}$ to $4.0 \log_{10}$ units by providing 2.5% lactose in the water or 7% lactose in the feed ration. The number of 42-day old market age broilers cecal culture positive for *Salmonella*, after repeated challenges on days 3 and 33, was decreased by 50% to 60% by adding 7% lactose to the feed ration. The pH of the cecal contents decreased by 1.0 to 1.9 units in the chicks provided dietary lactose and was accompanied by 5- and 25-fold increases in the concentrations of bacteriostatic undissociated volatile fatty acids pro-
duced by normal anaerobic bacteria flora in the ceca. The results of repeated experiments indicate that lactose provided in the diet of poultry with normal anaerobic intestinal flora, decreases the pH of the ceca, markedly increases the concentration of undissociated bacteriostatic volatile fatty acids in the cecal contents, enhances host resistance, and inhibits Salmonella colonization.

b. Dr. L. C. Blankenship, ARS-USDA, Athens, Georgia, presented an overview of the “International Symposium on Colonization Control of Human Bacterial Enteropathogens in Poultry.” The symposium was held September 27-29, 1989 in Atlanta, Georgia, at the Westin Peachtree Plaza Hotel. Sixteen invited speakers from around the world, who are actively engaged in “commensal” colonization research presented thirty-minute discussions on the current status of knowledge in their subject areas as well as their personal research. Dr. Blankenship outlined the major points made by each invited speaker. Also 20 poster presentations on colonization control were displayed. More than 200 people attended the Symposium with half or more registrants representing countries in the world. Proceedings will be published by Academic Press early in 1990. The cost is expected to be about $40.00. Additional information may be obtained by contacting Dr. L. C. Blankenship, USDA-ARS-Russell Research Center, P.O. Box 5677, Athens, Georgia, 306113.

c. Dr. G. H. Snoeyenbos, Chairman of the subcommittee, reported on increase funding of Salmonella research at USDA-ARS laboratories and state universities. A substantial increase in funding for salmonella research was made in 1989 through USDA-CSRS competitive grants. The Southeastern Poultry and Egg Association is currently funding 12 projects related to the control of salmonella in poultry and poultry products. This is a highly significant contribution by the poultry industry.

E. Education and Information
Dr. E. T. Mallinson, Chair of the subcommittee reported on activities of the Ad Hoc Governmental Interagency Committee. He initiated several meetings with personnel of the agencies in USDA and FDA involved in food safety leading to the resolution outlined below.

USDA-APHIS has completed the development of a series of eight video tapes on biosecurity and the poultry industry. The video tapes were available for further review at this meeting in the USDA Booth in the exhibit area. The video tapes will be available for loan from VS Area offices, Regional offices, and Emergency Programs in Hyattsville, MD.

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REPORT OF THE COMMITTEE

A tentative agreement has been made with American Association of Avian Pathologists, whereby they will have a convenient supply of the tapes for sale.

Dr. M. S. Cover outlined a course of action to get the USDA-APHIS video tapes on biosecurity and the poultry industry distributed to the broiler, turkey and egg industries.

Dr. G. W. Meyerholz, AES-USDA has proposed an extension service project for the development of biosecurity models and programs for food animal agriculture. These programs will help in quality assurance programs for food safety, profitability programs and in alternative agricultural practices.

Committee Action on one Proposed Resolution:

1. This resolution deals with the subject that USAHA request USDA and Health and Human Services make funds available to develop educational materials on food borne diseases and biosecurity and disease prevention programs for food animal production units.

SUBCOMMITTEE ASSIGNMENTS FOR 1989

A. Diagnostics, Data Collection and Epidemiology

B. Regulatory Programs

C. Industry
   1. Feeds and Feed Ingredients
      K. V. Nagaraja, Chairman, Augsburg, Bisplinghoff, Cover, Craig, Dubbert, Gonder, John, King, Kolb, McGovern, Peterson, Pomeroy, Robinson, Snoeyenbos, Vezey.
   2. Poultry Breeders
      D. A. Halvorson, Chairman, Benson, Bryant, Eckroade, Ghazikha-
SALMONELLA

nian, Johnson, Kolb, Kradel, Nagaraja, Peterson, Snoeyenbos, Tramel, Vezey, Weston.

3. Livestock Industry

D. Research
   1. Poultry, G. H. Snoeyenbos, Chairman, Beard, Benson, Bryant, Cover, Eckroade, Gonder, Ghazikhanian, King, McCapes, Nagaraja, Weston, Ziprin.
   3. Cattle and Small Ruminants
      R. A. Robinson, Chairman, Corrier, De Loach, Glock, Hancock, Hird, King, Pacer, Van Steenbergh, Vorhies, Welsh, McDonough.

E. Education and Information
   E. T. Mallinson, Chairman, Augsburg, Beard, Bryant, Cover, Craig, Dubbert, Eckroade, Griffith, Halvorson, Hancock, King, Kolb, Meyerholz, Peterson, Potter, Robinson, Rosenstein, Schwartz, Shane, Vorhies, Welsh, Weston.
   1) Ad Hoc Interagency Committee
      E. T. Mallinson, Chairman
   2) Ad Hoc Education Committee
      M. S. Cover, Chairman
JOINT MEETING COMMITTEES ON SALMONELLA AND TRANSMISSIBLE DISEASES OF POULTRY

Tuesday, October 31, 1989
1:30–5:30 PM
Meeting Room E
Riviera Hotel

Salmonella enteritidis Seminar

Presiding: Drs. B. S. Pomeroy and R. H. McCapes

1:30 pm Introductory Remarks—Dr. R. H. McCapes

1:35 pm 1. Public Health Impact
Dr. Lisa Lee, CDC, Atlanta, GA

1:50 pm 2. Current Information on SE
Naturally Infected Flocks and Pathogenic Characteristics
Dr. R. J. Eckroade, Univ. of Penn., Kennett Square, PA

2:10 pm Experimentally Infected Chickens
Drs. C. W. Beard and R. K. Gast
USDA-ARS, Athens, GA

2:25 pm 3. Impact on Egg Industry
Breeders Perspective
Dr. Gary Waters, DeKalb, IL

2:40 pm Egg Producer
Dr. David Kradel
Penn State University
University Park, PA

2:55 pm 4. Poultry Industry Perspective
Dr. Morris Cover—Foristell, MO

3:10 pm BREAK

3:25 pm 5. Status of Control Programs
USDA Policy and Status of SE
Dr. M. A. Mixson
USDA-APHIS-VS—Hyattsville, MD

Summary of S. enteritidis, Sources and Phage Types
David Miller
NVSL-USDA
Ames, IA

3:40 pm USDA-NPIP
Dr. Irvin Peterson
USDA-APHIS-VS—Hyattsville, MD
Background of the USAHA Salmonella enteritidis Seminar

R. H. McCapes

The seminar on *Salmonella enteritidis* is a function of a joint seminar meeting of the USAHA Committee on Salmonella and the Committee on Transmissible Diseases of Poultry and Other Avian Species.

In the winter of 1988, USAHA President Phil Bradshaw appointed an ad hoc Committee on *Salmonella enteritidis*, composed of Drs. B. S. Pomeroy, M. A. Van Buskirk, Jr., G. L. Waters, T. H. Woods, and R. H. McCapes (Chair), to recommend how USAHA might involve itself effectively in the national discussion and resolution of the *Salmonella enteritidis* issue. This committee recommended a joint seminar meeting of the Committee on Salmonella and the Committee on Transmissible Diseases of Poultry and Other Avian Species on the subject of *Salmonella enteritidis*.

The rationale for the joint meeting was that the membership of these two committees represented much of USAHA's and the Nation's expertise on the subject of salmonella infection in poultry and through the mechanism of a joint meeting, resolutions could be generated which would help guide USAHA and the Nation towards effective solutions. This recommendation was endorsed by President Bradshaw and the joint committee seminar on *Salmonella enteritidis* is the result.

The Public Health Impact of *Salmonella enteritidis* Infections in the United States

Lisa A. Lee
Enteric Diseases Branch
Centers for Disease Control
Atlanta, Georgia

Since 1976, epidemic egg-associated *Salmonella enteritidis* (SE) infections have affected the northeastern United States. Recently, increases in
SALMONELLA/TRANSMISSIBLE DISEASES OF POULTRY

the isolation rates of SE have been noted throughout the country and between 1985–1988, the annual number of outbreaks reported from outside the northeast has increased from 5 to 43 percent. Between January 1985 and December 1988, 140 outbreaks of SE were reported from 12 states. Of the 4976 cases, 18 percent were hospitalized, and 30 died. Highest case-facility rates were among the debilitated and elderly. Of the 24 SE outbreaks in hospitals and nursing homes, 3.6 percent of the patients died. A food vehicle was implicated in 89 of these outbreaks, and included grade-A shell eggs in 65 (73 percent). The likely egg source was traced in 43 outbreaks to distributors or farms in 12 states, including 5 outside the northeast.

Since January 1989, a total of 47 outbreaks of SE have been reported from 13 states. The annual number of reported outbreaks from 1985 to 1988 was 19, 34, 50, and 37, and it is likely that more outbreaks will be reported in 1989 than in any other previous year. Northeastern states reported 8 (17 percent) outbreaks and mid-Atlantic states reported 22 outbreaks (47 percent). Other states reporting SE outbreaks this year include Washington, Nevada, Tennessee, and Wisconsin. Of the 1,270 cases, 12 died. Eleven of the 12 fatalities were patients in nursing homes. Food vehicles were implicated in 29 (62 percent) of the outbreaks, and 20 (69 percent) were eggs or foods made with raw grade A shell eggs. A variety of vehicles were identified and included scrambled eggs, hollandaise sauce, homemade ice cream, baked pasta, puddings, raw cookie dough, and pastries containing meringue or custard. We conclude epidemic S. enteritidis infections continue to be a growing public health problem in the United States, particularly in the mid-Atlantic states, and that grade A shell eggs from many sources remain the principle source of infection.

Current Information on S. enteritidis Infected Flocks and Pathogenic Characteristics

Robert J. Eckroade and Charles E. Benson
University of Pennsylvania
School of Veterinary Medicine
New Boston Center
Kennett Square, PA 19348

This report briefly reviews some of the work we have been involved with over the past two years on flocks naturally infected with Salmonella serotype enteritidis.

Our studies on commercial layer flocks were initiated because of a “traceback” from a food-bourne outbreak thought to be due to eggs. The major effort in the First Flocks was to determine whether or not true egg transmission was occurring and if so, how often.
Birds from two “trace-back” farms and one laboratory submission were brought to New Bolten Center and housed individually in cages so that eggs could be identified by bird. Resident Flock #1 was found to be SE negative when eggs, both shells and contents, and necropsy tissues were culture negative. So much for the trace-back on this flock. Flock #2 came from a large complex with multiple trace-backs. These birds were studied for 17 weeks. SE was isolated from the yolk on 8 occasions, but not from the shell or albumin. Resident Flock #3 was studied following the isolation of SE from layers experiencing increased mortality. While SE was easily isolated from farm samples (egg yolk, egg belts, and cull chickens), eggs collected over 12 weeks were all negative and only one ovarian pool from necropsy was SE positive. A second series of experiments was done on ovaries collected by Drs. Chuck Southern and Larry Shipman at processing plants of trace-back flocks. Significant rates of SE isolation from these ovaries supported the hypothesis that current SE infections of commercial egg flocks may result in transovarian infection.

In the third series of studies two surveys were conducted on Leghorn Multiplier Breeders using dead-in-shell cultures to determine SE contamination. The first survey of 6 breeder flocks from one hatchery revealed two infected flocks. The second survey was state wide and involved 37 multiplier breeder flocks. All were dead-in-shell culture negative.

In order to evaluate the cleanout and disinfection procedures used to eliminate SE from depopulated contaminated poultry houses, four such houses were cultured for SE. All four houses had negative cultures for SE. All SE isolates should not be considered the same biologically. SE infection may cause mortality and decreased egg production in some laying flocks, but more are unaffected. SE can be isolated from normal as well as grossly affected ovaries. Some isolates are clearly more pathogenic for chickens and are transmitted to other chickens more readily than other isolates. We have use a Hela Cell Tissue Culture Assay to measure differences in the ability of different isolates to attach, invade and survive in this assay system.

We must be very careful in how we generalize about SE organism and SE infected flocks.

Current Information on SE: Experimentally Infected Chickens
R. K. Gast and C. W. Beard
USDA, ARS, Southeast Poultry Research Laboratory
Athens, Georgia

Experimental infections with *Salmonella enteritidis* (SE) were initiated in SPF laying hens of several different ages by oral inoculation or by horizontal contact transmission. Fecal shedding of SE strains by infected hens was examined by the collection of cloacal swabs. Swabs from most inoculated hens and some contact-exposed hens indicated the presence of
SE in the intestinal tract. In some of these hens, intestinal colonization persisted for as long as 18 weeks after the initial exposure to SE. SE was also recovered from several internal organ sites of both inoculated and contact-exposed hens. The effects of SE infections on total egg production were assessed by comparing infected hens to sham-inoculated control hens. Some SE strains caused significant decreases in egg production among infected hens.

The internal contents of eggs produced by infected hens were sampled for SE. Eggs were collected daily and held for 4 days at room temperature before the yolk and albumen of each egg were cultured separately. Contaminated eggs were produced by hens infected with some (but not all) SE strains. SE was found in a high percentage of the yolks and albumens of eggs laid by inoculated and contact-exposed hens during the first 2 weeks after inoculation, but from only 1 egg produced after the 3rd week post-inoculation. Specific culturing of the yolk contents by a method that excluded yolk membrane and albumen indicated no contamination by SE.

The serological response to SE infection was examined by the application of several conventional agglutination tests. A serological response, which peaked quantitatively at 2 weeks post-inoculation, could still be detected in most hens at 18 weeks post-inoculation. Both inoculated and contact-exposed hens became seropositive, the latter more slowly and at lower peak titers. The sensitivity and specificity of the tests compared were not significantly different, but the choice of antigen (S. enteritidis vs. S. pullorum) did influence the probability of detection of infected hens, particularly at long post-inoculation intervals.

The Breeder and Multiplier Aspect of the Salmonella Enteritidis Problem
Gary L. Waters
DeKalb Poultry Research
DeKalb, IL

The commercial egg production industry in the United States consists of approximately 234,000,000 leghorn hens. Approximately 2,700,000 multiplier breeders provide the day-old chicks for this industry. There are no statistics publicly available as to the number of grandparent and pedigree stocks utilized for this industry.

The pedigree thru multiplier breeder faction of this industry has worked for many years with the National Poultry Improvement Plan as administered by the U.S.D.A. to control specific diseases including Salmonella contamination.

The screening of multiplier breeders with Salmonella pullorum antigen (a Group D organism as is S.e.) over many years has served to show almost no positives. There is no statistical information available relative to com-
commercial flocks infected by horizontal spread or feed contamination, but total egg stocks isolation are believed to be far below other animal protein sources.

- Poultry meat 35% contam—5% of human cases
- Pork meat 12% contam—7% of human cases
- Beef meat 1.5% contam—19% of human cases
- Animal protein feed sources 27–34% contaminated

The focus of regulatory activity for the destruction of leghorn breeder flocks, which have historically exercised “Salmonella consciousness,” while failing to pursue known contaminated foodstuffs and implement a testing program for commercial egg production stocks, due to its overwhelming scope, is believed to be a defensive response to media and public outcry for action rather than a genuine scientifically designed effort to protect the public from S.e. This situation is further problem by improperly instructed and poorly informed laboratory staffs.

The genetics and multiplier breeder industry has a greater interest in Salmonella quality control for its own benefit than to comply with regulatory efforts. The inability to stop S.e. as a public health hazard should not be a reflection on this segment of the industry but an indictment for not wholistically approaching the problem.

**Salmonella enteritidis—Impact on Egg Producer**

David C. Kradel
Department of Veterinary Science
Penn State University
University Park, PA

The public health dimensions of the *Salmonella enteritidis* (SE) problem and the actual or potential governmental, media, marketing and/or consumer reactions have placed egg producers in a difficult if not at times critical situation. Producers are concerned because they do not want a product that may cause illness. Further they cannot afford the potential economic consequences of a significantly depressed egg market or the threatened costs of liability litigation. On the other hand they are faced with real economic, scientific and management dilemmas.

The economic fact is that at time of peak production the investment in an 80,000 layer flock may range from $250,000–$400,000. If this becomes classified as a “positive” flock it is depopulated or eggs must be sent to the breakers—without indemnity. Either option can be economically devastating. Many of the epidemiological questions needed for a rational, fair, and economically feasible control program remain unanswered. Some of the questions include: What is the prevalence of SE in the poultry population—might not the flocks caught up in tracebacks be really due to random chance selection? One risk factor associated with many of these flocks may be
putting a large number of eggs on the market—often into higher risk food handling situations.

What is an “infected” flock and what does this really mean from the standpoint of risk to public health? What is the importance of within flock prevalence rates in any risk analysis?

How can a flock owner be relatively certain a flock can be kept free of infection—i.e. what are the relative importances of the potential sources of infection—vertical, environmental contamination, feed, rodents, etc.? What can be done practically to significantly reduce the potential for any of these to serve as a source of infection?

Are the recommended egg washing and sanitizing procedures really reducing or are they possibly enhancing the potential for problems?

What are the responsibilities of those that handle eggs from production to consumption? It appears that the major responsibility and any economic penalty have been thrust upon the egg producers (and breeders). Producers may wonder where the educational arms of the universities and industry have been in developing innovative programs that would begin to familiarize the consumer with acceptable risk.

With many of the above questions incompletely answered and good field-oriented epidemiological studies needed to provide the answers, actual and implied actions by government and/or the legal system have forced producers and research workers to either not look or if looking being afraid to share the information.

None of these dilemmas have easy answers but in the interests of public health and problem solution, policies that do not discourage voluntary participation in epidemiologic research need to be developed. To not do this will only prolong the final solution to the SE problem and significantly increase rather than decrease the risk to public health.

Salmonella enteritidis
The Poultry Industry Perspective

Morris Cover
Foristell, MO

The presence of Salmonella in food products has been a recurring, but cyclic, problem. The poultry industry has always supported research directed toward ways of reducing the presence of Salmonella. An example of this philosophy is the program to reduce the presence of Salmonella pullorum which was started in 1930.

Following the unfortunate publicity concerning Salmonellosis in recent times, the Southeastern Poultry & Egg Association added one million dollars to the research program to fund research directed toward reducing this problem. A special advisory committee of experts was appointed to direct and monitor this research.

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The industry's response to the Salmonella enteritidis problem was similar. Southeastern's research immediately included research directed toward procedures and practices to reduce/eliminate this organism. The research is still continuing.

The serological testing program for Southeastern layer flocks has been accepted and was used rather quickly by the poultry breeder industry. To my knowledge, the breeder industry is clean and is actively proceeding to maintain this clean status.

Our research program included projects which addressed the following areas:

1. Survey of commercial shell eggs in several areas to determine its incidence.
2. Research into the possibility of transovarian passage and rate of shedding.
3. Vaccination of a positive flock to determine its feasibility as one method of control.
4. Survey of management procedures to indicate areas of contamination and ways to reduce the presence of S. enteritidis.

These are some of the areas in which industry has cooperated with research and regulatory areas to help solve this problem. It is not a one-faceted question. Such enigmas require all parts of the industry, the regulatory area and the consumer to participate and extend our knowledge by research and by regular use of those actions shown to be helpful.

Too little attention has been given to other areas where control can be a major factor. For example, the storage and handling of eggs after they leave the farm, retail store environment, restaurant and food-cooking establishments, i.e. breakfast bars, storage of omelet preparations, etc. This problem will be solved by activity of all phases of the industry, marketing, and the final handling and cooking of eggs for food.

**USDA Policy and Status of Salmonella enteritidis Serotype (SEE)**

M. A. Mixson
USDA-APHIS-VS
Hyattsville, MD

During Fiscal Year (FY) 1989, the Animal and Plant Health Inspection Service (APHIS), has been involved in resolving the human salmonellosis problem associated with Salmonella enteritidis serotype enteritidis (SEE) in table-egg production flocks. A full-time veterinary epidemiologist with training at the Centers for Disease Control has been working with field outbreaks to develop critical epidemiological data necessary for sound control programs. These efforts have been impeded by the lack of authority to make some investigations and by apprehensions and liability concerns of produc-
A veterinarian has been assigned to the staff to assimilate and disseminate data, provide coordination, and develop control strategies based on current research, epidemiology, and field studies. APHIS continues to offer laboratory support and epidemiologic assistance to States when requested. The pathogenicity of a swine field isolate of SEE was evaluated by experimentally inoculating weanling pigs. This isolate produced an extensive pneumonia with lesions in all inoculated pigs. Isolates of SEE from other species have been phage typed and monitored. Some isolates have been inoculated into susceptible chickens to determine pathogenicity. Due to potential liability, it has been difficult to determine the incidence of SEE in poultry flocks; however, there have been 60 human outbreaks reported to us in FY 1989, compared with 30 outbreaks in poultry flocks.

The National Poultry Improvement Plan of APHIS is getting support of the commercial-egg industry and most of the commercial egg-type chicken breeding flocks and egg-type hatcheries are participating in the "U.S. Sanitation Monitored" program. This program should provide protection from vertical spread and new introductions by this source to commercial-egg farms.

APHIS has been involved in developing priorities for phage typing, contacting laboratories and researchers concerning exotic types of SEE, conducting epidemiology when requested, developing action plans for use when requested by the poultry industry, and monitoring the incidence of serotypes throughout the United States in poultry and other species. In addition, efforts have been made to prevent the spread of SEE by enhancing biosecurity in poultry flocks and in developing model programs for use by poultry-producing States. Eight video tapes on biosecurity in poultry flocks have been developed and are being displayed at this meeting. These tapes are being made available to the poultry industry.

APHIS continues to protect the poultry industry from the importation of SEE phage-type 4 by import regulations and by the supervision and surveillance of imports of poultry and poultry products.

**Summary of S. enteritidis, Sources and Phage Types**

David Miller
NVSL—USDA
Ames, Iowa

From January, 1987—September, 1989—1116 *S. enteritidis* isolates were serotyped and 113 cultures were phage typed. Isolates were identified from several species of animals: 42% from chickens, turkeys 10%, avian (species not identified) 24%, cattle 8%, swine 3%, duck 2%, less than 1% goose, pigeon, sheep, horse, dog, rodent and zoo animal.

The predominate phage types were: 8-64%, 13A-15%, 28 and 34 5% each and 9B and 23 4% each and 9A, 11 and 14B less than 1%. Phage type 4
cultures were not identified.

Phage type 8 was identified in chickens, turkeys, cattle, swine, horse, sheep, dog, zoo animal and rodent and the second most common Phage type, 13A, was found in chickens, cattle, swine and zoo animal.

U.S. Sanitation Monitored Program for Egg-Type Chicken Breeding Flocks

Irvin Peterson
USDA, APHIS, VS
Hyattsville, MD

The voting delegates to the Biennial Conference of the National Poultry Improvement Plan approved changes to the "U.S. Sanitation Monitored" program for Egg-type chicken breeding flocks. These changes provide for a testing program to detect egg-type chicken breeding flocks infected with Salmonella enteritidis. The program was published in the Federal Register as final rulemaking in June and became effective on July 5, 1989.

This program provides for the following testing and culturing requirements for S. enteritidis:

1. Chicks for replacement breeding flocks must be cultured for Salmonella unless they originate from U.S. Sanitation Monitored hatcheries.

2. After four months and before breeding season.
   a. 300 birds per flock must be tested using pullorum antigen and reacting birds cultured for salmonella.
   b. composite samples of litter and other specimens are cultured for salmonella.

3. A sample of 30 dead germ eggs must be cultured every month for salmonella.

Egg-type breeding flocks and hatcheries participating in the "U.S. Sanitation Monitored" program are listed in the 1989 Directory of Participants in the National Poultry Improvement Plan (APHIS 91-41) or supplements to this publication. Almost all of the commercial egg-type chicken breeding industry is participating in this program.

Reports indicate that States and the egg industry are responding in a responsible manner to the requirements of this program. Our staff is not aware of any infected replacement chicks being placed which would indicate that no infected egg-type chicken breeding flocks exist.

Model State Program

Everett S. Bryant
The University of Connecticut
Storrs, CT

The proposed voluntary Model State Program was developed in response
to public health concerns regarding *Salmonella enteritidis* (SE) in humans and the role played by SE infected hens.

The purpose of this program is to:

A. Survey for SE infected flocks
B. To determine the most sensitive way to find SE flocks
C. To confirm or deny if an implicated flock is a risk to its customers

The levels of surveillance available to be monitored are:

A. Primary breeding flocks
B. Multiplier breeding flocks
C. Commercial egg producing flocks
D. Replacement growing flocks
E. Feed and feed ingredients
F. Egg processing, storage and transportation

The methods available to determine the presence of SE in flocks are limited. No clinical signs or significant losses signal a flock infection as with many other problems in the past with which we have had to deal. Blood testing, culture of the birds, the environment, and the eggs seem to be the only techniques available to us for table egg flocks. No one of these alone will do the job but when combined as in the Model State Program appear to find SE infected birds if present.

For commercial egg producing flocks, the NECAD Committee recommends:

A. Test 300 blood samples at 25 weeks of age with Pullorum antigen. The birds should be randomly selected and leg banded for future recovery.
B. Swab egg belts with 4 x 4 gauze sponge soaked in appropriate media.
C. Swab manure scrapers with 4 x 4 gauze sponge soaked in appropriate media.
D. Culture all routine diagnostic specimens for Salmonella.
E. Control rats, mice, flies and wild birds.
F. Establish strong program of maintenance, cleaning and sanitizing of egg handling equipment.

What are the present options for a commercial egg flock found positive to SE? It appears that no indemnity will be available so:

A. Immediate depopulation
B. Pasteurization of the eggs
C. Recertification after extensive testing
D. Vaccination and extensive testing

Probably the most practical location for SE surveillance is the replacement flock (day 1 to 20 weeks of age) and the empty building into which it goes at 1 day of age.
The Model Plan recommends:

A. Swab test the empty brooder facilities and equipment.
B. Culture all routine diagnostic specimens during the growing period for Salmonella.
C. Culture litter and/or feces on papers at 5 to 15 days of age.
D. Culture litter or manure at 12 to 18 weeks of age.
E. Test all routine blood samples submitted for immunity checks during the growing period with Pullorum antigen.

From May 1 to October 15, I have tested 13 replacement flocks with these results:

<table>
<thead>
<tr>
<th></th>
<th>SE positive</th>
<th>Non-D Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty building</td>
<td>3/13 (23%)</td>
<td>9/13 (69%)</td>
</tr>
<tr>
<td>Feces at 5 days of age</td>
<td>0/12 (0%)</td>
<td>9/12 (75%)</td>
</tr>
<tr>
<td>Manure at 15 weeks of age</td>
<td>0/3 (0%)</td>
<td>0/3 (0%)</td>
</tr>
</tbody>
</table>

The serotypes found from the manure on the papers (5 days) are usually quite different from those found in the empty building prior to chick placement.

So far I have found on a per farm basis:

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Empty Cages</th>
<th>Chick Papers</th>
<th>Same Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. agona</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. braenderup</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. cerro</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. gatuni</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. hadar</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Empty Cages</th>
<th>Chick Papers</th>
<th>Same Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. johannesburg</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. mbandaka</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. montevideo</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. schwarzengrund</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>S. thompson</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

From this limited experience, I feel that empty cages and buildings contain very different serotypes and a more varied assortment than what comes with the day old chicks.
Salmonella enteritidis Workshop: FDA Position

Joseph M. Madden
FDA, Washington, D.C.

In November, 1988, The U.S. Department of Agriculture and the Food and Drug Administration forwarded a "Model State Program for the Quality Assurance of Salmonella enteritidis" to the various States and Territories. This Program was an adaptation of that proposed by the Northeast Conference on Avian Diseases (NECAD), with some modifications, and called for the immediate testing of all Primary and Multiplier Grade A Whole Egg Chicken flocks. Commercial flocks are not tested under the Program unless they are derived from an infected breeder flock or implicated in an epidemiological investigation as providing eggs which caused human illness. A majority of the States and Territories have either accepted this Program or chose to test under the National Poultry Improvement Program (NPIP), an acceptable alternative. Some States are formally adopting the Program via legislation; at least one State has already conducted public hearings regarding adoption. FDA/USDA are prepared to test flocks implicated in human illnesses if States or Territories refuse to perform the required testing.

Despite the Model State Program and its implementation by a majority of States, sporadic outbreaks of illness continue to occur. The cause of this is multifaceted: some States and Territories are not testing; positive flocks remain in production due to the inability to identify them following human outbreak tracebacks; testing may not identify infected flocks either due to inadequate numbers of birds and samples tested, or due to the methodology employed in sampling and testing, or the isolation techniques used; and the mishandling of eggs at the food establishment level. Although single testing of a flock may not identify it as being infected, repeated testing will eventually identify the flock as being infected. Testing of flocks at the commercial level is not possible at the current time due to sheer numbers of birds found at this level; efforts are being concentrated at the breeder levels. Utilizing this approach, which clearly aided in the control of S. pullorum and S. galinarum in the poultry industry in the past, commercial producers can be assured of restocking their houses with pullets that are derived from breeders testing under either Program. The commercial producer must adequately disinfect his houses prior to repopulation with new pullets to prevent the horizontal transmission of S. enteritidis from the environment to the new pullets. Producers should be encouraged to await microbiological testing results of their houses prior to repopulation.

A public health problem remains in the Grade A Whole Shell Egg Industry today. Until this problem is solved, FDA has asked consumers to thoroughly cook their eggs, avoid the use of raw egg as an ingredient in recipes involving no further cooking, and to substitute commercially prepared items as replacements for routinely home-made products that contain raw eggs. Hospitals and Nursing Homes have been advised to utilize
pasteurized eggs where they may be substituted for Grade A Whole Shell Eggs. Commercial Food Establishments have been re instructed on the safe handling of Grade A Whole Shell Eggs to include emphasis on proper refrigeration of mixed eggs. It is anticipated that these measures will control the number of Salmonella enteritidis infections that result from the ingestion of contaminated and inadequately cooked or raw eggs/egg products, until control of this organism in the chicken population is attained.

Egg-Associated Salmonella Enteritidis—A State Agricultural Agency Perspective
Max A. Van Buskirk, Jr.
Director, Bureau of Animal Industry
Pennsylvania Department of Agriculture
Harrisburg, PA

Contamination of eggs by Salmonella enteritidis (SE) is a threat to public health that undermines consumer confidence in shell eggs and, consequently, is detrimental to farm economy. Scientific and technically sound solutions need to be developed that include accurate assessment and communication of egg-associated SE risk to the consumer, responsible producer and food handler measures to reduce risk and establishment of standards that will provide consumers assurance of egg quality and safety. Efforts by agricultural regulatory agencies to address SE control are handicapped by questionable statutory authority, inadequate technical knowledge, inadequate financial resources and a lack of industry support. Resolution of the egg-associated SE problem appears to prevent SE infection of egg-layer flocks, upgraded egg quality standards, restriction of eggs from flocks implicated in outbreaks of human salmonellosis and consumer education regarding proper handling and preparation of eggs.

Salmonella enteritidis—A State Agricultural Agency Perspective
Patton L. Smith
Assistant Director
Division of Animal Industry
Department of Food and Agriculture
Sacramento, CA

A summary of two S.E. episodes involving white leghorn flocks in California is presented. Investigations and subsequent testing of source breeding firms determined breeding stock not to be the source of infection. Environmental and/or feed were the most likely sources of infection; not egg transmission.

California continues to be a nonadvocate of widespread adoption of the proposed S.E. Model plan. The reasons being:
1. Although the salmonella public health problem is of major concern, S.E. represents only a small part of the problem. The model approach addresses
resolved only the S.E. segment. What Salmonella will be the next target of a model plan?

2. Egg transmission plays an insignificant role in disease spread when compared to environmental factors and feed contamination.

3. A precedent is being set to try to resolve a public health problem with a program directed toward an agent causing little economic impact to poultry. There are no measurements of program effectiveness other than reported human cases which could be environmental in origin or due to food abuse.

4. Full implementation of the Model plan would overload our veterinary diagnostic laboratory system resulting in precluding other important diagnostic efforts.

A more success oriented program would be to reduce the salmonella “load” of poultry products by addressing management factors involving the environment and feed.

Closing Remarks by R. H. McCapes

The presentations and discussions of today's seminar are setting the stage for precedent setting decisions regarding *Salmonella enteritidis*. These discussions will have significant implications for three broader areas:

1. The improvement of public health by reduction of certain zoonotic pathogens borne by raw human foods of animal origin.

2. Veterinary medicine's role in and strategy for the control of zoonotic pathogens borne by raw human foods of animal origin.

3. The orderly production of food animals in the United States.

In deciding what is the best strategy for the control of zoonotic pathogens, such as *Salmonella enteritidis*, there are three questions we must wrestle with simultaneously:

1. How can we effectively assure production of raw human foods of animal origin free of threat from such agents?

2. Will our control strategy result in an improvement of public health through the reduction of human illness?

3. Will our strategy be compatible with our supply of raw foods of animal origin?

The subject we are addressing today is *Salmonella enteritidis* in humans associated with table eggs. The underlying issue is much broader. It is, at least, salmonellosis in humans and raw foods of animal origin.

We are talking about embarking on a new era of animal/public health diseases control programs—the control of non-economically disruptive animal disease agents for primarily public health rather than animal health reasons. We are talking about animal/public health programs of staggering scope and complexity.
Veterinary medicine must maintain the leadership in the food safety area and is ideally capable of doing so. The control of zoonotic diseases is a "one medicine" issue, that is, it involves all of the animal kingdom including man. Veterinary medicine is trained to solve "one medicine" problems. It knows the animals, the food animal industries, the disease agents involved, their epidemiology and the public health issues.

This makes USAHA ideally suited and capable for a leadership role in the *Salmonella enteritidis* problem and the broader issue of salmonellosis in general.

**Salmonella enteritidis Seminar**

October 31, 1989
Las Vegas, Nevada

Committee Actions on Four Proposed Resolutions.

The Committee on Salmonella and Transmissible Diseases of Poultry and other Avian Species passed four resolutions pertaining to the Salmonella problem and referred them to the membership meeting of USAHA.

**Resolution No. 18**
1. This resolution deals with the subject that USAHA request USDA and Health and Human Services make funds available to develop educational materials on food borne diseases and biosecurity and disease prevention programs for food animal production units.

**Resolution No. 14**
2. This resolution requested that USAHA develop a Task Force in cooperation with USDA, Health and Human Services, State Agencies and the Poultry Industry to develop a control program for *S. enteritidis* infection in the commercial egg industry.

**Resolution No. 13**
3. This resolution stated that USAHA request USDA to develop a contingency plan for the eradication of *salmonella enteritidis* Phage Type 4 if it is introduced into poultry flocks in the U.S.

**Resolution No. 42**
4. This resolution stated that USAHA is strongly opposed to a premature mandatory testing program of all breeder, multiplier breeders and commercial egg production flocks until the scientific knowledge base is developed to allow construction of a program to permit the accomplishing of the goal of eliminating highly evasive SE from all flocks in the table egg industry.

Above Resolutions were passed by the membership of USAHA, October 29-November 3, 1989.
MOLECULAR APPROACHES TO THE CONTROL OF SCRAPIE

James Hope, Nora Hunter and James D. Foster
Institute for Animal Health, AFRC & MRC Neuropathogenesis Unit,
Ogston Building, West Mains Road, Edinburgh EH9 3JF, UK.

Scrapie, a neurodegenerative disease of sheep, is found in many countries of the world and its annual flock incidence in the USA has increased dramatically since 1978 (Foote & Pitcher, 1988). In the UK, a novel neurological disorder of cattle (bovine spongiform encephalopathy or BSE) has apparently resulted from the use of scrapie-contaminated feed supplements (Wells et al., 1987; Fraser et al., 1988; Hope et al., 1988; Wilesmith et al., 1988). This has increased awareness of the need to develop effective measures for controlling scrapie (Southwood et al., 1989). This paper outlines how molecular probes may help to monitor a scrapie control and eradication programme (Foote & Pitcher, 1988).

SCRAPIE-ASSOCIATED FIBRILS, PRP AND MARKERS FOR INFECTION

Scrapie is a transmissible disorder but the molecular structure of the transmissible factor or pathogen is not yet known. The pathogen has some of the properties of a conventional virus but can survive normal virucidal procedures such as prolonged exposure to formalin, dry heat and some regimes of autoclaving. Inactivation studies of scrapie infectivity have indicated that a protein may be the sole or an integral part of the infectious particle and a candidate host protein (PrP or prion protein) has been identified. Prion, virino and slow virus are among the many names used for the causative agent of scrapie and related diseases. Scrapie-associated fibrils (SAF), and other modified forms of PrP, accumulate in the brain and some peripheral tissues (spleen, lymph nodes) during the development of scrapie. To date, these aggregated forms of a normal host protein are the only candidate antigens which either specifically (as part of a virino or prion structure) or non-specifically (occluding a normal virus) co-purify with the infectious particle of scrapie.

NEED FOR PRE-CLINICAL DIAGNOSIS

Diagnosis of the disease is based on clinical signs and post-mortem brain pathology. These signs only develop late in the prolonged incubation period of the disease, which may last for months or even years. It is very difficult to detect those animals with scrapie which are not visibly affected by the disease, and it can persist undetected within a flock for several generations without causing clinical disease. The inability to detect the asymptomatic carrier of the disease has greatly hindered the effectiveness of approaches for the control and management of scrapie.

Present schemes incorporate mouse bioassay of tissues from sheep in order to assess the risk of the spread of scrapie. The cross-species bioassay
is time-consuming and expensive but there is as yet no reliable alternative for detecting the infectious particle. While SAF (and their major protein, PrP) are easily detected in brain extracts of experimentally-induced scrapie sheep (and rodents), they are much more difficult to find in the natural disease (Rubenstein et al., 1987; Gibson et al., 1987; Dawson et al., 1987; Scott et al., 1987). Similarly, analysis of peripheral tissues for these markers of infection is of limited value (Doi et al., 1987).

However recent improvements in the sensitivity of detection of the disease-specific accumulation of PrP in mouse models of the disease by immunohistochemistry suggest the application of this technique may be realistic in the medium-term future (Bruce et al., 1989; Farquhar et al., 1989; DeArmond et al., 1987). These changes in the scrapie-affected rodent brain precede the vacuolar degeneration used to diagnose the disease and are also seen in peripheral tissues where overt pathological changes do not occur (Bruce and McBride, personal communication). In the near future, it may be useful to include immunocytochemistry for ovine PrP as a component of the Scrapie Certified Flock scheme (Foote and Pitcher, 1988).

**GENETICS**

It has been known for over two hundred years that the development of natural scrapie in sheep depended on genetic factors (Anon, 1755). Similarly, the survival time of sheep inoculated with scrapie (SSBP/1, see Dickinson 1976) is mainly determined by a single genetic locus — *Sip*, with two alleles sA and pA. When injected intracerebrally, *Sip* sA/sA or sA/pA sheep die sooner than *Sip* pA/pA animals. If the inoculum is given sub-cutaneously to Cheviot sheep, only sA/sA or sA/pA animals develop scrapie while pA/pA sheep survive a natural lifespan (Dickinson et al., 1968). Isolates (and perhaps natural outbreaks) of scrapie can be classified according to their relative effects on sheep of the different *Sip* genotypes. Most isolates (Group A) produce the disease in carriers of the sA allele faster than in pApA sheep, but with at least one isolate (CH1641) (Group C) the ranking of *Sip* type (in respect to survival time) is not clear cut and may even be reversed (Foster and Dickinson, 1988; see Table 1). The incidence of natural scrapie also appears to be controlled by the *Sip* gene (Foster and Dickinson, 1988), and genetical methods offer one possible route to control and management of the disease (Kimberlin, 1979).

Genetic selection strategies for sheep which are resistant to a sub-cutaneous dose of scrapie have been used in the UK to produce nucleus flocks in which natural scrapie rarely if ever occurs (Dickinson et al., 1968; Nussbaum et al., 1975; Davies and Kimberlin, 1985). These selected flocks contain mostly “resistant” sheep (*Sip* pA/pA) in which replication or the deleterious effects of the scrapie pathogen may be sufficiently inhibited to allow survival. These flocks cannot be regarded as “scrapie-free” and may act as carriers of infection. These carriers of infection are not easy to detect either directly (by pathogen assay) or indirectly (by *Sip* typing), and this
CONTROL OF SCRAPIE

has greatly hindered understanding of the natural epidemiology of the disease and its eradication.

To provide an in vitro method for predicting the susceptibility or resistance of sheep to natural or experimental scrapie, various biochemical markers have been tested for their correlation with the alleles of Sip. For example, a correlation has been noted of OLA haplotypes with natural scrapie in Ile de France sheep (Millot, 1989), but this has been disputed (Cullen, 1989). Similarly, a lack of correlation has been noted between various phenotypes of albumin, pre-albumin, esterase, haemoglobin, transferrin, reduced glutathione and a-mannosidase and the alleles of Sip in Herdwick sheep (Collis and Millson, 1975; Collis et al., 1977), and between haemoglobin/potassium levels and scrapie susceptibility in Canadian Suffolk and Cheviot sheep (le Quercy and Avery, 1960).

We have searched for in vitro markers of scrapie resistance/susceptibility using two lines of (NPU) Cheviot sheep which were selectively bred for increased (positive line) or decreased (negative line) incidence of SSBP/1-induced scrapie. Paradoxically, a candidate product of the host control locus was discovered in studies on the molecular structure of the scrapie pathogen (see above). Mutations in or around the PrP gene are linked to the alleles of Sip in sheep (Hunter et al., 1989) and to homologous genetic loci in mouse (Carlson et al., 1986; Hunter et al., 1987) and man (Hsiao et al., 1989). These mutations can be detected flanking the sheep PrP gene by Southern blot analysis (Figures 1 & 2). Table 2 shows the correlation of PrP haplotype with the alleles of Sip in our Cheviot selection lines. Surprisingly, the same restriction length polymorphism can be used to detect “resistance” in other flocks of selected sheep (Herdwicks, Swaledales), and its value in predicting the response of unselected sheep to scrapie exposure is under investigation.

The DNA test for Sip alleles requires only a small blood sample and results are available within days rather than years. We suggest it may be useful to apply this test as a component of the Scrapie Certified Flock scheme (Foote and Pitcher, 1988), and we plan to work closely with Dr. Foote and his colleagues at Utah State University, USDA/APHIS/Veterinary Services and Utah Department of Agriculture to evaluate its usefulness in the USA.

REFERENCES


CONTROL OF SCRAPIE


Table 1. Incubation periods of SSBP/1 and CH1641 in Cheviot sheep of differing Sip genotype.

<table>
<thead>
<tr>
<th>Source of scrapie</th>
<th>Route of injection</th>
<th>Incubation period (days ± sem)*</th>
<th>Negative line (Sip^{sAAP})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBP/1</td>
<td>Intracerebral</td>
<td>197 ± 7</td>
<td>917 ± 90</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>313 ± 9</td>
<td>-</td>
</tr>
<tr>
<td>CH1641</td>
<td>Intracerebral</td>
<td>595 ± 122</td>
<td>360 ± 15</td>
</tr>
</tbody>
</table>

* Data adapted from Dickinson and others (1968) and Foster and Dickinson (1988a)
### Linkage of the PrP Gene and Sip

**NPU Cheviot Sheep**

<table>
<thead>
<tr>
<th></th>
<th>Negative Line</th>
<th>Positive Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Sip&lt;sup&gt;3&lt;/sup&gt;ApA)</td>
<td>18 PrP&lt;sup&gt;SS&lt;/sup&gt;</td>
<td>17 PrP&lt;sup&gt;LL&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 PrP&lt;sup&gt;LS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tested</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>66</td>
</tr>
</tbody>
</table>

**PrP EcoRI RFLP**

- L - 6.8 kb
- S - 4.4 kb

Table 2. Linkage of the sheep PrP EcoRI RFLP to Sip genotype in positive and negative line Cheviot sheep.
Figure 1. The EcoRI RFLP of the sheep PrP gene and its linkage to Sip.

Figure 2. Physical map of the region coding for PrP in the sheep genome.
U.S. BENEFIT FROM AN INTERNATIONAL RESEARCH PROJECT IN YUGOSLAVIA ON SHEEP PRODUCTION

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Abstract: The national multi-sheep project in Yugoslavia; Biotechnical Methods for Intensification of Sheep Production, jointly supported by the USDA–Office of International Cooperation and Development and the government of Yugoslavia provides a unique opportunity for enhancing the productive efficiency of sheep raising in both countries.

Sub-projects from four republics contribute to a common goal to improve the meat and milk production of the native sheep population. Three research locations have cooperated in the design of breed evaluation and mating plan studies to determine the importance of breed type by location. Quality characteristics of lamb meat, milk and wool are being evaluated. A second project has been recently initiated to improve the forage base for ruminant animal production throughout the year. Collaboration between scientists on an interdisciplinary basis has been established.

Technologies to improve production and efficiency of sheep raising are being developed for general application. Results from this project are expected to be of value in the United States and other countries of the world.

Introduction: Yugoslavia located on the Balkan Peninsula has been a crossroad for civilization through history. Hence the kingdom of Yugoslavia, established sixty years ago evolved from five nations into a country of six republics and two autonomous regions having two alphabets with three languages and religions. Since 1943, Yugoslavia has been governed as a Socialist Federal Republic with self-managing communities.

The land area of Yugoslavia is approximately the size of Wyoming with seventy-five percent covered by mountains and highlands and thirty percent forested. There are twenty-three million people living in the country with a relatively large portion directly involved with agricultural production. The country has five natural topographical regions with three major climatic types. Sheep raising has been an extensive and highly traditional enterprise for many years. The sheep population has been recently estimated at 7.5 million including 5.4 million ewes.

Project Development: Resource assessment is basic for the development of any production related research in agriculture. Therefore, a team of U.S. scientists visited Yugoslavia in 1984 to determine the importance and potential of sheep production in the country. The current sheep industry is based on a primitive breed structure with seasonal lamb production. Ninety percent of the sheep are of the Pramenka long-tail type from the group Ovis Vignei. Annual production of lamb per ewe is approximately 7.8 kg with an average fleece weight of 1.6 kg of low quality wool. Flocks are
INTERNATIONAL RESEARCH PROJECT

managed extensively and nutrition during the long winter months is limited and based on rather poor quality forage.

Three Yugoslavian scientists visited the U.S. in 1985 and developed a joint research project proposal. The project Biotechnical Methods for Intensification of Sheep Production was approved by USDA–Office of International Cooperation and Development and the government of Yugoslavia and initiated in 1986. The project is composed of four sub-projects located in the republics of Bosna-Hercegovia, Montenegro, Croatia and Macedonia. A major project objective is to improve the production performance for lambs and wool of the native type sheep. Breed evaluation and crossbreeding studies with the Wirtenberg, Romanov and Il-de-France breeds have been designed to determine the potential for improving productivity and importance of type adaptability among the diverse locations involved in the study. The technologies for embryo collection, freezing and transfer are being developed at one location. This sub-project will allow movement of genetic material into the country and enhance our knowledge of disease transmission and its control.

A second project Improvement of Forages for Livestock Production was initiated in 1989. This project will greatly complement the sheep project through efforts to improve forage production and quality for year-round nutrition systems. A sub-project of the forage project is to collect, identify, exchange and conserve plant germ plasm material. Yugoslavia appears to be a center of origin for many species of forage plants, especially legumes. Conservation of this germ plasm in a permanent gene bank would be an important contribution.

Cooperative Benefits: Collaboration on an international basis has the potential for many mutual benefits. Resource assessment and technical integration in the development of joint projects can expand not only the transfer of knowledge but stimulate creative ideas for further development. Well designed projects generally add new information to the bank of scientific knowledge. Ultimately, new technologies contribute to progressive change through the synthesis of more efficient systems of producing food and fiber.

Another feature of international project cooperation relates to humanitarian aspects. Cultural enrichment, cooperative enhancement of education and impact on living standards are positive yet somewhat intangible benefits that can improve the quality of life and make the world a better place to live.
The Sheep and Goat Committee met at 1:30 p.m. Monday, October 30, 1989. There were 20 members and 65 guests for a total of 85 people in attendance.

The Committee met as requested by the President of USAHA to consider the business of the Committee and submit the following report:

Mr. Bert Hawkins, representing the Llama Association, presented his concerns regarding the importation of Llamas from Chile and the need for changes in the federal regulations to require quarantine at the Harry S. Truman Facility prior to entry into the U.S.

Dr. Jerry Callis, USDA-ARS (Retired), took issue with the need for lengthy quarantine in view of the extensive testing to certify Chili free of FMD.

Sheep and Goat Committee member Olin Timm stated that this issue should be addressed by the Import/Export Committee or the Committee on Diseases of Cattle, Bison and Llamas. Therefore, no action was taken by the Sheep and Goat Committee.

Dr. Charles Parker, Chairman, Department of Animal Science, Ohio State University, Columbus, Ohio, illustrated how coordinated research on foreign sites in collaboration with other scientists can be highly beneficial to the U.S. sheep industry. Carefully designed experiments to discover and develop technologies to improve the productive efficiency of lamb, milk and wool is a common need throughout the world.

The United States-Yugoslavia Sheep Research Program supported by IRD/OICD is a multi-republic project based on this premise. Aspects of the research include breed evaluation; meat, milk and wool quality evaluation; embryo manipulation, involving collection, freezing and transfer; disease control and technology transfer to private producers.

A separate project on improvement of forage production has been initi-
SHEEP AND GOATS

ated with a sub project proposed for plant germ plasm collection and conservation.

Mr. John Finlay of Vermont discussed establishment of a Dairy Sheep Industry in the United States. A nucleus of producers across the United States is involved in sheep dairying with the objective of capturing a market now being supplied by imports.

The required technology is now available in the United States to develop a viable dairy sheep industry that would be competitive with foreign source products. The critical need is the importation of germ plasm to develop high producing dairy flocks. Such imports would also contribute genes that could be used to increase milk production in existing breeds of sheep in the United States, thus increasing total lamb production.

Based primarily on results of the cooperative studies on embryo transfer, it is now possible for USDA to import dairy sheep germ plasm from foot and mouth disease free countries.

Dr. Alex Thiermann, Deputy Administrator, International Diseases, USDA, APHIS, reported on a meeting held in Washington, D.C., last April, sponsored by ARS, on Bovine Spongiform Encephalopathy (BSE) in the U.S. and the United Kingdom, where Dr. Gerald Wells, leading BSE scientist in the United Kingdom, presented the most recent epidemiological studies in the cattle industry in the U.K.

As a result of this meeting, the BSE task force recommended that USDA and Universities initiate research on transmission of the scrapie agent in cattle and transmission of the BSE agent in mink.

Professor R. E. Marsh, Department of Veterinary Science, University of Wisconsin, reported that the first affected cattle in the U.K. were observed in 1985, with the incidence gradually increasing since then. Investigators estimate that the disease has now leveled off at 100 cases per week. Epidemiological evidence, aided by computer modeling, indicated that exposure was via a feed ingredient and began in 1982. This feed ingredient was believed to be rendered meat and bone meal from scrapie infected sheep.

BSE has not been officially reported in the United States at this time.

Dr. Dave Herrick, USDA-APHIS, discussed new regulations regarding the export of animals to Mexico. Although very restrictive regulations were first proposed, USDA has been successful in negotiating changes in the requirements to permit easier access to this important export market.

Dr. James Hope, Head, AFRC and MRC Neuropathogenesis Unit, Institute for Animal Health, Agriculture and Food Research Council, Edinburgh, Scotland, discussed Molecular Approaches to the Control of Scrapie.

While the molecular structure of the pathogen is unknown, it is very resistant to inactivation. This pathogen can only be detected by transmis-
sion to other animals. The development of natural and experimental scrapie in sheep depends on genetic factors. The sip gene determines the survival time of sheep following exposure. This survival time can be a few months or several years.

A simple blood test developed at Dr. Hope's unit can now determine which of the Sip alleles is present in an animal and, if so, whether the animal is susceptible or resistant to the known field strains of scrapie.

Dr. Hope plans to work closely with Dr. Foote and his group at Utah State University to evaluate the usefulness of this methodology in the USA.

Dr. Wilber Clark, USDA-APHIS, reported on studies of scrapie virus infection in cattle. Ten cattle were inoculated with scrapie virus at the USDA Scrapie Field Trial, Mission, Texas. The origin of the virus inoculum was infected Suffolk sheep and Angora goats. Three of the 10 inoculated cattle developed signs of a subacute degenerative disease of the central nervous system. Histopathological studies and biochemical analysis for prion protein indicated that the test cattle were experimentally infected with scrapie virus. Laboratory studies are continuing to define the role of scrapie virus in cattle.

Dr. Arthur Andersen, USDA-ARS, reported on current research on the diagnosis and control of chlamydial infection in sheep. Chlamydia is widespread in the sheep population where it is considered to be a major cause of abortions and polyarthritis. The diagnosis of chlamydia, in the case of abortion, can be difficult because isolation is not always possible. Dr. Andersen presented data to show that immunohistochemical staining of histological sections can be used to rapidly and accurately identify the agent in tissues. Dr. Andersen also emphasized the need for continued research on methods to control chlamydial infections in sheep.

Dr. Chester Gipson, USDA, APHIS, VS, gave an overview of the subcommittee hearing on scrapie which addressed HR 799, sponsored by Congressman Leach of Iowa, concerning scrapie eradication, indemnification and research, with total funding of $11 million for FY 1990-1991. Nine million dollars were allocated for indemnification and $2 million for research. (This bill did not pass out of committee).

Although APHIS was not in support of this bill, Dr. Gipson said APHIS does support the industry and the principles and establishment of the Negotiated Rule Making Process.

Dr. Gipson also stated APHIS would continue the current Scrapie program, which came out of conference with approximately $1.1 million in funding. These funds would be used primarily for indemnity and research, as in the past. The biggest change was scrapie has now become a line item in the budget.

Dr. David Galbreath, APHIS, Policy and Program Development, gave a brief presentation on the principles of negotiated rulemaking. He reported on the progress which has been made in getting the scrapie negotiated
rulemaking process established. The selection of the Advisory Committee is in progress, funding has been established and an independent convener is under contract. Dr. Galbreath introduced Mr. Howard Bellman of Madison, Wisconsin, who will meet with industry representatives and act as a convener/mediator for the scrapie negotiated rulemaking.

Mr. Howard Bellman, an attorney and professional arbitrator, discussed his role as the convener and mediator for this procedure. He listed some of the necessary steps to be taken in selecting the people who will represent the various interests involved in the complex process of the negotiated rulemaking.

Dr. Tom Walton, USDA-ARS-ABADRL, discussed the occurrence of several recent outbreaks of bluetongue in sheep. The most severe outbreak involved a flock of 3000 sheep in Utah with 100 to 150 deaths. Studies are in progress to determine the serotype of the virus involved. Additional cases were reported in Colorado and Wyoming, and Dr. Walton said that the absence of a hard freeze has prolonged the insect activity in these areas.

The Committee supports the principle and establishment of the Scrapie Negotiated Rule Making Process and feels it strengthens the Scrapie Resolution passed by USAHA in 1988. The committee passed resolutions concerning Bovine Spongiform Encephalopathy (BSE), Chlamydia research and support of dairy and cheese sheep projects in the United States.

NEGOTIATED RULEMAKING PROCESS AND THE FUTURE OF THE SCRAPIE PROGRAM

David Galbreath
Animal and Plant Health Inspection Service
Policy and Program Development

Let me begin by saying that I am pleased to be able to address the committee this afternoon and express my thanks to Michele Howard, the chairman of the committee for putting me on the agenda. It’s not often that I have the opportunity to share a program with such distinguished colleagues.

I want to discuss with you this afternoon some of the basic principles of the regulatory negotiation or REGNEG, and as it has become known in recent years, the negotiated rulemaking process. I also want to share with you some of the complexities we have been dealing with in Hyattsville to get REGNEG on scrapie going. Then I want to introduce to you the person who has been selected as convener for the REGNEG and, with the chairman’s permission, relinquish part of my time to him.

Rulemaking, the process of developing, publishing and effecting a regulation based upon legislative authority was established by the Administrative Procedures Act of 1946. That Act defined the ways by which Federal
agencies must write, publish and react to public comments when they make regulations. The point is that it is very difficult and expensive. And then when a rule is put into effect, it may go through years of legal battles, even litigation, adding to the expense. During the mid 1970's there began to be more interest in the informal rulemaking process using conflict resolution techniques. Utilizing input from interested parties early in the process, negotiators found it easier and less expensive, resulting in more appropriate rules and these methods became known as negotiated rulemaking.

In 1983, the Federal Aviation Administration was the first Federal agency to try using negotiated rulemaking. REGNEGs have been used by:

- Department of Transportation
- Environmental Protection Agency
- Department of Labor
- Nuclear Regulatory Commission
- Department of Agriculture (Forest Service)
- APHIS has used it only once for the Varroa Mite rule last year.

During the early 1980s the Federal Advisory Committee Act of 1972 was used and is still used to set up negotiation sessions. We must follow the guidelines of this Act to set up the scrapie REGNEG.

There is currently vast interest in furthering the use of REGNEG to the point that legislation was introduced this year in Congress to codify important procedural protections, clarify some provisions of existing law, and provide funding to support development of the procedures.

In concept, the REGNEG process sounds hypothetically pretty simple. Let me make it quite clear that, as it is now accomplished, this is far from the reality. Those of us on the project team have been working since April to get motion set to reach a solution to the scrapie problems. I'll mention the three major steps generally and then if you have specific questions I'll be around this afternoon and try to answer them.

First, there is the problem of contracting. In order to have an independent party to act as a convener and mediator, as is recommended, a contract must be written, approved and negotiated. In this case we were able to use an existing contract with EPA while we are developing one of our own.

Second, you have to obtain funding. There were no funds budgeted for scrapie REGNEG in FY 1989 or 1990 so this had to be established as an item. The funds for this will come out of Departmental Advisory Committee allocations. We may yet have to ask for industry support.

Third, the Advisory Committee has to be established. This is a long and complicated procedure requiring approval at about ten agency and departmental levels. The documents were submitted in July and when I checked on Friday, it was at the last departmental step and then will go to GSA and that will take another 15 days.
SHEEP AND GOATS

Many people have been involved in bringing the REGNEG to where it is now. All this effort has been to work toward giving negotiations the best chance of success. Hopefully we can all work together toward the goal that we need a scrapie program that we can all live with based on the technical knowledge available at this time and keep the door open for future developments. I’m not going to try to predict what type of scrapie program will result, that will be the job of the negotiators.

The big question is when this will happen. The best answer I can give is in 60 days. At this stage, the most critical part of the regulatory negotiation is to try to get the correct groups or organizations represented at the negotiation table. Mr. Bellman is an independent party who will try to do that. He was selected for this duty by contract through a non-profit organization, Conservation Foundation.

Mr. Howard S. Bellman is an arbitrator and mediator with nearly 25 years’ experience, mainly in labor relations, environmental negotiations and other large scale multi-party disputes. He conducts his practice in Madison, Wisconsin, and is a Senior Fellow at the Conservation Foundation as well as a Senior Consultant to Endispute, Inc. He has served as Secretary of the Wisconsin Department of Industry, Labor and Human Relations and as Commissioner of the Wisconsin Employment Relations Commission.

Mr. Bellman is a member of the National Academy of Arbitrators, currently serves on the Board of Directors of the Society for Professionals in Dispute Resolution and is a former Chairman of the State Bar of Wisconsin, Labor Law Section. He is a graduate of the University of Cincinnati where he earned Bachelor of Arts and Bachelor of Laws degrees and of New York University where he earned a Master of Laws degree. He has also authored several publications on mediation and arbitration.

Thank you. I would like you to welcome Howard Bellman.
BLUE EYE PARAMYXOVIRUS INFECTION IN PIGS IN MEXICO

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Pig Production Department
Veterinary School, University of Mexico, Mexico 04510

The Blue Eye is a new disease of pigs associated with a paramyxovirus infection, and characterized by central nervous disorders, reproductive failure and corneal opacity.

HISTORY

The disease emerged in 1980 in central Mexico with numerous outbreaks of encephalitis and corneal opacity in piglets, from which an hemagglutinating virus was isolated,1 this virus was identified as a member of the paramyxoviridae group2,3,4 and was shown not to be serologically related to previously described paramyxoviruses;5 we named this virus the “Blue Eye Paramyxovirus” (BEP).

The first outbreak was observed in a commercial farm with 2500 sows located in La Piedad, Michoacan. That year similar outbreaks were observed in other farms of the same area as well as in the states of Jalisco and Guanajuato. In 1982 the disease was diagnosed in the Estado de Mexico, in 1983 in the Federal District and in the states of Nuevo Leon, Hidalgo, Tlaxcala and Queretaro. Also in that year pigs with the disease were identified in the slaughter house in the states of Tabasco and Yucatan (these pigs apparently were brought from central Mexico but no clinical outbreaks have been identified in these states); in 1984 in Tamalipas and in 1988 in Puebla and Campeche there were also outbreaks. The main focus of the disease, however, is in central Mexico in the states of Michoacan, Jalisco and Guanajuato where there is a dense population of pigs.

In 1983 the disease was reproduced experimentally by inoculating one-day old piglets with the BEP by different routes.2 In 1984 reproductive failure was induced by inoculating the BEP in pregnant sows with different lengths of gestation.3,6

Differences in clinical signs had become evident; in 1980 in the first outbreaks, the piglets were mainly affected and mortality with nervous system disorders in pigs older than 30 days were uncommon; since 1983 in fattening farms, severe outbreaks of encephalitis with high mortality in pigs 15 to 45 Kg of body weight has been observed frequently (they were severe during 1984 to 1985 in central Mexico).7,8 It was not until 1983 that the reproductive failure in the sow and transient infertility in boars was identified,9 but in 1987 a severe problem in the boar became evident, with orchitis, epididymitis and testicular atrophy. Differences in strain virulences have been described.10
INFECTION IN PIGS IN MEXICO

This disease has not been reported elsewhere, nor have clinical signs similar to those described in Blue Eye been observed in other species.

ETIOLOGY

The Blue Eye Paramyxovirus is easily recovered from the brain, tonsil and lung of affected animals in outbreaks of Blue Eye.\textsuperscript{11,12}

All primary cultures and cell-line monolayer cultures used, as well as chick embryo support virus replication. It has been growth in monolayer cultures of primary pig kidney, bovine thyroid, bovine embryo, equine dermis, swine testicle, cat kidney, baby hamster kidney\textsuperscript{21} and vero cell lines. In the primary pig kidney and in the PK 15 monolayer cultures, syncytium formation occurred.

Erythrocytes from the following animal species: chicken, guinea pig, mice, rat, rabbit, hamster, cow, horse, pig, goat, cat and dog; as well as human cells of the four groups tested for haemagglutination with the supernatant fluid proved to be positive. Spontaneous elution at 37°C occurred after 30 to 60 minutes. The infected PK 15 cells were also positive to haemadsorption with chicken erythrocytes.

The infectivity of the virus was abolished by treatment with ether, chloroform, formalin and B propiolactone but it was resistant to Actinomycin D. Formalin treatment was able to inactivate both the replication activity and the haemagglutination properties. Virus inactivation was detected at 56°C after 4 hours of heat treatment. The virus showed a buoyant density of 1.21 g/ml in sacarose gradients.

The electron microscopic examination showed particles similar to paramyxovirus, measuring from 135 x 148 nm to 257 x 360 nm. The virion was pleomorphic but usually more or less spherical and no filamentous forms have been observed. The envelope was covered with a layer of closely spaced surface projections or spikes. Nucleocapsids from disrupted virus particles were frequently seen as a single entity with a diameter of 20 nm and a length of 1000 to 1630 nm or more. In PK 15 cells the virus was seen growing in the cytoplasm, occasionally with the formation of inclusion bodies.

The serological analyses showed that specific antiserum prepared against paramyxovirus 1,2,3,4,6, and 7, parainfluenza 1,2,3,4a,4b and 5 were not able to affect the BEP infectivity.\textsuperscript{5}

EPIDEMIOLOGY

The disease has only been confirmed in pigs. Experimentally the BEP affects mice and chick embryos; rabbits, dogs and cats do not show clinical signs, but the rabbit produced antibodies.\textsuperscript{11,12}

Pigs subclinically infected are the main source of the disease; the virus may be disseminated by people and vehicles. Other sources of infection
have not been demonstrated but wind and possibly birds could be incrimi-
nated.

Farms located in a valley and separated from 1 to 5 km, all suffer from
blue eye whereas one farm close to them (3 km) located up in a hill remains
free of the disease. There were no other contacts except for wind and birds.

It has been proved that the disease is self-limiting in close herds. Sentinel
pigs introduced to the farm 6 to 12 months after the outbreak remain
asymptomatic and do not produce antibodies against BEP.\textsuperscript{13}

Naturally infected animals develop antibodies that usually persist
throughout their life. Affected farms within an enzootic area have become
affected three years later. Farms with a continuous system of production
may have cases periodically.

Blue eye is more common from March to July, which are the driest and
hottest months of the year in this area, but outbreaks are observed
throughout the year.

**CLINICAL SIGNS**

In commercial breeding units the problem may start in any area, usually
it is first observed in the farrowing house, with central nervous system
signs and high piglet mortality. At about the same time, the farmer may
observe corneal opacity in some weaned or fattened pigs.\textsuperscript{4,12,13}

The disease appears to be self-limiting. The mortality rate rises rapidly
and falls within a short time. Once the initial outbreak is over no new
clinical cases appear unless susceptible pigs are introduced to an infected
farm, as has been observed on farms which operate on a continuous flow
pattern.

The clinical signs are available and depend mainly on the age of the pig.
Piglets two to 15 days old are most susceptible, and the clinical signs are
sudden in onset. Healthy piglets suddenly become prostrated and de-
pressed or show nervous system signs. Fever, a starting hair coat and an
arched back are sometimes accompanied by constipation or diarrhoea,
followed by progressive nervous signs; ataxia, weakness, rigidity mainly of
the hind legs, muscle tremor and abnormal posture (sometimes a sitting
position). Anorexia does not occur while the piglets can still walk. Some
piglets are hyperexcitable, showing squealing and paddling movements
when handled. They become prostrated generally in lateral recumbency.
Other signs include lethargy, with some involuntary movements, dilated
pupils, apparent blindness and sometimes nystagmus. Some piglets suffer
from conjunctivitis with swollen eyelids and lacrimation. Often the eyelids
are closed and adherent with exudate. In 1 to 10 percent of the affected
piglets either unilateral or bilateral corneal opacity is present. Frequently
corneal opacity can be seen in piglets without other signs and is commonly
observed to resolve spontaneously. In the first cases observed, piglets
usually die within 48 hours of the appearance of clinical signs, but in later cases death occurs after four or six days.\textsuperscript{12}

Of the litters farrowed during an outbreak, 20 percent to 65 percent are affected. In these litters the piglet morbidity is between 20 percent and 50 percent and the mortality of the affected pigs between 87 percent to 90 percent. The piglet mortality lasts from 2 to 9 weeks depending mainly on the system of management.

Most of the sows of affected litters are clinically normal. Some of them show moderate anorexia one or two days before the appearance of clinical signs in the piglets and corneal opacity has also been observed in the sows in the farrowing house during outbreaks.

After weaning, pigs more than 30 days old show moderate and transient clinical signs such as anorexia, fever, sneezing and coughing. Nervous system signs are rare but when present they consist of depression, ataxia, circling and swaying of the head. As in piglets, unilateral or bilateral corneal opacity and conjunctivitis are seen without other signs and continue to appear on the farm for another month. Only 1 percent to 4 percent of pigs older than 0 days are so affected and the mortality is low.

Since 1983, in addition to Blue Eye, a 20 percent mortality with severe central nervous system manifestations has been observed in 15 Kg to 45 Kg pigs, but only in badly managed farms, practicing a weaner fattening system and receiving animals continuously from different sources. Various other diseases have also been diagnosed on these farms. Corneal opacity was present in up to 30 percent of pigs.\textsuperscript{7,8}

Gilts and other adult pigs also occasionally develop corneal opacity like the farrowing sow. In pregnant sows an increase in the number of animals returning to oestrus is observed. This sign lasts six to eight months. Abortion has been observed in some dams. During outbreaks there is also an increase in stillbirths and mummified fetuses up to 24% and 5% respectively.

In boars there is a reduction in fertility associated with an increase in the size of the testicle and epididymus usually unilateral, and later, testicular atrophy with hardness of the epididymus, 14 to 40 percent of the boars in the farm are so affected.

**GROSS LESIONS**

There are no specific gross changes. A mild pneumonia is frequently observed at the ventral tips of the cranial lung lobes. Mild gastric distension with milk (in piglets), distension of the urinary bladder with urine and small accumulation of fluid with fibrin in the peritoneal cavity were observed. Brain congestion was also a feature. Conjunctivitis, chemosis and varied degrees of corneal opacity have been confirmed; vesicle formation, ulcers and queratocono has been observed in the cornea as well as
exudate in the anterior chamber. Recently pericardiac and kidney haemorrages have been observed.\textsuperscript{4,12}

In boars there are orchitis, epididymitis and later, testicular atrophy with or without granulomatouse formations in the epididymus.

**MICROSCOPIC LESIONS**

The main histological changes were located in the brain and spinal cord. There was a non-suppurative encephalomyelitis affecting mainly the grey matter of the thalamus, mid-brain and cerebral cortex and characterized by multifocal and diffuse gliosis, perivascular cuffing with lymphocytes, plasma cells and reticular cells, neuronal necrosis, neuronophagia, meningitis and choroiditis.\textsuperscript{14} Intracitoplasmic inclusion bodies were found in neurones. There were variations in the severity and extent of these lesions.\textsuperscript{11,12,15}

The lungs had localized, scattered areas of interstitial pneumonia characterized by thickened septa with mononuclear cell infiltration.

Changes in the eye are mainly observed in those animals with corneal opacity and consist of corneal oedema, and anterior uveitis with neutrophils, macrophages and mononuclear cells infiltrating mainly the iridocorneal endothelium; the external sheet of the cornea is often with cytoplasmic vesicles and in some, intracitoplasmic inclusions are observed in the epithelial cells near to the corneo-scleral angle.\textsuperscript{11,12,15}

Many animals showed a mild tonsillitis with desquamated epithelium and inflammatory cells in the crypts.

**DIAGNOSIS**

Clinical signs such as encephalitis, corneal opacity and reproductive failure in the sow or orchitis and epididymitis in the boar gave the basis for a diagnosis. The non-suppurative encephalitis, anterior uveitis, keratitis, orchitis and epididymitis also contributed.

Serological tests such as haemagglutination-inhibiting antibodies (HI), neutralizing antibodies and ELISA have been developed to identify positive animals. Until now HI is the most reliable. Also a direct immunofluorescence test has been performed in tissue section and monolayers, using a conjugate prepaarated with rabbit or pig serum.

Virus isolation is easily performed in monolayers of PK 15 cells with brain and tonsil samples. A citopathic effect characterized by syncytium formation occurred.

Differential diagnosis with other encephalitis and causes of reproductive failure need to be performed, specially against Aujeszky's disease. Until now only the BEP produces corneal opacity in up to 30% of pigs.\textsuperscript{11,12,16}
INFECTION IN PIGS IN MEXICO

CONTROL

Elimination of the blue eye paramyxovirus from infected herds has been performed by management practices such as: closing the herd, cleaning and disinfecting, all-in all-out, elimination of clinically affected animals (nervous signs or infertile boars), followed by serological testing, herd performance analyses and sentinel BEP seronegative pigs to confirm the elimination of the BEP.17

A vaccine of killed virus, elaborated in cell monolayer cultures is being developed, and preliminary trials are encouraging.

REFERENCES

STEPHANO


INTRODUCTION


STATE SURVEY

The statistical survey of Illinois swine herds has been completed and discontinued effective December 30, 1988.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Number swine tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>19,456</td>
</tr>
<tr>
<td>1985</td>
<td>20,265</td>
</tr>
<tr>
<td>1986</td>
<td>14,526</td>
</tr>
<tr>
<td>1987</td>
<td>9,534</td>
</tr>
<tr>
<td>1988</td>
<td>6,290</td>
</tr>
<tr>
<td>TOTAL (1984-88)</td>
<td>70,071</td>
</tr>
</tbody>
</table>

Number tested in previous survey

Total swine diaphragms examined in Illinois from

Fifteen infected herds were disclosed from 1971 to 1975. No new infected herds were disclosed in the period 1984-1988.

GEORGETOWN SITE

WILDLIFE TRAPPING — GEORGETOWN

October 1987-June 2, 1988

<table>
<thead>
<tr>
<th>Species Trapped</th>
<th>Total Number Tested</th>
<th>Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opossums</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Raccoons</td>
<td>29</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Rats</td>
<td>44</td>
<td>1* (2%)</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

*First positive rat since early 1970s.
A table for wildlife trapping in Georgetown was published in the summary (Page 108, USAHA) of “Illinois Trichinosis Control Program” article for 1987 and was entitled October-November 4, 1987. The preceding table is a revision of that table with data for October 1987 to June 2, 1988.

**GEORGETOWN AREA**

Wildlife Trapping by Commercial Trappers in Georgetown Area November 16, 1987 through January 12, 1988

<table>
<thead>
<tr>
<th>Species Trapped</th>
<th>Total Number Tested</th>
<th>Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opossums</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Raccoons</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>Coyotes</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Minks</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Foxes</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>131</strong></td>
<td></td>
</tr>
</tbody>
</table>

**EAST ST. LOUIS SITE**

**RODENT TRAPPING AND POISONING (2)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Number Tested</th>
<th>Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>255</td>
<td>0</td>
</tr>
<tr>
<td>Cat</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Mice</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

The trichina disclosed in the cat were dead.

**POST-DEPOPULATION MONITORING OF PREMISES**

*Georgetown Farm*

In August, 1987, eight replacement swine were tested using the ELISA (1) after being on the premises several months. The results were negative.

In February, 1988, 51 replacement swine from the Georgetown farm were tested by ELISA. Seven were classified as trichina positive. Since a portion of these swine were tested at a slaughter market, only two of the seven swine were available for necropsy. The two positive swine were captured, euthanized and tested by the digestion technique. One animal yielded 1.78 LPG live trichina.

In May, 1988, all of the swine on the Georgetown farm were serologically tested. Of 376 swine tested by the ELISA, 26 were positive. Tongues from the 26 serum positive animals were tested by the digestion technique. Trichina were disclosed in 17 of the 26 tongues. The 17 positive swine yielded .011 to 1000 larvae per gram from 52 grams to 100 grams examined.
The 26 serum positive animals were euthanized and processed by a dead animal rendering establishment into grease and feed tankage. The negative animals remained on the farm.

In November, 1988, 179 swine were tested using the ELISA. Five of 179 swine were ELISA positive for trichinella. Tongues were recovered and tested from three of the five positive swine. One of the three positives yielded trichinella larvae on the pooled sample digestion test. Three larvae (.07 LPG) were disclosed to 45 grams of tissue. Statistically, this means that if the normal 5 gram sample had been tested, it probably would have been scored as negative. The other two positive swine could not be identified and were shipped to slaughter. Another group (275) large swine were shipped to slaughter without being tested. Approximately 670 swine remain on the farm under quarantine.

In August, 1989 blood samples were collected from 120 swine at a market where the swine were enroute to slaughter. Four animals were classified as positive on the ELISA test with optical density of 25%, 35%, 44%, and 52%. Since these animals were enroute to slaughter, no tissue was collected for pooled sample digestion.

East St. Louis

In September, 1987, 23 repopulated swine belonging to 11 owners in the East St. Louis complex were tested. One infected sow was identified. In November, 1987, four additional swine, from the owner of the infected sow, were tested, and all were negative.

In September, 1988, 67 swine belonging to 19 owners, were tested by the ELISA. One animal was positive with a very high reading on the ELISA (63%). The pooled sample digestion of 100 grams each of the tongue, diaphragm and ham revealed 67-400-94 larvae per gram respectively. The 66 negative swine had very low ELISA readings. The owner stated this sow had been purchased as a feeder pig through a licensed feeder pig sale. Traceback to the farm of origin of the feeder pigs failed to disclose evidence of trichinosis. Four of the 67 negative swine were from the owner of the positive sow identified in September 1987.

In May, 1989, 22 swine belonging to seven owners, were tested by ELISA. Six swine were positive by ELISA. The six swine and one cat were examined by the pooled digestion method. Five swine belonging to one owner and the one cat were positive. The larvae recovered from three swine and the one cat were too numerous to count. The other two hogs had LPG values of 0.02 and 1.0.

In October, 1989, sixty swine belonging to fifteen owners, were tested. Two owners each had three animals that were classified as positive on the ELISA. Four of the six swine yielded larvae on pooled sample digestion. One of the owners of the infected swine owned the infected sow disclosed in September, 1988 and was the owner of an infected sow disclosed in May,
1989. The three herd owners of the May and October infected swine are on adjacent premises.

<table>
<thead>
<tr>
<th>SWINE ID</th>
<th>ELISA OD%</th>
<th>Larvae in Tongue (LPG)</th>
<th>Larvae in Diaphragm (LPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Herd owner #1</td>
<td></td>
</tr>
<tr>
<td>906</td>
<td>20</td>
<td>None Observed</td>
<td>None Observed</td>
</tr>
<tr>
<td>909</td>
<td>20</td>
<td>2.94</td>
<td>1.8</td>
</tr>
<tr>
<td>911</td>
<td>39</td>
<td>*TNTC</td>
<td>54.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Herd owner #2</td>
<td></td>
</tr>
<tr>
<td>912</td>
<td>27</td>
<td>.3</td>
<td>.28</td>
</tr>
<tr>
<td>913</td>
<td>18</td>
<td>None Observed</td>
<td>None Observed</td>
</tr>
<tr>
<td>916</td>
<td>44</td>
<td>2.98</td>
<td>3.68</td>
</tr>
</tbody>
</table>

*TNTC — Too numerous to count.

In June, 1989, rat poisoning was conducted using Assault.² Seventy-three dead rats were tested by pooled digestion using 7.0 grams of tongue from each rat. The 55 adult and 18 juvenile rats were identified according to specific swine locations. Pooled digestion disclosed three positive adults. All the juvenile rats were negative. The positive rats were killed in the general area of the positive swine. The three positive rats had LPG values of .2; .2; and .6.

On re-survey of the area, three cats (1 adult, 1 juvenile and 1 kitten) and three adult rats were examined by the pooled digestion method. The kitten was negative, the juvenile cat had 11 trichinella in 10 grams of tissue (1.1 LPG), and the adult cat had 1,000 trichinella in 10 grams of tissue, (100 LPG). The three adult rats were negative.

In summary, a total of 58 adult rats were tested and 5% were found to be infected.

**SUMMARY**

The eradication of trichinellosis from Illinois swine herds has been an elusive activity. Trichinellosis was either not eradicated or has been reintroduced on both premises. In East St. Louis, an infected sow was disclosed in 1987, one year after depopulation and another sow was disclosed in 1988, two years after depopulation. In May, 1989, five swine were positive to pooled sample digestion.

In February, 1988, two years after depopulation, the Georgetown farm was tested and infected swine were discovered. In May, 1988, additional infected swine were disclosed and in November 1988, one infected hog was disclosed with a low larvae count.

The incidence of infected rats in the East St. Louis complex declined following the depopulation in 1986, but has returned to the former level. Infected wildlife at the Georgetown farm has declined since depopulation in 1986.
ILLINOIS TRICHINELLOSIS CONTROL PROGRAM

Extensive traceback has failed to disclose evidence of trichinosis on the farms of origin of the repopulated swine.

The role of rodents and wildlife relative to the disease on these premises has not been established. Infection in rats disappeared after the swine were depopulated and increased when a number of infected swine were disclosed.

Depopulation-repopulation of trichinosis infected swine herds has not eliminated the infection.

REFERENCES
2. Purina Assault Rat Place Pack, Purina Mills, Inc., St. Louis, Missouri.

ACKNOWLEDGEMENTS
Special thanks to Ronald Ogden, State Director, Animal Damage Control, United States Department of Agriculture, for his assistance with poisoning and trapping of rodents.

Special thanks to James Austin for his assistance throughout the program. He served as manager of the special task forces that conducted the farm operations.

Special thanks to Guy Simmons, who conducted the trapping activities on the Georgetown farm and collected the wildlife heads from commercial trappers.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

Chairman: Dr. J. P. Kluge, Ames, IA
Vice Chairman: Dr. D. G. Thawley, St. Paul, MN

G. A. Anderson, KS; G. W. Beran, IA; L. G. Beihl, IL; N. Black, NM; P. E. Bradshaw, IL; J. Castaneda, SA; J. R. Cole, GA; A. M. Creswell, TN; P. B. Doby, IL; J. A. Dornard, IN; G. A. Erickson, IA; D. Galbreath, MD; A. M. Gallina, WA; D. P. Gustafson, IN; D. L. Harris, KY; G. W. Hausman, IA; H. T. Hill, IA; H. S. Joo, MN; W. L. Kadel, KY; C. L. Kanitz, IN; F. J. Mulhern, MD; P. A. O'Berry, IA; R. E. Omohundro, CO; R. R. Ormiston, MD; R. Polen, NJ; C. Rogers, NE; L. Schlater, IA; L. W. Schnurrenberger, AZ; R. Schultz, IA; J. E. Slauder, MO; W. C. Stewart, MD; R. E. Thompson, NM; H. W. Towers, Jr., DE; M. W. Vorhies, KS; D. L. Weiss, IA; J. C. Wright, AL.

The Committee met at 1:30 p.m. on Monday, October 30, 1989 at the Riviera Convention Center, Las Vegas, Nevada. Fifteen committee members and forty-five guests were present.

The committee heard a report by Dr. Paul Doby (Illinois Department of Agriculture), and Dr. Darwin Murrell (USDA) on the Illinois trichinosis program. They reported on the observed epidemiology of trichinella on farms where eradication had been attempted. It would appear that even following depopulation of swine, the parasite often is maintained in the rodent population, and later infected the repopulated swine herds. They concluded that their evidence indicated that depopulation alone is not effective and it must be combined with a program of aggressive rodent control.

Dr. Lynn Joens (University of Arizona) presented a paper for Dr. D. L. Harris (Pig Improvement Co., KY) on eradication of swine dysentery. Dr. Harris proposed that swine dysentery is a good candidate for a national eradication effort. He described procedures that may be used when attempting to eradicate the disease from individual herds of swine. He charged the committee to consider forming a combined committee with representatives from LCI, AASP, NPPC & USAHA with the objective of examining the feasibility and economics of swine dysentery eradication.

Dr. Lynn Joens together with Dr. Neil Jensen of the National Animal Disease Center discussed the development and use of DNA probes for the detection of Treponema hyodysenteriae in clinical samples. Dr. Joens reported a test sensitivity of approximately 10 organisms per gram of feces with a DNA probe from a plasmid. Dr. Jensen also reported a high sensitivity with a DNA probe that is complementary to T. hyodysenteriae ribosomal RNA. These methods using DNA probes have many advantages over the conventional culture method which has a low sensitivity, takes 4-6 days for a result, and is interfered with by other bacteria present in clinical samples. The probes are at least as sensitive as culture and are
TRANSMISSIBLE DISEASES OF SWINE

highly specific in differentiating *T. hyodysenteriae* from other non-pathogenic species.

Dr. Alberto Stephano from Mexico presented a paper on Blue Eye paramyxovirus infection in Mexican swine. The disease was first recognized in 1980 in young piglets. In 1984 it appeared to increase in virulence and is now present in 14 states in Mexico. It is characterized by central nervous signs, corneal opacity and high mortality in nursing and weaned pigs. In sows there is reproductive failure with return to estrus and increase in number of stillbirths and mummified fetuses and some abortions. In boars, there is orchitis and epididymitis which results in testicular atrophy and reduced fertility. Serological studies have shown that the Blue Eye paramyxovirus is different from previously described members of the paramyxovirus group. The disease was produced experimentally using purified isolates from field cases.

Two papers were presented of the possible cause or causes of the new reproductive disease which has hit the U.S. swine industry over the past two years. Dr. Carole Bolin from the National Animal Disease Center reported on the association between the culture and serology of *Leptospira bratislava* and the occurrence of the disease. She reported that she currently needs to conduct studies to produce the disease in experimentally infected swine. Isolations from stillborn and weak pigs have included genotype 1 which had not previously been reported from pigs. Successful isolation of *L. bratislava* requires inoculation of several types of media and cultures must be incubated for up to 26 weeks. Fetal antibody levels and FA technique on fresh fetal tissues and placenta are used in addition to culture to diagnose the disease. It appears that antibodies are not effective in eliminating the infection in carrier animals.

Dr. H. S. Joo from the University of Minnesota reported on his observed association between the disease syndrome and presence of Encephalomyocarditis virus antibodies. Because of the unknown etiology of the condition it has become known as the mystery disease of 88/89. In affected herds the clinical signs observed are sick sows that have fever and are off feed and outbreaks of late abortions, stillbirths, and weak pigs that die shortly after birth.

Following discussion, the committee passed three resolutions to be transmitted to the Board of Directors of the USAHA. These included: 1) That USAHA, LCI, AASP and NPPC set up a committee to examine the feasibility and economics of the elimination of swine dysentery from individual herds of swine and to better publicize means of prevention and control; 2) USAHA support the proposed additions to the Program Standards for Pseudorabies Eradication relating to feral swine movement and changes in the Uniform Methods and Rules for Porcine Brucellosis Eradication as written by the Feral Swine Subcommittee; and 3) USAHA request that the USDA immediately place swine Blue Eye Paramyxovirus infection on the official list of foreign animal diseases.
PROBABLE INCIDENCE OF TUBERCULOSIS TRANSMISSION
FROM MEXICAN STEERS

D. L. Thompson, D.V.M., M.P.V.M.
Animal Health Branch
Department of Food and Agriculture
State of California

In December, 1988, federal inspectors and veterinarians at a slaughter establishment in California submitted tuberculous tissues from 3 steers. The steers all had the same owner and all appeared to be of Mexican origin. One had an official Mexican metal ear tag in its ear (SARHCTB-C /7053).

The tissues were submitted to the National Veterinary Services Laboratory in Ames, Iowa (NVSL) where pathologists declared that the lesions were compatible with tuberculosis. A "6-35" investigation was then initiated. The Area Veterinarian-in-Charge for Veterinary Services, U.S.D.A., was notified. He forwarded the information to our State offices with a request that an investigation be started. Dr. Robert Hargreaves was assigned to this. His work revealed that the company listed as the owner at the time of slaughter had purchased them in July and September, 1988. Dr. Hargreaves then contacted the previous owner and discovered that all cattle that had commingled with or been exposed to these steers had already been sent to slaughter. Normally this would be the end of an investigation. However, Dr. Hargreaves did an excellent job of educating and raising the concerns of the herd manager. This coupled with the herd manager's memory of many tuberculous herds in that area during the 1960s led that manager to volunteer a small purebred herd for testing. This herd, comprised of 66 cattle, was a showpiece for the ranch. They were like pets that stayed very near the headquarters of the ranch and had commingled with and been exposed to thousands of Mexican steers. These steers were processed through, and pastured on the ranch during the preceding four years. The purebred cattle had been purchased in Texas four years before, shipped directly to the ranch in California, and had not left the ranch since that time.

Since this herd was in a much different part of the district than where Dr. Hargreaves began the investigation the testing was assigned to Dr. Dorothy Davidson-York. In early February, 1989, she conducted a caudal fold test on the herd. She was thorough and tested all cattle six months and older. Two animals had 20 mm responses. They were 17 month old unbred heifers. The subsequent comparative cervical test on these two yielded responses of: 2 mm avian, 8 mm bovine and: 1.5 mm avian, 8 mm bovine. Both were branded as reactors and a Hold Order was issued. I might add that Dr. Davidson-York was also the testing veterinarian for the last tuberculous herd diagnosed in CA in 1986.

Dr. Davidson-York, her supervisor Dr. Gary Montgomery, and Dr. Adel Malik, a very helpful veterinarian with MPIP (Meat and Poultry Inspection
TUBERCULOSIS FROM MEXICAN STEERS

Program, FSIS, U.S.D.A.) inspected the carcasses when they were slaughtered at a local abattoir. They found calcified lesions in the suprathyroidal and hepatic lymph nodes of both carcasses. They also found tuberculous lesions in mesenteric lymph nodes. Tissues were submitted to NVSL where histopathologists categorized the lesions as compatible with mycobacteriosis. Hold Orders were then issued for the entire purebred herd and about 400 Mexican steers that had commingled with the purebred herd.

As more was discovered regarding the exposure history of this herd, the potential of establishing Mexican steers as the source of infection increased. This history strongly indicated that the herd had a very limited potential sources of infection other than Mexican steers. There was also an unusual opportunity to test and examine virtually all possible sources of infection other than the Mexican steers. The discovery of these facts greatly increased our desire to depopulate this herd. A meeting with the owner was arranged. There was some delay because he lived in another state. However, hopes were high because the herd manager was telling us that the owner was very interested in depopulation. Our hopes were dashed at the meeting. The owner requested further testing when he realized that the indemnity for depopulation was significantly less than for his two reactors. There was then some delay at the request of the owner. During this time NVSL informed us that attempts to culture mycobacteria were negative, i.e. no growth on synthetic media. Inoculation into guinea pigs was requested and begun.

In early May, 1989, the herd was tested with the single cervical test. This yielded sixteen reactors and resulted in the owner reconsidering depopulation. He agreed to it and the fifty-four nonreactors were slaughtered in California on June 1. No lesions were detected in those cattle. At about the same time 15 reactors were slaughtered at a plant in another state which had facilities for cooking. The sixteenth reactor was a young bull with a serious injury. It was destroyed at the ranch. Lesions were found and tissues were collected from six of the reactors. Almost all lesions were in the gastrointestinal tract. None were found in the thoracic cavity. Pathologists at NVSL reported that tissues from three of them were compatible with mycobacteriosis. Subsequently M. bovis was cultured from all three of these reactors. In addition the guinea pigs were positive for M. bovis after inoculation with tissues from the original two reactors. All animals with compatible lesions were less than two years old.

The following items greatly support the concept that M. bovis was transmitted from Mexican steers to cattle native to the United States:

1. No known exposure to any other cattle except Mexican steers since they were purchased 4 years before;

2. No sales except bull calves. Therefore all but three of the original herd were still available for testing and examination of the carcasses. The 3 exceptions were: a. A bull which was sold to slaughter 3 years before
and; b. A cow that died of complications from a prolapsed uterus and; c. an "old cow" that was found dead in the pasture. Both of these cows died over 2 years before;

3. Lesions compatible with M. bovis and/or from which M. bovis was isolated were only found in cattle less than 2 years old;

4. No nonnatural additions to the herd except one bull purchased three years before (tested negative and carcass yielded no lesions).

Work still remaining to be done includes monitoring the steers under Hold Order. They’ve been shipped to a feedyard in another state and will be shipped to slaughter during the last months of 1989. Federal officials in that state have been notified and requested to assure special examination of the carcasses. Also, there are two neighboring herds which possibly had some “fenceline exposure” to the infected herd. Information to date continues to indicate that no “fenceline exposure” occurred, however more will be done to confirm this and/or test if appropriate.

This case is by far the best example of transmission of bovine tuberculosis from Mexican steers to cattle native to the United States. Hopefully it will be of assistance in evaluating whether current policies for livestock imported from Mexico need to be revised.

Finally an additional comment. As you know the Tuberculosis Program is a State-Federal Cooperative Program. The work involved with this herd was a good example of that cooperation. We thank Drs. Mitchell Essey, Evelyn Williams, and James Roswurm for their assistance and support.
CANADA'S CAPTIVE WILD UNGULATE PROGRAM
W. S. Bulmer, Ottawa, Canada

Introduction
Canada eradicated brucellosis from the national cattle herd in 1985 and the eradication of tuberculosis is imminent.

In recent years, an increasing number of wild ungulates such as bison, elk, deer, etc., have been introduced into the more heavily populated areas of Canada and, as a consequence, are coming into close contact with domestic livestock and people.

These animals may be infected with brucellosis and/or tuberculosis. Game farms and bison ranches situated among cattle ranches or farms are potential sources of infection to domestic cattle. Also, Agriculture Canada is expected to provide the public with protection against zoonotic threats, especially in petting zoos or roadside exhibits where young children may have intimate contact with animals.

Policy
All premises such as game ranches and zoos on which wild ungulates are held in captivity have been identified and classified as Type A or Type B wild ungulate premises. Type A premises are subjected to disease eradication programs for brucellosis (Brucella abortus) and tuberculosis (Mycobacterium bovis). Type B premises designation is applied to zoological parks. They are monitored but are not routinely tested.

Type A Premises
These are premises engaged in the commercial rearing of captive wild ungulates, where their presence is considered to pose a threat to domestic livestock because of potential transmission of disease. The threat of transmission may be due to the situation of the premises among livestock farms, the possibility of direct or indirect contact across, through or over fences or the possibility of these animals escaping or being released into rural areas.

Included in this category are premises which sell to or trade with Type A premises. Examples of Type A premises are: game farms, bison ranches, or wild animal dealers. Travelling zoos because of their itinerant nature are included in the Type A premises designation.

The program (test and slaughter with compensation) applies to captive wild ungulates and any domestic species of cattle, sheep or goats maintained on the same premises. It does not apply to other susceptible species such as monkey, bear, lion or camel, etc. maintained on a Type A premises.

Type B Premises
Type B premises include publicly or privately owned premises where captive wild ungulates are kept and which meet the following conditions:
(a) Located in an urban area with no domestic livestock operation adjacent to the perimeter fence of the zoological garden, or

(b) Located in a rural area with a continuous perimeter fence enclosing the zoological garden and an inner fence not less than two meters from the perimeter fence forming a permanent buffer zone in which animals are not permitted.

(c) If a petting zoo is one of the zoological exhibits, all domestic species (cattle, sheep and goats) used in the petting zoo must be sold directly to slaughter or used in carnivore diet preparation when these species are removed from the petting zoo.

(d) Restrict the entry of farm vehicles into the zoo.

(e) Restrict the access of zoo vehicles into domestic livestock operations.

(f) The disposal of manure from the zoo must constitute no hazard to domestic livestock species.

Testing Methods

For brucellosis, the card test is applied at the time of sampling and the buffered antigen plate test (BAPT), complement fixation test (CFT) and ELISA tests are applied at the laboratory.

For tuberculosis, the caudal fold tuberculin test, using standard dosage for cattle, is employed in bison and other bovidae. The mid-cervical intradermal tuberculin test is employed for cervidae.

An initial test of all bovidae and cervidae is conducted. Except in cases where all animals are adequately covered by screening programs, a complete retest of all such animals present is conducted at regular intervals not exceeding 3 years. Such intervals are established by a District Veterinarian in consultation with his/her Regional Office and takes into account factors such as, the degree of contact with domestic livestock, security measures and facilities designed to prevent contacts or escapes, population turnover, number of sales and additions, source of additions, clinical signs and other epidemiological factors. Additions without test are permitted from premises of equal status (i.e. tested negative at least once). Additions from Type B premises are tested negative before being added to tested herds.

Screening Programs

Herds are monitored by the regular screening programs (eg. tuberculosis-like lesion monitoring at slaughter) and can be exempted from test if adequately covered by these programs. This applies in particular to bison herds reared for meat production.

When other traditional detection methods such as reports from diagnostic laboratories, practicing veterinarians, owners or Medical Health Officers give rise to suspicion of the presence of brucellosis or tuberculosis, the origin of the animals is traced and herdmates are tested.
Reactor Animals and Infected Herds

Brucellosis or tuberculosis test reactors are evaluated for compensation and ordered slaughtered.

Compensation at full market value is awarded for all animals ordered slaughtered.

Injuries

When a test eligible ungulate is killed or dies of injuries as a direct result of handling for purposes related to the captive wild ungulate program, compensation may be awarded. No compensation is awarded for mortality that results from the use of a nonapproved chemical restraint drug.

Domestic Livestock

Any cattle, sheep or goats maintained on Type A premises will be subjected to the same requirements as captive wild ungulates.

Special Situations

When there are susceptible non test eligible species (e.g. monkey, bear, lion, camel, etc.) in addition to captive wild ungulates on a type A premises, only the wild ungulates are tested. If the test eligible captive wild ungulates are negative for tuberculosis and brucellosis, the susceptible non test eligible species are also considered negative. If reactors occur among the captive wild ungulates, all species of animals on the premises are tested. If either brucellosis or tuberculosis is confirmed, all susceptible animals on the premises are depopulated.

Type B Premises

Type B premises are not tested unless either brucellosis or tuberculosis is suspected because of association with another infected premises. If infection is confirmed, all animals susceptible to the particular disease are depopulated.

These premises are inspected twice yearly to determine the number of captive wild ungulates in the zoological park. Any additions to or transfers from the park since the previous visit are recorded. An assessment of the animal health risk is made.

If deficiencies identified in the assessment are not corrected, the Type B designation reverts to a Type A designation.

Implementation

The program has been implemented in phases beginning in 1987. In each Agriculture Canada District Veterinary Office, captive wild ungulate premises were identified and an inventory of Type A and of Type B premises was created. The premises were visited, the source(s) of the animals was determined, handling facilities were assessed and a testing date was arranged determined, handling facilities were assessed and a testing date was arranged. Phase I has been completed in all seven Regions. Phase II,
the actual testing is nearly completed in Manitoba, 25% completed in Alberta and has been started in the other Regions.

To date (1989), tuberculosis has been detected in a New Brunswick bison herd of 166 animals (1985) and brucellosis (biotype 4) was detected in an Ontario bison herd of 160 animals (1988). Both herds were depopulated.

Figure 1. DISTRIBUTION OF CANADIAN GAME FARMS
1989
Procedures for the identification of mycobacteria of veterinary interest were developed to facilitate the processing of tissues and the identification of isolates. The goals of the project were to (1) simplify laboratory isolation procedures for mycobacteria, (2) evaluate mycobacterial media that could be easily prepared from readily available materials, and (3) prepare a simplified method for identification of mycobacteria using procedures amenable to minimally equipped laboratories.

The processing of animal tissue was simplified by using commonly available equipment. Selection of primary isolation media was limited to those not requiring inspissation since this procedure is difficult to do in most laboratories. Two agar-based media, Herrold egg yolk and Middlebrook 7H10, were used.

Identification of mycobacterial isolates was modified by reducing the number of tests from 14 in the conventional method to 7 in the simplified method. Inoculation of test media was reduced from 14 tubes in the conventional method to 5 tubes in the simplified procedure.

One hundred and seven tissues of animal origin suspected of mycobacterial infection were processed using the new system and compared to duplicate tissues processed by conventional methods used at the National Veterinary Services Laboratories. Isolates from tissues processed by the new system and other sources were identified using the simplified method and compared to conventional procedures.

There was 98.75% agreement of results between isolation methods and 98.75% agreement between the two identification methods.

Additional tests may be used in more advanced laboratories to extend the ability to identify additional species.

INTRODUCTION

Conventional methods for isolation and identification of mycobacteria have changed little over the past 20 years. The complexity of these procedures has varied between laboratories depending upon technical ability, expertise, and other resources.

New techniques involving high technology have started to make inroads
into the procedures for isolation and identification of mycobacteria in laboratories with the interest and necessary resources. DNA probes and radiometric methods are being used in many laboratories and may well represent the wave of the future. A problem, however, exists in many countries where even conventional methods are hard to perform. Logistical and budgetary support may be limited, and the performance of many routine procedures are impossible because of the lack of equipment, trained personnel, and readily available laboratory supplies.

The purpose of this study was to evaluate a simplified method for the isolation and identification of mycobacteria. The method studied is not necessarily new but has taken existing information and formulated it into a method which, if effective, could be useful in developing countries. The methods described will allow identification of most mycobacteria to group level and most major veterinary pathogens to species level.

**MATERIALS AND METHODS**

One hundred and seven animal tissues known to be tuberculous were processed by the simplified procedure. Portions of the same tissues were also processed by conventional methods to evaluate the efficacy of the procedure. Identification was performed on 101 mycobacterial isolates using the simplified procedure. All procedures were performed in a biological safety cabinet.

Tissue portions of approximately 1 cm² were macerated in a mortar and pestle using 10 ml of nutrient broth containing 0.04% phenol red as a diluent. The macerated tissue suspension was added to a 20 × 125 mm screw cap test tube and treated with 5.0 N NaOH. Exposure to the alkali was not allowed to exceed 10 minutes. The suspension was neutralized by adding 6.0 N HCl until the mixture turned yellow. Adjustment to pH 7.0 (a pale pink) was made by addition of 1.0 N NaOH. Mycobacteria present in the suspension were concentrated by centrifugation at 1650 × g for 20 minutes.

The pellicle and 90% of the supernatant were decanted. With a sterile cotton swab, 4 tubes of Herrold egg yolk media and 4 tubes of Middlebrook 7H10 media were inoculated with tissue sediment resuspended in the residual fluid. All tubes were incubated at 37 C for 8 weeks. Tubes were examined weekly for growth.

Colonies typical of mycobacteria were confirmed as acid-fast by stained smears using the Ziehl-Neelsen procedure. A portion of the colony was also inoculated into 8.0 ml of Dubos Tween albumin broth (DTA) and incubated for 1–2 weeks at 37 C.

Identification methods were based on appearance time, presence or absence of pigment, growth in Proskauer and Beck medium, presence and type of cording, niacin production, and sodium chloride tolerance (Fig. 1).
IDENTIFICATION OF MYCOBACTERIA

One-tenth of a milliliter of the 1- to 2-week DTA cultures were inoculated onto each of 3 tubes of Herrold egg yolk agar, 1 tube of Middlebrook 7H10 agar with 5.0% NaCl, and 1 tube of Proskauer and Beck medium. One tube of Herrold egg yolk agar was covered with heavy paper or aluminum foil and incubated at 37 C, one tube was incubated uncovered at 37 C, and one tube was incubated uncovered at 45 C. Tubes were observed every 2–3 days for the appearance of growth and pigmentation. The Middlebrook 7H10 medium with 5.0% NaCl was incubated at 37 C and observed weekly for growth for a period of 30 days. The Proskauer and Beck medium was incubated for 1–2 weeks at 37 C and observed for granular or uniform growth. A smear was made from the tube sediment and stained by the Ziehl-Neelsen method. The smear was observed microscopically for cell morphology and the presence of cording. Mycobacterium bovis cells were 2.0–4.0 μm x 0.5 μm and tended to produce uneven staining with a “washed out” pinkish cast in one end of the cell. Mycobacterium tuberculosis cells were 2.0–3.0 μm x 0.5 μm and stained uniformly.

Microscopic observation of standard M. bovis smears made from Proskauer and Beck cultures revealed corded cells with loose ragged ends. The corded cells tended to be short. Mycobacterium tuberculosis cords were usually very extensive with long serpentine cords with tightly bound cells.

The niacin test was performed on the Proskauer and Beck culture by adding 1.0 ml of 4.0% aniline in 95.0% ethyl alcohol followed by the addition of 1.0 ml of 10.0% aqueous cyanogen bromide. The appearance of a yellow color within 5 minutes was considered a positive test for niacin.

RESULTS

Mycobacteria were isolated from all tissues processed by the standard method. Isolations were made with the simplified method from 106 of 107 tissue samples (Table 2). One specimen containing M. bovis was not detected by the modified method.

The simplified method was very nearly as accurate as the conventional method for identification of mycobacteria including the major pathogens of veterinary interest (Table 3). One M. bovis isolate was identified as M. avium, but all other isolates examined were correctly identified. With the simplified isolation and identification method, there was 98.75% agreement with conventional procedure.

DISCUSSION

The use of the simplified isolation and identification procedures may hold promise. As can be seen in Tables 2 and 3, no significant differences were observed when compared to the conventional system. The modified method of isolation reduces the number of media required from 4 to 2. Herrold egg
yolk and Middlebrook 7H10 were selected for the simplified isolation method because (1) *M. bovis* is known to grow well on each and, (2) they require no inspissation step in their preparation which eliminates the need for special autoclave settings. The use of the mortar and pestle for macerating tissue eliminates the need to purchase electric blenders in countries where these devices might not be common. The other components of the system such as neutralizing reagents should be available in most countries. Simplification of the identification methods reduces the number of tests from 14 to 7 and the number of tubes inoculated from 14 to 5.

The Proskauer and Beck medium is very versatile since several tests producing valuable information can be performed with the medium. Most mammalian mycobacteria grow with a granular texture in this medium.

Safety in the mycobacteriology laboratory is a subject of prime concern because of the infectious nature of most mycobacteria. Where possible, most laboratories perform all procedures in biological safety cabinets. These may range from laminar flow systems to closed port glove cabinets. For many developing countries, this safety equipment is an unobtainable luxury. An inexpensive glove box should be obtained to provide at least minimal protection of laboratory personnel.

The simplified system described here may be expanded to allow further differentiation of mycobacterial species. Depending on the technical capability of the laboratory, the addition of a few simple tests will result in identification of additional isolates.

The use of the Tween 80 hydrolysis test as described will differentiate most saprophytic mycobacteria from pathogenic species. It will also differentiate *M. kansasii*, which is positive, from the negative reactors *M. tuberculosis*, *M. bovis*, and *M. simiae*.

The nitrate test is easy to perform and will differentiate *M. tuberculosis* from *M. bovis* and *M. kansasii* from *M. marinum*. Among rapid growing mycobacteria, *M. fortuitum*, which is nitrate reductase positive, may be differentiated from *M. chelonei*. *Mycobacterium szulgai*, a reactor, may also be separated from *M. scrofulaceum* by the test.

The arylsulfatase test may be used to separate *M. fortuitum* and *M. chelonei* from *M. phlei* and *M. smegmatis*.

Thin-layer chromatography has been used to differentiate between *M. bovis* and other mycobacteria. It can also be used to separate genera and confirm an isolate as *Mycobacterium* sp. Although it would require the purchase of some equipment, it should not be beyond consideration by a developing laboratory.
REFERENCES


Table 1. Comparison of Simplified and Conventional Methods.

<table>
<thead>
<tr>
<th>Tissue processing</th>
<th>Conventional Method</th>
<th>Simplified Method</th>
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<tr>
<td>Media used</td>
<td>Electric blender</td>
<td>Mortar and pestle</td>
</tr>
<tr>
<td></td>
<td>Lowenstein-Jensen</td>
<td>Herrold egg yolk</td>
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<td></td>
<td>Herrold egg yolk</td>
<td>Middlebrook 7H10</td>
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<td>Middlebrook 7H10</td>
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<td></td>
<td>Stonebrink</td>
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<td>Identification tests</td>
<td>Proskauer and Beck medium</td>
<td>Proskauer and Beck</td>
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<td>Isoniazid sensitivity</td>
<td>Niacin production</td>
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<td></td>
<td>Thiophen-carboxylic hydrazide sensitivity</td>
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<td>Niacin production</td>
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<td>Pyrazinamidase</td>
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<td>Acid from arabinose</td>
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<td>Arylsulfatase</td>
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<td>Middlebrook 7H10 w/NaCl</td>
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<td>Thin-layer chromatography (Opt.)</td>
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IDENTIFICATION OF MYCOBACTERIA

Table 2. Comparison of Simplified Isolation Method with Conventional Procedure on 107 Animal Tissues

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. Examined</th>
<th>Isolation Method</th>
<th>Percent Correlation</th>
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<tr>
<td></td>
<td></td>
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<tr>
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<td>M. tuberculosis</td>
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<td>1</td>
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<td>M. neoaurum</td>
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<tr>
<td>M. flavescens (IV)</td>
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Table 3. Comparison of Simplified Identification Method with Conventional Method on 101 Mycobacterial Isolates

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<th>Conventional Method</th>
<th>Percent Correlation</th>
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<td><strong>Total</strong></td>
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Figure 1
Flow Chart
Simplified Isolation and Identification of Mycobacteria
Three years have now passed since meetings began with Mexico to resolve problems arising from the international movement of bovine tuberculosis infected and exposed cattle. The United States Animal Health Association's (USAHA) 1986 Resolution No. 36 called for regular meetings of animal health officials, tuberculosis experts, and cattle industry representatives of both countries to consider these problems and make recommendations. There has emerged from these meetings the recognition and deep conviction that the true solution of these problems lies in the joint effort leading to the eradication of bovine tuberculosis from both countries.

Mexico has since presented a plan for improved control leading to the eradication of tuberculosis in their six border States with Chihuahua State taking the lead. The program would extend southward at the appropriate time to include the balance of those States from which cattle are assembled for export to the United States. This plan was included in the 1988 report. Area testing has begun in Chihuahua State and a very low prevalence of tuberculosis in its beef herds has been reported. This is consistent with the rate of tuberculosis detected on regular slaughter of steers traceable to Mexico which in fiscal years (FY) 1988 and 1989 was approximately 0.03 percent of the number of steers imported the previous fiscal years.

There were two United States-Mexico meetings for bovine tuberculosis eradication held during FY 1989. The first was held April 26–27, 1989, at El Paso, Texas, which included animal health officials and cattle industry representatives from both countries in accordance with 1986 Resolution No. 36. The purpose of this meeting was to develop plans for coordinated tuberculosis control and eradication in the border States and other related activities included in the broad recommendations approved by the Secretary of Agriculture on November 20, 1987.

The United States contingency was led by Dr. Saul T. Wilson, Jr., and included eight Animal and Plant Health Inspection Service personnel. The USAHA and the cattle industry were represented by J. O. Pearce, J. B. Finley, and J. Hagler. The State veterinarians (or their representative) of
the border States were Drs. L. C. Vanderwagen of California, E. R. Hinshaw of Arizona, S. England of New Mexico, and J. Lindstrom of Texas. The Mexico group was led by Dr. Luis Fernandez Zorrilla, Mexico's new Director of Animal Health, and included nine Federal veterinarians and three representatives of the Confederacion Nacional Ganadera; Enrique Dominguez, Gerardo Caraveo Mullen, and Simon Chavez Lozano.

Implementation plans were made for the development of slaughter surveillance in Mexico, preparation of laboratories for tuberculosis examinations, training, publicity and education, and animal identification. Problems were addressed that were caused by differences in the tuberculins, testing procedures and test interpretation in cattle tested for international movement.

A summary of the recommendations of this meeting follows:

That Mexico implement slaughter surveillance in its Federally inspected establishments.

That two laboratories be equipped and personnel be trained as soon as possible for mycobacterial examination with the emphasis placed on histopathology.

That projects be developed to familiarize animal health officials and cattle industry representatives of both sides with the field procedures and cattle industry practices of the counterpart country.

To develop animal identification procedures permitting traceback to the herds of origin of animals found tuberculous.

That all veterinarians in Mexico approved to conduct the tuberculin test receive training to achieve uniformity in testing techniques and followup procedures. This would involve about 150 veterinarians which would receive 1 week training to be given at four regional locations in Mexico.

That a meeting be held in about 6 weeks to resolve problems resulting from differences in the tuberculins used by the two countries and differences also in testing procedures and test interpretation.

That a tuberculosis expert be detailed in Mexico for 1-year as a resource person for implementation of field activities. During this period, a permanent position for this function should be created in Mexico City.

That a small committee be formed to meet frequently to oversee and guide tuberculosis control and eradication activities in the border States.

That future meetings include other cattle diseases of mutual interest as well as tuberculosis.

The second meeting, which resulted from the previous discussions regarding field problems seen with differences in tuberculins used by the two countries, was held September 26-27, 1989, in Mexico City. This was a technical meeting held at PRONABIVE, the National Veterinary Biologics Production Laboratory of Mexico. The meeting was led by Dr. Luis Fernan-
dez, Director, Salud Animal, and Dr. Juan Garza Ramos, Director of PRONABIVE. Dr. Granville H. Frye led the United States delegation. Also present were Drs. M. A. Mendez, Assistant Director, Salud Animal, L. Gual, Chief Epidemiologist for Mexico and PRONABIVE staff members. Other United States attendees were Drs. Mitchell A. Essey and Evelyn Y. Williams, Veterinary Services, R. Dale Angus, Science and Technology Services, and John Mason, Juan Lubroth, and Eduardo Serrano of the Foot and Mouth Disease Commission in Mexico.

Projects were proposed to: (1) compare existing tuberculins in cattle to determine the consequences, if any, of these differences, and (2) produce and evaluate new experimental batches of tuberculin utilizing production methods and seed cultures as variables. The group wishes to invite CEPANZO, Pan American Health Organization (PAHO), to participate in the proceedings related to these trials. CEPANZO is PAHO's regional laboratory located in Buenos Aires, Argentina. CEPANZO is a world recognized authority on tuberculin standardization.

Early in the joint effort for tuberculosis eradication the United States made a commitment to improve its slaughter surveillance to enhance Mexico's ability to detect infected herds by the slaughter traceback mechanism. The United States Food Safety and Inspection Services (FSIS), Meat and Poultry Inspection Operations (MPIO) has responded to this need by increased efforts to recover identification devices and otherwise improve the detection rate and identification of tuberculous cattle from Mexico. In FY 1988 the 2,418 suspicious lesions submitted by MPIO exceeded that of any previous year. In FY 1989 the figure jumped to 4,493, an increase of 89 percent over the previous high. Records show that the increase was largely made up of lesions from feedlot cattle. One reason for the marked increase in suspicious lesions submitted may be the record number of 946,002 steers imported from Mexico the previous year. In FY 1989 the number of imported steers dropped to 615,000.

There were 270 slaughter positive investigations of feedlot cattle initiated in FY 1989 of which an estimated 167 cases (62 percent) traced to Mexico. This compared to 191 out of 258 (74 percent) feedlot cases traced to Mexico last fiscal year and an overall average of 69 percent feedlot cases traced to Mexico from 1982 through 1988. To add perspective to this information it should be pointed out that feedlot cases make up approximately 95 percent of all tuberculosis cases detected on slaughter surveillance.

Last year the "M" brand was reported in a total of 202 suspicious lesions submitted of which 23 (11.4 percent) were found positive for tuberculosis on laboratory examination. Official Mexico eartags were recovered from a total of 135 suspicious cases of which 21 (15.6 percent) were confirmed tuberculous.

In 1988 and 1989 there was a total of 25 positive cases with eartag
identification forwarded to Mexico for investigation. Mexico reported that 2 infected herds were discovered to date as the result of its investigation of these cases. It appears that problems remain in the process of ear tag identification of cattle to their herd of origin. Cattle identification has been identified as a major problem for priority attention in both countries.

Mexico has stated its intent to initiate slaughter surveillance in 4 of its 54 Federally inspected slaughtering establishments. These four establishments are approved by FSIS to export meat to the United States which means that the inspection is equal to United States slaughter inspection standards. Traceback with epidemiological case development for finding additional infected herds will be an important element of Mexico’s emerging program. Mexico faces major problems in the development of slaughter surveillance because most of its slaughter is conducted at establishments not under Federal inspection.

The United States will assist in equipping laboratories and training personnel for histopathological and mycobacteriological examinations for tuberculosis. The first two laboratories selected for this diagnostic capability are located in Ciudad Chihuahua and Mexico City. The sites were selected by Mexico to best serve its field needs and slaughter surveillance. Equipping these laboratories is underway utilizing the General Services Administration (GSA) excess property system with the completion goal set for December 31, 1989. The Cattle Diseases and Surveillance Staff which has Veterinary Services’ responsibility for this activity, has had much cooperation and support from Science and Technology Services, International Services, Agricultural Research Services, and GSA in this endeavor.

A major role for the United States is providing training and technical support for Mexico’s developing program. Both countries have agreed to appeal to appropriate governmental and international agencies for support. The Office of International Cooperation and Development (OICD) has provided funds for training for about 20 Mexican personnel in the United States since 1986, and continues its interest in this program. We are pleased to report that the Agency for International Development (AID) will include tuberculosis training for Mexican personnel under their technical assistance short term training program. Plans are in progress for three specific training projects for which AID will provide transportation costs, and OICD the subsistence costs.

The First priority has been given to training 6 laboratory personnel at National Veterinary Services Laboratories (NVSL) for a 3 week period in January 1990. The training will be in mycobacteriology (4 persons) and histopathology (2 persons) to provide tuberculosis diagnostic services for the Chihuahua and Mexico City laboratories. NVSL’s Diagnostic Bacteriology Laboratory and Pathobiology Laboratory have designed courses specifically for Mexico and have set time aside for this project.

The other training projects include: (1) cross training of a small group of
UNITED STATES – NEW MEXICO JOINT INITIATIVE

animal health personnel and cattle industry representatives of each country for a 2 week training period in the counterpart country for familiarization in the other's cattle industry procedures. There will be special regard to animal identification, including visits to grazing operations, feedlots, saleyards, slaughtering establishments, etc., and (2) data management training for three personnel in the Tuberculosis Information Management System, a stand alone microcomputer based system with major potential for tuberculosis program management at all stages of development. This will be at the National Center for Animal Health Information Services located in Fort Collins, Colorado.

Mexico has implemented a nationwide training program designed to include all veterinarians approved to conduct official tuberculin testing in Mexico; approximately 150 veterinarians. Four regional courses of 1 week each are in progress (Guadalajara, Veracruz, Torreon, and Merida) with 35-40 veterinarians attending each course. Three courses have been completed and the final course is scheduled for completion in November 1989. United States tuberculosis experts are participating in these training courses.

A project was completed during 1987-88 in response to a 1987 recommendation to develop an effective cattle identification method acceptable in lieu of the “M” brand required for all feeder steers entering the United States from Mexico. This project utilized a “passive transponder” the approximate size of a rice grain. The microchip was implanted in various locations of the head to make it recoverable at the time of visceral inspection at which time in most slaughtering establishments the hide and all other integument had been removed from the animal. The implants were made into feeder sized calves and removed at slaughter about 10 months later. It was found that injection of the microchip into the postorbital fossa gave 100 percent survival and recovery results with no apparent pain, adverse effects, or edible product adulteration. Though the implant has the disadvantage of being externally invisible, it gives the potential of providing accurate traceback identification in virtually all tuberculosis cases found in cattle from Mexico. With the exception of the tuberculin test itself, no single procedure could contribute more to providing a sound foundation for an effective tuberculosis eradication program.

We are pleased to report that the revised Guide for Bovine Tuberculosis Projects, PAHO’s Technical Note No. 15, was accepted by the Inter-American Meeting at the Ministerial Level, on Animal Health (RIMSA) at their meeting in Washington, D.C. on April 21, 1989. The revised publication is now published in Spanish and in English. The revision was a project of the United States Animal Health Association, multinational Subcommittee to Develop Guidelines for the International Control of Bovine Tuberculosis, chaired by Dr. Lowell R. Barnes. The revised guide is an important source of information for all countries developing programs for improved control leading to the eradication of bovine tuberculosis. It provides the best
prospect for blocks of countries achieving uniform procedures thereby reducing the potential for international movements of tuberculosis infected and exposed cattle.

The International Symposium on Tuberculosis and Paratuberculosis was held in Mexico City on November 15-17, 1988. The symposium was co-hosted by the United States and was the first symposium on bovine tuberculosis to be held in the western hemisphere in almost two decades. All sessions were very well attended by veterinarians, students, and laboratory personnel from Mexico, Canada, United States, Guatemala, Argentina, Venezuela, Cuba, Netherlands, Australia and other countries. We commend Mexico for its conception, planning, and successful culmination of the symposium.

Area testing results in Mexico and our own slaughter surveillance indicate that the prevalence of bovine tuberculosis is relatively low in the northern part of Mexico from which cattle are exported to the United States. United States slaughter surveillance alone could be sufficient basis for implementing an effective eradication program based on slaughter traceback and epidemiological case development of herds found infected. However, this would require that cattle identification problems be corrected and efforts be redoubled for the collection of official eartags from “M” branded cattle. Interest and concern prevail throughout animal health agencies, other involved governmental services, and the cattle industries of both countries for actively pursuing the goal of eradication. The recommendations forwarded are sound and the implementation plans are realistic. Lack of funds, however, severely deter the implementation process. It is unrealistic to believe that implementation of slaughter surveillance, epidemiological case development and a test and slaughter program can occur without at least modest funding. And no country has ever achieved meaningful progress in tuberculosis eradication without at least partial compensation of owners for animals destroyed because of tuberculosis.

Further, history shows that any country embarking upon a meaningful eradication program can expect to meet remarkable success shown by dramatic reduction of disease prevalence in the early years of the program. Three years of discussions and followup actions now require commitments by governments and industry for funding field activities, laboratory support and owner indemnity if a meaningful program is to be achieved.

We submit that in this joint effort lies the most realistic potential for rapidly reducing the prevalence of bovine tuberculosis in the northern States of Mexico to acceptable levels and eventually to the eradication of bovine tuberculosis from both countries.
STATUS OF THE STATE-FEDERAL
TUBERCULOSIS ERADICATION PROGRAM
FISCAL YEAR 1989
Ralph L. Hosker, D.V.M.
Hyattsville, MD

There was a total of 15 tuberculosis herds found during fiscal year (FY) 1989, a number that could seem discouraging after 3 years of declining infected herds nationally. Upon closer examination, however, it is apparent that this year as in FY 1988, only three newly infected herds were detected, with the remaining 12 either exposed herds or those carried over from FY 1988. The first new infected herd this year was in California and was located through the traceback of imported feedlot steers with lesions at slaughter. This small beef herd was apparently exposed by the imported steers that were on the same premises. A report of this case has been made to you. The second was a large dairy that had previously been detected during the FY 1985 outbreak near El Paso, Texas. It was again found to be infected when a cull cow sent to slaughter in April had lesions that were confirmed as tuberculosis by the laboratory. The last tuberculosis infection detected in FY 1989 was in a purebred accredited dairy herd located in Pennsylvania, an Accredited Free State.

Two infected herds, both dairies, were carried over from last fiscal year. One of these has since been depopulated and the other was released from quarantine in March.

A total of 10 exposed herds were reported in FY 1989, all of which were depopulated. Eight of these were identified during the FY 1988 North Dakota outbreak in the Cheyenne Grazing Association. The depopulation of these eight was deferred until funds became available in early FY 1989.

Two exposed herds were small beef cattle operations that were detected by a direct slaughter traceback of an affected bull. These operations were under the same management and mixing and exchange of animals had taken place.

Surveillance tissue submissions greatly increased in FY 1989, and a record number of slaughter traceback investigations of positive cases were conducted. The tuberculosis incentive awards program was amended on July 13, 1989, to increase the monetary award that United States Department of Agriculture (USDA) Meat Inspector personnel receive for the submission of suspicious lesions from adult animals. The secondary award that inspectors receive when an infected herd is located in the United States as a result of the lesions and identification they submitted was also increased. It is anticipated that these award changes will further increase slaughter submissions in the future.

The Tuberculosis Information Management System (TIMS) was installed in the Cattle Diseases & Surveillance Staff (CDSS) during the year to manage data generated by slaughter surveillance and resulting epidemiological investigations. This same program had previously been installed
in a number of States for use in stand alone computers. This system has proven extremely useful in handling information on the testing of infected and exposed herds and the tracing of cattle movements into and out of infected herds. TIMS may also be used by the field for maintaining accountability of animals in herd accreditation and by CDSS to periodically provide useful tuberculosis information for program management and for other purposes of concern to interested user groups.

In FY 1989, 615,087 head of cattle were imported from Mexico, almost all of which were steers used for rodeo, grazing and feeding purposes. This was a decrease from the record number 946,002 imported in FY 1988. This year, there were 2,828 suspicious tissue specimens submitted from feedlot cattle at slaughter, of which 270 were found positive for tuberculosis and investigated. The investigations completed in FY 1989 established that 62 percent of feedlot cases were steers of Mexican origin. This compares with the 1982-1988 average of 67 percent. Imported Mexican steers normally spend 6 to 18 months on pasture and on feed in the United States prior to being slaughtered. Additionally, a survey in March 1989, indicated that Mexican steers are used for rodeo sports in 31 States. Each of these situations provide an opportunity for infected Mexican steers to spread tuberculosis to domestic livestock. The best long-range solution to this exposure problem is to reduce or eradicate the disease in Mexico.

For the past 3 years, meetings on tuberculosis have been taking place between the United States and Mexico in response to 1986 USAHA resolution No. 36. The first meeting this year was held in El Paso, Texas, in April 1989 and was to further discuss mutual concerns, and to plan future actions to resolve the tuberculosis problems of the two countries. It included members of the USAHA, cattlemen of Mexico and the United States, Mexican and United States animal health personnel and representatives of other governmental agencies. A second meeting in Mexico City took place in September 1989. This was primarily a technical meeting that addressed the tuberculin production, skin testing procedures and testing interpretations of the two countries. Field trials were proposed to help clarify some of the issues discussed. As part of the joint initiative, Mexico with USDA,APHIS, VS, assistance, is planning to equip two laboratories to provide diagnostic capability for tuberculosis. Mexico has planned a national tuberculosis control program that will begin first in its six northern States and from there be expanded to encompass the whole country.

From 1980 through 1989, a total of 80 tuberculosis infected herds have been detected, 69 (86 percent) of which were depopulated. Of the 11 herds not depopulated, 1 was a beef herd and 10 were dairy herds. It is apparent that dairy herds owners have often chosen a test and slaughter program to free their herds of infection rather than employ whole herd depopulation. Many owners are concerned that the compensation they received for depopulation through salvage and indemnity will not permit them to replace their animals and return their herd to an economically sound
operational level. A decision not to depopulate is often unfortunate since historically, many herds on a test and slaughter program return to infected status in 2 to 5 years. Such reinfection has occurred during the last 2 years in two herds that were involved in the El Paso milk shed outbreak of 1985 and a third herd is currently under investigation. This emphasizes the wisdom of the program policy that gives first priority to depopulation in the handling of tuberculosis affected herds.

Figure 1 — In FY 1989, four States became Accredited Free. The total number of accredited free States at the years end was 41 plus the U.S. Virgin Islands. The newly accredited free States are: Oregon (1/12/89), Alabama (1/31/89), Florida (5/16/89) and Mississippi (6/30/89). One State, Idaho, has not had an infected herd in more than 5 years and could soon qualify for Free status. The Pennsylvania outbreak resulted in the temporary suspension of that State's free classification. Its operational status for interstate and export movements during the suspension period is that of a Modified Accredited State. If additional cases are not found, the State's Accredited Free status will be reinstated when the epidemiological investigation of the outbreak is completed.

Figure 2 — There was a total of 15 tuberculous herds (infected and exposed) in the United States in FY 1989. In North Dakota, eight exposed beef herds identified in FY 1988 were depopulated in FY 1989. Texas and Louisiana each carried over one infected herd from FY 1988. The herd in Texas has been released from quarantine and the herd in Louisiana was depopulated, both were dairies. Of the five tuberculous herds found in FY 1989, two were small beef herds in Texas that have been depopulated. The three new infected herds detected in FY 1989 were located in California (beef), Texas (dairy) and Pennsylvania (dairy). The dairy herd in Texas was not depopulated. The testing and depopulation of exposed animals from the Pennsylvania herd has not shown that there has been spread of the infection.

Figure 3 — This pie graph shows that the methods of locating the 15 tuberculous herds were: traceback from regular slaughter 4, high-risk herd test - 1, contact and adjacent herds - 9 and routine herd test - 1. The chart includes carry over herds, new herds, infected herds and exposed herds.

Figure 4 — This graph, similar to the proceeding, shows the methods by which 13 new herds, infected and exposed were located: traceback from regular slaughter - 3, (two infected and one exposed), routine herd test - 1, (one infected herd) and contact and adjacent herds - 9. This last section was made up of 8 exposed herds in North Dakota and 1 exposed herd in Texas.

In FY 1989, a total of 1,918,838 cattle were tested in 80,211 lots with a reactor rate of .02 percent. It is noteworthy that this year, as in FY 1981, 1982, 1983, 1985 and 1986, an infected herd was found during routine testing by an accredited veterinarian.

Figure 5 — This graph illustrates another way to evaluate the methods for detecting tuberculous herds under quarantine each year. It shows in FY
1989, 2 herds were initially located through tuberculin testing procedures. One was a follow-up high-risk herd test of a previously quarantined infected herd and the second resulted from an accreditation herd test. In the remaining, 13 herds epidemiological procedures were carried out prior to identification of the herd.

Figure 6 — This graph compares affected herds found vs. herds depopulated during recent years. The 13 depopulations carried out in FY 1989, included one infected dairy in Louisiana detected in the previous year. The depopulation percentage in FY 1989, was 87 percent compared to an average of 69 percent for all herds (infected and exposed) over a 10-year period.

Figure 7 — This map represents the proportions of herds depopulated in 5 States. The two herds not depopulated were large dairies in Texas.

Figure 8 — These bar graphs emphasize the value of animal identification in 253 regular slaughter traceback cases. There was a 2 percent success rate in 199 unidentified cases and 20 percent in 54 identified cases.

The low success in identified cases is due to the inclusion of feedlot steers with metal eartags, M brands and any other device or brand as part of the 54 identified cases. Metal eartag numbers applied for importation from 17 positive feedlot steers were reported to Mexico animal health authorities in FY 1989. Mexico reports that two infected herds have been located as a result of this information.

Figure 9 — This graph illustrates suspicious tissue submissions in FY 1989 by month from State and Federal slaughtering establishments. The FY 1989 total of 4,493 is almost double that of last year. The peak months for submissions closely compare with submissions over a period of years. The total includes 1,665 tissue submissions from adults and 2,828 from feedlot cattle.

Figure 10 — This figure shows that the remarkable increase in suspicious tissue submissions from regular slaughter that occurred in FY 1989. The 4,493 submissions, which includes 99 from State plants, was 89 percent greater than last year’s total. The number of cases of tuberculosis (281) was 6 percent greater than last year’s total. The positive cases included 11 adults and 270 feedlot cattle. The increase in submissions is the result of an all time high in imported feeders in FY 1988, the use of M branding as an external identification, the increased tuberculosis awards and, most significantly, closer liaison between meat inspection and animal health personnel.

The low level of tuberculosis in the United States is evidence that the tuberculosis program is based on sound epidemiological principles. The goal of USA Tuberculosis Free is realistic and, as the cumulative data of this and previous years show, we continue to move closer to the eradication of this complex disease. Final eradication, however, will require continued improvement and intensification of the basic program elements that have brought us to where we are today.
Tuberculosis Eradication

Bovine Tuberculosis Area Status

September 30, 1989

- Accredited Free States (41) Plus Virgin Islands
- Modified Accredited Areas (9) Plus Puerto Rico
- No M. Bovis for Over 5 Years (1)
Tuberculosis Eradication
Location of 15 Tuberculous Herds
FY 1989
Tuberculosis Eradication
Methods of Locating 15 Tuberculous Herds Under Surveillance During FY-89

- Traceback of Regular Kill Slaughter Animals (4)
- High Risk Herd Test (1)
- Routine Test of Herds (1)
- Contact/Adjacent Herd (9)
Tuberculosis Eradication

Methods of Locating 13 Newly Detected Tuberculous Herds During FY-89

- Traceback of Regular Kill Slaughter Animals (3)
- Routine Test of Herds (1)
- Contact/Adjacent Herd (9)
Tuberculosis Eradication

Tuberculosis Eradication

Herds Found vs. Herds Depopulated

FY 1980 - 1989
Tuberculosis Eradication

Proportion of Tuberculous Herds Depopulated

FY 1989

13 Herds Depopulated
15 Tuberculous Herds
Tuberculosis Eradication
Traceback of 253 Tuberculous Investigations
Closed (Regular Kill Animals) FY 1989

199 Unidentified
98% Unsuccessful

54 Identified
80% Unsuccessful
Tuberculosis Eradication

(State and Federal Establishments)

Number of 6-35's Submitted FY 89

Submitted

Number of 6-35's

Tuberculosis Eradication
Tuberculosis Traceback Investigations Submitted (Regular Kill) FY 1989

Cases Not Tuberculosis

Cases of Tuberculosis

* (State and Federal Submissions)

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(State and Federal Submissions)
REPORT OF THE COMMITTEE ON TUBERCULOSIS

Chairman: Dr. V. P. LaBranche, Boston, MA
Vice Chairman: Dr. T. J. Hagerty, St. Paul, MN

L. R. Barnes, IN; T. F. Conner, IN; M. A. Essey, MD; G. H. Frye, MD; D. E. Hensel, CO; B. R. Hillman, ID; E. M. Himes, TX; R. L. Hosker, MD; S. B. S. Hurley, WI; A. T. Kimmel, KS; C. L'Ecuyer, CAN; H. Lloyd, FL; C. W. McGinnis, NH; A. R. McLaughlin, WI; M. E. Oetting, MO; W. J. Owen, IA; J. B. Payeur, IA; J. O. Pearce, Jr., FL; N. Stirling, SD; C. D. Stumpff, KS; R. L. Tharp, MO; C. O. Thoen, IA; D. L. Thompson, CA; D. K. Thorpe, SD; R. Velure, ND; D. L. Whipple, IA; B. Widger, NY; E. Y. Williams, CO.

The meeting of the Tuberculosis Committee of the USAHA convened on November 1, 1989, at 1:30 P.M., at the Riviera Hotel in Las Vegas, Nevada. Fifty-eight guests and members were present.

Dr. Ralph Hosker, USAPHIS USDA, of Hyattsville, MD, was unable to attend. His report on the status of the state-federal tuberculosis eradication program for fiscal year 1989 was given by Dr. Granville Frye of the same department.

There were fifteen herds affected with tuberculosis, three new herds, with the others being exposed or carryovers from previous years. One newly infected herd was in California, infected by exposure to infected Mexican steers. The second was a large dairy that had been previously detected during the FY 1985 outbreak near El Paso, Texas. The last tuberculosis infection detected in FY 1989 was in a purebred accredited dairy herd located in Pennsylvania, an accredited Free State. A more detailed report will be included in the proceedings book.

Dr. Conrad L 'Ecuyer of Canada reported on the Bovine Tuberculosis Eradication status in Canada. Slaughter surveillance is the most important part of the program. One million nine hundred thousand cattle were slaughtered with one submission per thousand head slaughtered. Canada is divided into ten regions. Eight have been free of M. bovis for five years, the other two are nearing accreditation. There is also a program for game farms; testing for brucellosis and tuberculosis.

Dr. Mitchell Essey, USAPHIS USDA, spoke on the Mexico/United States Bovine Eradication Effort. There were two meetings of US and Mexican officials in 1989. One in El Paso, Texas, and one near Mexico City. The following recommendations were presented; 1. immediately offer training and technical support; 2. aid in development of laboratories in Mexico; 3. have key personnel from both countries meet regularly; 4. establish a committee for eradication of TB in Mexico; and 5. to explore means of identification, other than the 'M' brand which would be visible from several feet. A more detailed report will be included in the proceedings book.

Dr. Dennis Thompson of the Department of Food and Agriculture from Sacramento, CA, gave a very interesting report on the probable cause of
REPORT OF THE COMMITTEE

tuberculosis transmission from Mexican steers to native California cattle. A small herd of cattle consisting of 66 head had been in contact with several thousand Mexican steers that were processed or pastured on the ranch during the preceding four years. These were tested as a result of tuberculosis being detected in 3 steers at slaughter. This herd was tested and depopulated. An interesting report will be included in the proceedings book.

Dr. Lowell Barnes reported on the adoption and publication of International Recommendations for bovine tuberculosis eradication programs. He stated that International Tuberculosis Programs in the Americas are very important to achieving and maintaining the eradication of tuberculosis in the US.

The publication of the recommendations are important to assure availability of information and to serve as a valuable communication medium. These recommendations are the result of initial work done by the USAHA Tuberculosis Committee and its subcommittee, and represents the opinions of internationally recognized authorities on tuberculosis programs.

This document was accepted by USAHA in 1986, and adopted by the Pan American Health Organization at its VI Inter American Meeting on April 27, 1989.

Dr. Barnes summarized the most important points of the program which included providing a legal basis for the program, and developing an adequate staff and administration to carry out an effective program.

Dr. Barnes made the following motion. That the Tuberculosis Committee, through its chairman, Dr. Victor P. LaBranche, strongly recommends that Guide Lines for Preparation of Plans for Programs of Bovine Tuberculosis Eradication be published in the Annual Proceedings of the United States Animal Health Association for the year 1989.

The motion was seconded and unanimously passed.

Dr. Robert Velure, State Veterinarian, presented a summary and update of the 1988 tuberculosis outbreak in North Dakota.

Dr. Velure stated that between July 1, 1988, and December 2, 1988, 36,420 animals in 104 herds were tested. 99 herds were placed under quarantine. 333 Reactor animals were found. 7 lesioned animals, all in the index herd, were cultured positive for M. bovis. A total of 11 herds were depopulated. All had been exposed to the index herd. 4,934 animals were depopulated at a cost of $1,584,385.54.

Testing in adjacent herds and in the 10 herds that have repopulated, has resulted in no reactor animals.

North Dakota remains a tuberculosis free state.

Dr. Harold McCoy, APHIS area tuberculosis epidemiologist, gave a detailed report of an outbreak of tuberculosis in a Pennsylvania herd.

This herd had been a closed herd with a show string for several years. It
TUBERCULOSIS

was a purebred Guernsey herd consisting of one hundred twenty six head of which one hundred nine were declared as tuberculosis reactors. This case is still open, and we will hear more in 1990. A very interesting summary is included in the Proceedings.

A simplified system for Identification of Mycobacterium of veterinary interest in developing countries was outlined by Mr. Jerry Jarnigan of N.V.S.L., Iowa.

His report consisted of short cut laboratory procedures that would give the same results as our more modern methods do, in countries with less sophisticated laboratory equipment. A detailed report will be included in the Proceedings.

Dr. Philip Anderson of Idexx of Portland, Maine, reported on an in vitro test for the diagnosis of *M. bovis* infection in cattle. This is a serological test designed as an aid or supplemental test to diagnose tuberculosis. A detailed summary will be included in the Proceedings.

Preliminary evaluation of a Cell Wall Protein Antigen as a skin test for the diagnosis of Bovine tuberculosis. Dr. R. Dale Angus, N.V.S.L.

Dr. Angus discussed an insoluble cell wall protein extracted from *M. bovis*. The antigen was injected intradermally into guinea pigs and cattle either sensitized to or infected with *M. avium* or *M. bovis*. The cell wall antigen was more active per microgram than PPD tuberculin in both species. A detailed report will be included in the Proceedings.

Dr. Janet Payeur of National Veterinary Services Laboratories at Ames, Iowa, reported on a study on microchip implants for cattle identification at slaughter. Because of inadequate cattle identification at slaughter, and also with the advent of “rapid kill” slaughter establishments as few as only 2% of lesioned animals have been successfully traced. The microchip is a viable alternative to present identification methods and provides the possibility of much greater traceback capability.

Dr. Robert Meyer, epidemiologist from APHIS in the southeast region, discussed a tuberculosis training manual being reproduced for distribution to Food Safety and Inspection Service personnel. The manual contains information on how to recognize tuberculous lesions and their submission to National Veterinary Service Laboratory for diagnosis of tuberculosis. He also gave an update on the Tuberculosis Information Management System (TIMS), a microcomputer program to manage data from the tuberculosis eradication program. TIMS has been implemented in approximately half of the states, and at headquarters.

Discussion on an amendment proposed last year to change the UM&R was held. This would require a written agreement prior to formal quarantine release. It will stipulate that all herd members are to be inventoried and permanently identified. Further, that all of those identified shall be removed by slaughter prior to the final annual test. (Which would be the fifth annual test.)
REPORT OF THE COMMITTEE

Following discussion, a motion was made and the proposal defeated. A new amendment was proposed and tabled. It was to return to the use of a two fold test, in lieu of the single cervical test in affected herds. The meeting was adjourned at 5:30 P.M.

PRELIMINARY EVALUATION OF AN INTRADERMAL TUBERCULIN TEST UTILIZING CELL WALL PROTEINS EXTRACTED FROM MYCOBACTERIUM BOVIS

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National Veterinary Services Laboratories
Science and Technology
Animal and Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa 50010

INTRODUCTION

Few significant changes in the methods of tuberculosis diagnosis in domestic animals have been made since Koch produced the first tuberculin, now known as Old Tuberculin (OT). The first major advance was the use of all synthetic media\textsuperscript{4} in the 1920s and 1930s which the USDA adopted in 1934. The next major changes (1940s) were the preparation of Purified Protein Derivative (PPD) by either trichloracetic acid (TCA)\textsuperscript{5} or ammonium sulfate (AS)\textsuperscript{10} precipitation of the culture filtrate tuberculoprotein. The USDA in 1974 changed from \textit{M. tuberculosis} OT to PPD produced by AS precipitation utilizing the same \textit{M. bovis} and \textit{M. avium} strains as used in Europe.\textsuperscript{1,7}

The presently available tuberculins can have serious limitations relative to potency or sensitivity or specificity. Other types of tuberculin-active products may have significant benefit in supplementing the role of present products, especially in infected animals.

More recently, the emphasis has been to separate or extract various fractions from the different cellular components of the mycobacteria. These fractions are being used as the antigen in several diagnostic procedures including the intradermal skin test, the lymphoblastogenic response assay, and in numerous serologic test systems, especially enzyme-linked immunosorbent assay tests (ELISA).\textsuperscript{3,6,12}

This report provides information on preliminary tests conducted with two products of such an extraction process and one chemically synthesized peptide product.\textsuperscript{5,9}
Animals

Guinea pigs were Hartly strain weighing 500 to 700 gm when sensitized. Cattle were of predominantly Holstein and other mixed breed weighing from 400 to 1200 lbs. Control animals were neither sensitized nor infected with mycobacteria.

Sensitization

Both guinea pigs and cattle were sensitized by injection of 0.5 ml heat killed mycobacteria suspended in oil of *M. bovis* strain AN-5 (AN-5) or *M. avium* strain D-4 (D-4). The guinea pigs received intramuscular (IM) injections and the cattle received intradermal (ID) injections.

Infection

Eight cattle were infected by intravenous injection of 0.01 mg of viable *M. bovis* cells in a 5 ml volume prepared as described. Proof of injection was by observation of lesions at autopsy and isolation of *M. bovis* from the animals.

Tuberculin Tests

Intradermal (ID) injections (0.1 ml) were made in sensitized guinea pigs as outlined. Intradermal injections of cattle (0.1 ml) were made at 2, 3, 4, or 5 sites on both sides of the lateral cervical area using a Latin square design similar to methods described previously. The concentrations used in the standard guinea pig test (20 animals) were based on a preliminary guinea pig study in six animals. The initial concentrations of all products used in cattle were based upon the respective guinea pig studies. The later tests in cattle were based upon the results obtained in the initial cattle tests using the respective products. All measurements of tuberculin reactions were made independently by two individuals, and the results were taken as the average of the two readings. The guinea pig response was the diameter (mm) of erythema, and the cattle response was increase in skin thickness (mm).

Tuberculins

The reference *M. bovis* strain AN5 tuberculin was prepared for the USDA under commercial contract by the ammonium sulfate precipitation method. Two of the experimental products were produced by a slight modification of chemical extraction. Procedures reported by Melancon-Kaplan et. al. using the live cells from an *M. bovis* strain isolated at the National Veterinary Services Laboratories (NVSL) from a naturally infected cow. These two products, the cell wall protein (CWP) and the less refined cell wall protein-peptoglycan complex (CWP-PG), were obtained from P. J. Brennan, Colorado State University, Fort Collins, Colorado.

The fourth product was a thirteen amino acid synthetic peptide (SP) produced as described and which has been reported to have tuberculin-like activity when used in ID tests in sensitized guinea pigs. This product was
REPORT OF THE COMMITTEE

provided by Percy Minden, Research Institute of Scripps Clinic, La Jolla, California.

RESULTS

Guinea Pig Assays

The preliminary tests of CWP and CWP-PG in guinea pigs were done to establish appropriate dosage levels. Results are given in Table 1 and Graph 1. Standard Assay procedures for these products gave results as shown in Tables 2 and 3 and Graphs 2 and 3. Both CWP and CWP-PG appeared to give stronger tuberculin responses in *M. bovis* and *M. avium* sensitized guinea pigs than the reference PPD when based on the total protein content injected. However, reactions were not as strong in *M. avium* sensitized animals as the reactions at the same dosages in the *M. bovis* sensitized animals, and these relationships are shown in Graph 4.

Cattle Assays

These assays were very limited in animal number for all products tested. The initial amount of SP applied (4 sites/animal) was based upon published reports of response in guinea pigs. The dosage used in the second and third groups of animals (2 products at 2 sites/animal) was based on the results of the first SP cattle tests. The results are summarized in Tables 4, 5, and 6; however, the product in cattle was essentially non-reactive as a tuberculin even in very high doses.

The dosages in the first trial (2 products at 3 sites/animal) using CWP and CWR-PG were based upon the results of the guinea pig assays using the same relative ratios between guinea pigs and cattle as used when ID testing with standard PPD tuberculins. These data are given in Table 7 and Graph 5. The dosage used in the second cattle trial (3 products at 5 sites/animal) was based upon the results of the first CWP and CWP-PG cattle study.

DISCUSSION

The SP lacked sufficient tuberculin activity in cattle to be considered as a reasonable candidate for further investigation. The reported response of this product in guinea pigs sensitized with sonicates of mycobacterial cells may involve responses to antigens that are not adequately exposed to the immune system when sensitization is with whole killed cells or in infections with viable mycobacteria.

Both the CWP and CWP-PG showed tuberculin-like activity, both in guinea pigs and in cattle. However, the potency level per microgram of protein compared to the reference *M. bovis* PPD tuberculin was generally lower. The guinea pig tests indicate perhaps a better *M. bovis* to *M. avium* specificity than was evident in the cattle assays. In the second cattle trial, when additional products were evaluated together on each individual animal, even though the total protein load was the same, there was a marked decrease in the degree of reactions elicited by all the experimental
TUBERCULOSIS

products tested. The reason or reasons for this decline are not directly evident. There may have been an increased interference with the cell mediated response mechanism by these new products (CWP and CWP-PG) as compared to the products (CWP, CWP-PG, and SP) tested in the first cattle trial. Since one of the initial products (SP) did not demonstrate definite observable responses, the volume of immunologically active protein applied in the second study may have been an excessive volume for the immune system to handle, and this caused the decreased tuberculin response noted. This situation in cattle is analogous to the preliminary tests in guinea pigs resulting in decreased responses with increasing dosage as demonstrated in Table 1 and Graph 1.

One problem that is evident, and it may be a very serious and perhaps difficult one to solve, is the high degree of heterologous reactions that are seen in *M. bovis* sensitized and infected animals as compared to *M. avium* sensitized animals which occur with the CWP and CWP-PG. This is quite evident in Tables 2, 3, and 4 and Graphs 1 and 2. Additional investigations must be made to address the specificity and potency aspects of these new products, as well as products similarly produced from cells of *M. avium* or other mycobacteria.

SUMMARY

Protein fractions extracted from the cell wall of a *Mycobacterium bovis* field isolate have been shown to possess strong tuberculin-like activity in both guinea pigs and cattle. This occurs whether the products are applied to guinea pigs and cattle sensitized with heat-killed mycobacteria cells or are applied to experimentally *M. bovis* infected cattle. Data is given on the relative potency evaluation of these products in homologous and heterologous tuberculin test systems.

REFERENCES

REPORT OF THE COMMITTEE


Table 1. Response in *M. bovis* Sensitized Guinea Pigs when Tested with Various Doses of CWP and CWP-PG.

<table>
<thead>
<tr>
<th>Dose (microgm)</th>
<th>Mean Response</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CWP</td>
<td>CWP-PG</td>
<td>CWP-PG</td>
</tr>
<tr>
<td>0.1</td>
<td>11.51</td>
<td>18.42</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>5.44</td>
<td>12.06</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>4.82</td>
<td>7.95</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>2.63</td>
<td>6.74</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Response in *M. bovis* and *M. avium* Sensitized Guinea Pigs when Tested with CWP and CWP-PG.

<table>
<thead>
<tr>
<th>Dose (microgm)</th>
<th>Mean Response</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bovis</td>
<td>Avium</td>
<td>CWP-PG</td>
</tr>
<tr>
<td></td>
<td>CWP</td>
<td>CWP-PG</td>
<td></td>
</tr>
<tr>
<td>0.004</td>
<td>1.94</td>
<td>1.02</td>
<td>1.42</td>
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<td>0.011</td>
<td>4.57</td>
<td>4.01</td>
<td>2.26</td>
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<tr>
<td>0.033</td>
<td>4.49</td>
<td>4.53</td>
<td>1.79</td>
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<tr>
<td>0.100</td>
<td>8.39</td>
<td>8.45</td>
<td>6.15</td>
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Table 3. Response in *M. bovis* and *M. avium* Sensitized Guinea Pigs when Tested with *M. bovis* PPD.

<table>
<thead>
<tr>
<th>Dose (microgm)</th>
<th>Mean Response Bovis</th>
<th>Mean Response Avium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>10.84</td>
<td>4.03</td>
</tr>
<tr>
<td>1.2</td>
<td>11.44</td>
<td>4.57</td>
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<tr>
<td>2.4</td>
<td>13.40</td>
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</tr>
<tr>
<td>4.8</td>
<td>17.98</td>
<td>9.29</td>
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</table>

Table 4. Results of Initial Intradermal Testing With Synthetic Peptide and *M. bovis* PPD in 1 Control and 2 *M. bovis*-Sensitized Cattle 72 hours Post Injection. (4 Sites per animal).

<table>
<thead>
<tr>
<th>Increase in Skin Thickness (mm)</th>
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</thead>
<tbody>
<tr>
<td>Animal Status</td>
</tr>
<tr>
<td>Sensitized</td>
</tr>
<tr>
<td>Sensitized</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

Table 5. Results of Intradermal Testing with Synthetic Peptide and *M. bovis* PPD in Control, Sensitized, and Infected Cattle 72 hours Post Injection. (2 Sites per Animal).

<table>
<thead>
<tr>
<th>Increase in Skin Thickness (mm)</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
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<tr>
<td><em>M. bovis</em> infected</td>
</tr>
<tr>
<td><em>M. bovis</em> infected</td>
</tr>
<tr>
<td><em>M. bovis</em> infected</td>
</tr>
<tr>
<td><em>M. bovis</em> infected</td>
</tr>
<tr>
<td><em>M. bovis</em> sensitized</td>
</tr>
<tr>
<td><em>M. bovis</em> sensitized</td>
</tr>
<tr>
<td><em>M. bovis</em> sensitized</td>
</tr>
<tr>
<td><em>M. bovis</em> sensitized</td>
</tr>
<tr>
<td><em>M. avium</em> sensitized</td>
</tr>
<tr>
<td><em>M. avium</em> sensitized</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>None</td>
</tr>
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Table 6. Results of Intradermal Testing with Synthetic Peptide and *M. bouis* PPD in Control, *M. bouis* Sensitized, and Infected Cattle 72 Hours Post Injection. (3 sites per Animal).

<table>
<thead>
<tr>
<th>Animal Status</th>
<th>SP (12 mg)</th>
<th>PPD (0.1 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Sensitized</td>
<td>0</td>
<td>25.0</td>
</tr>
<tr>
<td>Infected</td>
<td>0.5</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Table 7. Results of Intradermal Testing with CWP, CWP-PG, and *M. bouis* PPD in Control, *M. avium* Sensitized, *M. bouis* Sensitized, and *M. bouis* Infected Cattle 72 Hours Post Injection.

<table>
<thead>
<tr>
<th>Animal Status (No. of Animals)</th>
<th>CWP 0.1 mg</th>
<th>CWP-PG 0.033 mg</th>
<th><em>M. bouis</em> PPD 0.1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (3)</td>
<td>0.5 (1)</td>
<td>0.5 (1)</td>
<td>0.5 (3)</td>
</tr>
<tr>
<td>Avian Sens. (5)</td>
<td>17.7 (3)</td>
<td>13.0 (3)</td>
<td>13.7 (2)</td>
</tr>
<tr>
<td>Bovine Sens. (5)</td>
<td>15.5 (2)</td>
<td>9.0 (2)</td>
<td>20.7 (2)</td>
</tr>
<tr>
<td>Bovine Inf. (4)</td>
<td>17.7 (2)</td>
<td>21.2 (2)</td>
<td>21.8 (4)</td>
</tr>
</tbody>
</table>

Table 8. Results of Intradermal Testing with CWP, CWP-PG, and *M. bouis* PPD in 1 Control and 4 *M. bouis* Infected Cattle.

<table>
<thead>
<tr>
<th>Animal Status</th>
<th>CWP 0.05 mg 0.02 mg</th>
<th>CWP-PG 0.05 mg 0.02 mg</th>
<th><em>M. bouis</em> PPD 0.1 mg</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-0.5</td>
<td>-1.0</td>
<td>-0.5</td>
</tr>
<tr>
<td>Infected</td>
<td>1.0</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Infected</td>
<td>5.5</td>
<td>6.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Infected</td>
<td>3.0</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Infected</td>
<td>11.5</td>
<td>8.5</td>
<td>18.0</td>
</tr>
<tr>
<td>Infected (avg.)</td>
<td>5.3</td>
<td>4.8</td>
<td>8.4</td>
</tr>
</tbody>
</table>
Graph 1. Response in *M. bovis* Sensitized Guinea Pigs when Tested with Various Doses of CWP and CWP-PG

Graph 2. Response in *M. bovis* Sensitized Guinea Pigs when Tested with CWP and CWP-PG
Graph 3. Response in *M. avium* Sensitized Guinea Pigs when Tested with CWP and CWP-PG

Graph 4. Response in *M. bovis* and *M. avium* Sensitized Guinea Pigs when Tested with CWP and CWP-PG
Graph 5. Response in Control, \textit{M. bovis} Sensitized \textit{M. avium} Sensitized, and \textit{M. bovis} Infected Cattle when Tested with SP, CWP, CWP-PG, and \textit{M. bovis} PPD

Graph 6. Response in Control and \textit{M. bovis} Infected Cattle when Tested with CWP, CWP-PG, and \textit{M. bovis} PPD
VACCINATION OF ELK IN WYOMING WITH REDUCED DOSE STRAIN 19 \textit{BRUCELLA:} CONTROLLED STUDIES AND BALLISTIC IMPLANT FIELD TRIALS.

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Box 3312, University Station, Laramie, WY  
H. A. Dawson  
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SUMMARY

The presence of brucellosis has been documented in 15 of 23 elk (\textit{Cervus elaphus}) herds supplementally fed during winter months in western Wyoming. Prevalence among adult females was 16-50\% on the five most extensively tested feedgrounds. Controlled studies of the effectiveness in elk of reduced dose strain 19 \textit{Brucella abortus} vaccine was tested and field trials of a ballistic implant delivery system were conducted. An airgun was used to propel a methylcellulose biobullet containing lyophilized vaccine, which implants in the muscle tissue, dissolves, and is absorbed. Five hand-held syringe and biobullet-delivered vaccine trials were conducted with challenge at six to 106 weeks postvaccination. No abortions or positive hemocultures were observed following vaccination and prior to challenge. Elk vaccinated with biobullets containing $5.6-7.6 \times 10^9$ cfu showed higher antibody titers than elk vaccinated by hand with $1.3-1.5 \times 10^8$ cfu; low dose ($3.7 \times 10^7$ cfu) biobullet vaccinates showed titers similar to hand vaccinates. This indicated that the effective dose of biobullet-delivered strain 19 was near the dose loaded into the biobullet. Reduced dose strain 19-induced titers were less persistent in cow and calf elk than those due to standard dose vaccine. Calving success following challenge was 23 (62\%) of 37 among vaccinates and was 3 (33\%) of 9 among nonvaccinates. At necropsy, 19 (45\%) of 42 vaccinates and 9 (69\%) of 13 controls were infected. Indices of infection of vaccinate groups were lower than their matched controls. In field trials, elk were marked with an airgun propelled, gelatin-encased paint ball and a biobullet was immediately implanted in the hind quarter. Cow and calf elk were marked and vaccinated from a feed sled or trailer as they fed on hay. Field trials were begun in 1984 and approximately 8,000 doses have been administered to elk on seven feedgrounds. An estimated 3,824 elk were vaccinated during winter 1989, including 88-100\% of calves and 71-91\% of cows on each feedground.

INTRODUCTION

Brucellosis is a bacterial disease of cattle caused by \textit{Brucella abortus}. Infection may result in 25 to 85 percent abortion among cows in a newly exposed herd (Bellver 1986). Brucellosis is readily transmitted to suscep-
VACCINATION OF ELK IN WYOMING

tible cattle by exposure to reproductive products or ingestion of material contaminated by reproductive products of an infected cow. The disease is the target of a nationwide cooperative eradication program begun in 1940 involving the U.S. Department of Agriculture, state departments of agriculture, and livestock producers. The eradication program has been successful in eliminating brucellosis from livestock in 27 states, including Wyoming and Montana (Anon. 1988). It is expected that Idaho will receive brucellosis-free status during late 1989 or early 1990.

Brucellosis is known to occur among approximately 22,000 elk in western Wyoming which are supplementally fed during winter months to reduce winter mortality and depredation on agricultural crops. Brucellosis was first recognized in elk of the supplementally fed herd at the National Elk Refuge in 1930 (Murie 1951). The infection has since been documented in elk at 15 of 23 state and federally operated feedgrounds. Prevalence of infection among adult females is approximately 40% at the National Elk Refuge and Horse Creek and Camp Creek feedgrounds near Jackson, 50% at Greys River Feedground near Alpine, and 16% at the Soda Lake Feedground north of Pinedale (Herriges et al. in press, Thorne et al. 1981a). Other herds in the state which are not supplementally fed are essentially free of brucellosis: none of 428 hunter-killed elk blood samples collected statewide in 1969 tested positive (Thorne 1970) and limited subsequent blood sampling of non-feedground elk has failed to reveal any reactors (unpublished data).

Research using captive elk at the Wyoming Game and Fish Department's Sybille Wildlife Research and Conservation Education Unit demonstrated that the disease resulted in 50-70% calf loss during the first pregnancy following infection, through abortion and birth of weak calves which died. Aborted fetuses, nonviable calves, fetal membranes, and fetal fluids were the primary sources of infection to susceptible animals. The disease was shown to readily transmit from elk to elk (Thorne et al. 1978) and from elk to cattle when confined together (Thorne et al. 1979).

Evaluation of standard dose *Brucella abortus* strain 19 vaccine in captive elk demonstrated the serologic response of elk to vaccination was similar to that in cattle and that calving success following challenge was higher among vaccinated elk than non-vaccinated controls (Thorne et al. 1981). A significant drawback to the vaccine was a strain 19-associated abortion rate, possibly as high as 27%. Though stress likely predisposed elk to abortion in these studies, strain 19-induced abortions have been documented in cattle vaccinated with a standard dose (Stableforth and Gal loway 1954). A reduced dose of strain 19 vaccine is now recommended for cattle and this paper will report on the effectiveness of reduced dose strain 19 in elk.

A system for remote delivery of vaccine was developed while reduced dose strain 19 trials were being conducted. BallistiVet, Inc. (White Bear Lake,
HERRIGES, THORNE, ANDERSON, DAWSON

MN) manufactured an airgun which fires a vaccine containing methylcellulose implant, termed a biobullet. A remote system of delivery was felt to be superior over a program of capture and vaccination with hand-held syringe. Therefore controlled studies of biobullet-delivered strain 19 vaccine in captive elk were initiated and, simultaneously, field trials begun. The objectives of these studies have been to determine the effectiveness of reduced dose strain 19 vaccine in elk and to determine the feasibility of biobullet delivery to free-ranging elk on feedgrounds, with the eventual goal being control of the disease in feedground elk. Control will result in increased calf production and reduced potential for transmission of the disease to domestic animals.

METHODS AND MATERIALS

Controlled Studies

Five trials were conducted at Sybille from 1981 through 1988. Elk were obtained by trapping on the National Elk Refuge in January, February, or March of the year they were vaccinated. Also, in 1981 a group was formed from elk already held at Sybille; and, in 1985, a group of elk captured from the National Elk Refuge was supplemented with elk calves born at Sybille. Freedom from brucellosis was determined by negative reactions to serologic tests on at least two samples collected over a 4 to 9 week period. Elk were vaccinated with either 5 ml of *B. abortus* strain 19 vaccine injected subcutaneously by syringe at the base of the neck or with a biobullet containing strain 19 vaccine ballistically implanted by airgun into the large muscle mass of the rear leg. Strain 19 vaccine was lyophilized in a lactose pellet and loaded into biobullets at National Veterinary Services Laboratory, Ames, Iowa. Some animals in each trial were not vaccinated to serve as controls. Dates of vaccination and challenge, vaccine dose, and number and age of elk in each trial are listed in Table 1. Vaccine doses ranged from $3.6 \times 10^7$ to $7.6 \times 10^9$ colony forming units (cfu) *B. abortus* strain 19. Vaccinated and non-vaccinated control elk were challenged with $7.5 \times 10^6$ cfu (1981-1987) or $4.5 \times 10^6$ cfu (1989) virulent *B. abortus* type 1 strain 2308 in 0.1 ml physiologic saline solution placed in the left conjunctival sac.

Calving success of vaccinated and control elk following challenge was determined. Fetuses of newborn calves lost to causes other than brucellosis or undetermined causes were not considered in calculating group calving success.

To measure serologic responses to vaccination and challenge, blood samples were obtained at frequent intervals from all elk and tested by the standard plate agglutination test (SPT), complement fixation test (CFT), buffered *Brucella* antigen rapid card test (BBA), and rivanol test (Riv). Standard procedures of the U. S. Department of Agriculture, Animal and Plant Health Inspection Service were used to conduct the SPT, BBA, and Riv tests (U. S. Department of Agriculture, not dated, a and b). The CFT was
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patterned after the microtiter technique for the diagnosis of bovine ana-plasmosis (Martin and Ritchie 1973).

Hemocultures were prepared from blood samples collected following vaccination in three of the five trials and following challenge in all trials to detect Brucella in circulating blood (Thorne et al. 1981). Bacteriologic cultures for Brucella were prepared from stomach content, spleen, liver, and lung of aborted fetuses and nonviable calves. Most challenged elk were necropsied; tissue collection and culture for Brucella followed procedures of Thorne et al. (1981).

Elk were considered to be infected with Brucella if the organism was recovered from any tissue. Each infected tissue was given a score of 1 to 4 based on the number of colonies produced on media plates, and an index of infection for each animal was calculated (Jones et al. 1964, Ghosh et al. 1968). The index of infection was calculated for each group according to Jones et al. (1964) where:

\[
\text{Index of infection} = \frac{\text{Total of scores recorded for all tissues of all animals within a group}}{\text{Total number of tissues cultured from group X 4 (maximum possible score per tissue)}} \times 100
\]

Field Trials

The biobullet airgun was powered by compressed air contained in a diving tank and regulated at 900-1200 pounds per square inch (psi). Ten biobullets were loaded in a plastic clip, sealed with cellophane, and placed in a plastic/foil pouch in which air was replaced with nitrogen. Biobullets used in field trials during 1986 and 1987 contained approximately $20 \times 10^9$ cfu B. abortus strain 19; in 1988 and 1989 the dose was approximately $7 \times 10^9$ cfu. Loaded biobullets were stored at $\leq 0^\circ \text{C}$ until immediately before use. The surface of the biobullet was rifled, as was the airgun barrel. Bullets were propelled at 800-925 feet per second (fps) and implanted in the large muscle mass of the rear leg. Some elk were shot in the neck during 1984-1986.

Gelatin-encased, oil-base paint balls (Nelson Paint Co., Iron Mountain, MI) were used to mark vaccinated elk. During 1984 one double-barrelled gun which shot paint balls as well as biobullets was used; only single-barrelled biobullet airguns were available beginning in 1987 and separate CO$_2$-powered paint ball pistols were used to mark elk (Nelson Paint Co., and Pursuit Marketing, Northbrook IL). In 1988, paint ball pistols were attached to biobullet rifles by means of a bracket and were converted from CO$_2$ to compressed air power.

Elk were first marked with a paint ball on the shoulder or chest and a
biobullet was immediately implanted ballistically in the hind quarter. All elk were consistently marked on the same side to facilitate future identification. Vaccination was conducted from a horse-drawn feed sled or tractor-drawn feed trailer. Feed was distributed around a stationary feed sled and elk vaccinated as they approached, or feed was distributed in lines and elk vaccinated as the tractor or sled drove back along the line of feeding elk. Adult and yearling females, as well as calves of both sexes, were vaccinated. Tally meters were used to record the numbers of doses administered which were later compared with the numbers of cows and calves counted on the feedgrounds by Wyoming Game and Fish biologists. Vaccination was conducted during January through early April. Elk feeders generally vaccinated elk for 1-1.5 hours, three days per week over a 2-6 week period.

Elk on some feedgrounds did not normally come onto the feedground in the presence of the feeder. To encourage them to do so, the feeder distributed hay late in the afternoon and waited at a distance. Once elk were accustomed to feeding in the presence of the feeder, he fed only near the stationary sled. When elk routinely approached the feed sled or allowed the feed sled to closely approach them, they were gradually accustomed to the sound of the airgun. A pneumatic (pump) air pistol with a soft report was dry fired and then the biobullet airgun itself was dry fired at gradually increasing volumes.

RESULTS AND DISCUSSION

Controlled Studies

Five reduced dose vaccination trials were conducted — three by hand injection and two by biobullet delivery (Table 1). Adult elk in two hand vaccination trials were challenged at short intervals (6 or 10 weeks postvaccination) to simulate elk vaccinated on a feedground in winter and exposed to infection in late winter or spring. Another hand-vaccination trial included elk vaccinated as calves and challenged two years (106 weeks) later as first calf heifers. Elk in two biobullet vaccination trials were challenged at one year (46 weeks) or two years (104 weeks) postvaccination. Trials are referenced by length of period from vaccination to challenge (Table 1).

No abortions or positive hemocultures for strain 19 were observed during the period following vaccination with reduced dose strain 19 and prior to challenge. In earlier trials of elk vaccinated with standard dose strain 19, as many as 27% may have experienced strain 19-associated abortions (Thorne et al. 1981). Stress associated with recent capture and captivity was believed to have predisposed these elk to strain 19-induced abortions. In the current studies, a reduced dose did not cause abortion in lightly or highly stressed elk. Elk challenged at 6 weeks postvaccination had been held at Sybille for 3 years and were considered lightly stressed by confinement and handling. Elk challenged at 10 weeks postvaccination were
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recently captured at the National Elk Refuge and were considered more highly stressed.

The serologic response of elk to vaccination with reduced dose strain 19 delivered by hand-held syringe or biobullet (Tables 2-5) was similar to that of the elk vaccinated with a standard dose by hand-held syringe (Thorne et al. 1981). All elk became seropositive following vaccination. Antibody titers of reduced dose vaccinates peaked at nearly the same levels as standard dose vaccinates but declined sooner and remained at lower levels for 20 to 104 months postvaccination.

Antibody response of biobullet vaccinates and hand vaccinates were very similar, though high dose (5.6-7.6 X 10^9 cfu) biobullet vaccinated elk had higher peak titers than hand vaccinates (1.3-1.5 X 10^9 cfu). In addition, low dose (3.7 X 10^7 cfu) biobullet vaccinates showed peak antibody titers similar to or higher than hand vaccinates. This was probably dose related and indicated that the effective dose of vaccine delivered by biobullet was very near that loaded into the biobullet. Doses loaded into biobullets were chosen based on the assumption that some mortality of B. abortus strain 19 organisms occurred (Angus in press). Theoretically, mortality might occur after the implant reaches body temperature but before the strain 19 organisms are absorbed into body fluids. However, mortality of lyophilized strain 19 stored at temperatures as high as 25°C for several weeks was slight (Angus in press). The serologic response observed in elk in these studies also indicated that mortality between implant and absorption is slight.

Low dose biobullet-vaccinates showed lower peak antibody titers than high dose biobullet vaccinates but did show similar titers at 20 to 46 weeks (Table 5). Elk vaccinated as calves showed peak antibody titers similar to elk vaccinated as adults but experienced lower subsequent titers. Antibody response to challenge by all elk was generally higher than the response to vaccination.

Reduced dose strain 19-induced titers were less persistent than those due to standard dose vaccine. With the sample obtained nearest February 20 (40-53 weeks postvaccination) among trials that extended one year or more postvaccination, 9 of 11 calves and 6 of 15 cows showed no reaction on any tests, 4 cows showed some reaction on one test but were considered negative by our criteria, and 2 calves and 5 cows were considered positive. At 98 or 101 weeks postvaccination, eight of ten elk vaccinated as calves and 3 of 4 vaccinated as cows showed no reaction on any tests, 1 cow was negative but showed some reaction, and 2 calves were positive.

Seventeen elk were eliminated from trials because of positive reactions on tests prior to vaccination or, among control elk, prior to challenge. Twelve non-vaccinated elk which were negative on at least one sampling date prior to mid-March seroconverted in March, April, or May, possibly indicating a seasonal variation in antibody titer related to developing
pregnancy. Antibody titers of some vaccinated elk also showed a small rise at this time of year. Two of 4 nonvaccinated control calves in the 106 weeks postvaccination study tested negative on all tests of samples collected from February, 1982 through at least late 1983. One seroconverted in November 1983 and the other in March 1984.

Overall calving success following challenge of reduced dose strain 19 vaccinates (Table 1) was equivalent to that of standard dose vaccinates (62%) and higher than nonvaccinated controls in reduced dose (33%) or standard dose (31%) trials (Thorne et al. 1981). Calving success was higher among hand-vaccinates (72%) than biobullet-vaccinates (42%), but there were only 12 biobullet vaccinated elk and only one nonvaccinated elk to serve as a matched control (Table 1). Small sample sizes made comparisons of calving success between individual trials difficult. Sample sizes were reduced by excluding newborn calves or fetuses lost to undetermined causes or to causes other than brucellosis (4 in vaccinate groups, 2 in control groups) and by eliminating elk which seroconverted prior to vaccination or challenge.

At necropsy, number of elk infected, number of tissues infected per infected elk, and index of infection were generally lower in vaccinated than matched nonvaccinated controls (Table 6). Overall, 19 (45%) of 42 vaccinates and 9 (69%) of 13 controls were infected at necropsy. Among biobullet-vaccinates, 9 (56%) of 16 were infected at necropsy and the one matched control was also infected. The average bacteriologic score of infected tissues varied little between vaccinate and control groups or between trials.

These studies indicated that reduced dose strain 19 provided protection from abortion and, to a lesser degree, protection against infection. Both are important in controlling the disease in elk, though a reduction in abortions alone among vaccinated elk on feedgrounds would decrease the opportunity for transmission to susceptible elk and livestock.

Field Trials

Initial field trials of biobullet vaccination were conducted on the Greys River Feedground near Alpine, Wyoming during winter 1984; 122 elk were implanted with placebo biobullets. In 1985, strain 19 loaded biobullets were used and, though detailed records were not kept, an estimated 70% of cow and calf elk were vaccinated (Table 7). (Subsequent experience indicated that the proportion of calves shot was probably higher than 70% and the proportion of cows lower.) During 1986, vaccination was again conducted at Greys River with a double-barrelled biobullet and paintball gun and initiated at Green River Lakes Feedground using a biobullet airgun and a separate CO₂-powered paint ball pistol. Vaccination was repeated at these two feedgrounds during 1987 using separate paint ball guns at both feedgrounds.

During these first three years of field trials, efficiency and effectiveness...
were limited by the paint ball delivery system and because vaccination was attempted in late-winter or early spring when elk were less dependent on supplemental feed. The effective range of paint balls fired by CO$_2$-powered pistols was short and decreased with decreasing temperatures. The use of two separate guns made it necessary for two operators to be present or decreased the shooting efficiency of one operator. Beginning in winter 1988, paint ball guns attached to biobullet airguns and powered by compressed air were used. However, problems with accuracy of biobullet airguns delayed initiation of vaccination until late February at Greys River and early March at Forest Park and Green River Lakes feedgrounds and, therefore, limited the number of elk which were vaccinated. Numbers of elk vaccinated in 1989, a winter of near-normal snowfall, were probably more representative of future vaccination efficiency.

Approximately 3,825 elk were vaccinated on seven feedgrounds during winter 1988-89. Proportions of elk estimated to have been vaccinated ranged from 71% to 91% of adult and yearling females on each feedground and 88-100% of calves. The number of doses administered to calves on a feedground often exceeded the number of calves counted on that feedground, indicating that some calves were vaccinated twice; probably as a result of lost or inapparent paint marks. Where this occurred, the difference was used to estimate the amount of revaccination. For example, 280 calves were counted at Greys River Feedground in 1988 but 317 doses were administered to calves. Therefore, at least 37 doses (12%) were revaccinations. Applying this same proportion to cows, an estimated 413 cows were vaccinated rather than 469, the number of doses administered to cows. In some cases, part of the differences between number of doses administered and number of calves on a feedground may have resulted from movement of elk on or off the feedground in the period between classification counts and completion of vaccination or from misidentification of cows and calves.

Paint marks were lost over time through fading, rubbing, or shedding; and, therefore, the amount of revaccination which occurred was probably related most closely to the length of the vaccination period, which generally was 2-6 weeks. Vaccination occurred over a 6-week period at Greys River Feedground in 1988 and revaccination of calves was estimated at 12%. In cases where the number of doses administered did not exceed the number of calves counted on a feedground, a weekly revaccination rate of 2% was assumed in estimating the proportion of cows and calves vaccinated (Table 7).

Vaccination was conducted for the fifth year during 1989 at Greys River Feedground. Only calves were vaccinated because all adult females that were likely to come within range of the biobullet airgun would probably have been vaccinated in at least one of the previous four years. In addition, limiting vaccination to calves would reduce problems with retained vaccinal titers when sero-tested in future years. Also, calves were easier to vaccinate because they were more likely to approach within airgun range,
which allowed all calves to be vaccinated more rapidly than adults. This factor, combined with lower numbers of calves in the population, made vaccination of calves on Greys River Feedground possible in seven days of work.

Vaccination of elk on the National Elk Refuge was tested during winter 1989, and was conducted at only one of four feeding sites and included only calves. Elk numbers on this feeding site varied from about 2,000 to 3,500 elk (21-37% of the herd) during the 2-week vaccination period. Approximately 29% of the calves on the Refuge were vaccinated at this site, indicating that most calves present on this feedground were vaccinated.

Only one documented mortality due to biobullet-induced injury has occurred — a calf which was hit in the lungs in 1986. Seven calf mortalities suspected to be due to biobullet injuries occurred in winter 1989. Three showed wounds that were possibly due to biobullet injury, and lung tissue from another showed blood within the alveoli and bronchioles indicating blood aspiration from a possible head or neck wound. The other three reported mortalities were observations of paint-marked calves found dead with no obvious cause of death. These were difficult to evaluate because some mortality of calves occurs on most feedgrounds in most winters, and most calves will have been marked within a few weeks of the initiation of vaccination. Some mortality due to biobullets was expected given the limited accuracy of the system, the crowded conditions on the feedgrounds, and the necessity of occasionally vaccinating elk which were moving after being hit by the paint ball. If all seven of the above-mentioned mortalities were due to biobullet injury, less than 0.2% of the estimated number of elk vaccinated in 1989 were killed. Low mortality due to biobullets should be acceptable if the program is successful in preventing far greater mortality due to brucellosis.

Prior to initiation of vaccination on a feedground, elk were habituated to human activity and to the report of the airgun. This required from 2 weeks at several feedgrounds to 1.5 seasons at Forest Park Feedground. Elk at Forest Park were originally thought to be too timid for successful biobullet vaccination so a program of trapping and hand-vaccination was tested. It was very labor intensive and, for a variety of reasons, only marginally successful (77 elk vaccinated during two winters). Consequently, we decided to attempt to use the biobullet system which was then being used on two other feedgrounds. During the 1987 and 1988 feeding seasons, elk were encouraged to closely approach the sled by feeding only around the sled, and they were acclimated to the report of the airgun by repeated dry-firing. Vaccination was initiated late in the second season and a high proportion of cow and calf elk were vaccinated (Table 7).

Elk at the Jewett Feedground rarely approached the feedground in the presence of the feeder, normally travelling onto the feedground in late afternoon after the feeder departed. During winter 1987, feeding was often
delayed until late afternoon and the feeder remained on the feed sled on the feedground. By the end of the season most elk were travelling onto the feedground in the presence of the feeder; some were within 50 meters of the feed sled. Experience on these two feedgrounds indicated that biobullet vaccine delivery is feasible on all feedgrounds, including the few feedgrounds with relatively timid elk.

On feedgrounds where elk were accustomed to the activities of the feeder, elk were acclimated to the report of the airgun and to closer approach by the feed sled for 2-8 weeks before vaccination began. On feedgrounds where elk had been vaccinated the previous year, little or no acclimation to vaccination activities was necessary unless new draft horses were used or elk were present which had apparently not been on the feedground the previous year.

Success of the program will be measured in the short term by the number and proportion of elk vaccinated, thus relying on controlled vaccination studies to demonstrate that those immunizations were effective. Ultimately, changes in calf production and sero-prevalence will be used to evaluate effectiveness of the program. Increases in calf:cow ratios, however, are expected to be small relative to the annual variation in these ratios. For instance, the number of calves per 100 cows at Greys River Feedground between 1982 and 1989 has varied between 27.3 and 45.7 (mean=37.5, s=7.8) and was only 16.5 in 1981. We have estimated that one out of every eight calves are lost to brucellosis in highly infected herds such as Greys River.

The prevalence of infection, as measured by serology, also may be inconclusive in the short term due to probable retention of vaccinal titers by cows, especially those which may have been vaccinated in successive years. Vaccination of only calves in the near future should reduce this problem.

Lack of an immediate quantitative measure of the effectiveness of the vaccine administered under field conditions should not delay expansion of the program. Rather, controlled studies should be relied upon to demonstrate vaccine effectiveness, and field trials continued until long-term measures of success are available.

ACKNOWLEDGEMENTS

Appreciation is expressed to Carl Engstrom, Brad Meyer and others of the Sybille Wildlife Research and Conservation Education Unit who daily cared for research animals and assisted in all handling and sampling procedures. Many Wyoming Game and Fish elk feeders vaccinated elk via biobullet. We sincerely appreciate their interest and participation, which made the field studies successful. Game Division District 1 and Habitat and Technical Services Division personnel assisted greatly in elk trapping and
blood sampling. Dr. Doug Woody, APHIS, USDA, assisted in blood sampling and testing at trap sites. Personnel of the National Elk Refuge assisted in trapping of elk and in acclimation and vaccination of elk. Steve and LuAnn Kilpatrick assisted in many ways. Fred Paul, Terry McDermott, and Marty Chevalier of Ballistivet, Inc., provided technical assistance, and Dr. Dale Angus, NVSL, APHIS, loaded biobullets and monitored vaccine viability. Drs. Granville Frye and John Kopec, Veterinary Services, APHIS gave administrative assistance. The assistance of Ella Nelson, Veterinary Services, Wyoming State Veterinary Laboratory, who conducted laboratory serologic tests, and all of those named above was greatly appreciated. Funding for this project was provided by Veterinary Services, APHIS, USDA; U.S. Fish and Wildlife Service, USDI; and Wyoming Game and Fish Department under Cooperative Agreement No. 12-16-028.

Table 1. Calving success of reduced dose *Brucella abortus* strain 19 vaccinated and nonvaccinated elk following challenge with *B. abortus* type 1 strain 2308.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Age at vaccination</th>
<th>Vaccine dose</th>
<th>Vaccination date</th>
<th>Challenge date</th>
<th>Calving success ¹ (a/b)</th>
<th>Vaccinates</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand Vaccinates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 weeks Adult</td>
<td>1.34 x 10⁹ cfu</td>
<td>5-Mar-81</td>
<td>12-May-81</td>
<td>5/5</td>
<td>1/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks Adult</td>
<td>1.46 x 10⁹ cfu</td>
<td>2-Mar-82</td>
<td>13-Apr-82</td>
<td>9/12</td>
<td>2/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>106 weeks Calf</td>
<td>1.46 x 10⁹ cfu</td>
<td>2-Mar-82</td>
<td>14-Mar-84</td>
<td>4/8</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biobullet Vaccinates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104 weeks Adult</td>
<td>7.6 x 10⁹ cfu</td>
<td>21-Mar-85</td>
<td>26-Mar-87</td>
<td>2/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>7.6 x 10⁹ cfu</td>
<td>21-Mar-85</td>
<td>26-Mar-87</td>
<td>0/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 weeks Cow</td>
<td>3.7 x 10⁷ cfu</td>
<td>5-May-88</td>
<td>23-Mar-89</td>
<td>2/5</td>
<td>0/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>5.6 x 10⁹ cfu</td>
<td>5-May-88</td>
<td>23-Mar-89</td>
<td>1/4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Calving success: Number of viable calves (a), potential calves (b); calves lost to undetermined causes or to causes other than brucellosis were disregarded (4 in vaccinate groups, 2 in control groups). Eight elk seropositive before vaccination and 9 non-vaccinated elk seropositive before challenge were excluded.

²Two cows which were seropositive before challenge were included.
**Table 2.** Serologic response of adult female elk vaccinated with *Brucella abortus* strain 19 and challenged with *B. abortus* type 1 strain 2308 at six or 10 weeks postvaccination.

<table>
<thead>
<tr>
<th>Weeks post-vaccination</th>
<th>Challenged at 10 weeks&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Challenged at 6 weeks&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPT&lt;sup&gt;3&lt;/sup&gt;</td>
<td>BBA&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>83</td>
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<tr>
<td>6&lt;sup&gt;5&lt;/sup&gt;</td>
<td>23</td>
<td>67</td>
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<tr>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>17-18</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>36-42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Five adult females in group.

<sup>2</sup>Twelve adult females in group.

<sup>3</sup>SPT=Standard plate test, Riv=rivanol test, CFT=complement fixation test; geometric mean antibody titer, expressed as the reciprocal of a serum dilution.

<sup>4</sup>BBA=buffered *Brucella* antigen rapid card, expressed as the percent producing a positive reaction.

<sup>5</sup>Sample obtained prior to challenge.

**Table 3.** Serologic response of eight female elk vaccinated as 7- to 9-month old calves with reduced dose *Brucella abortus* strain 19 and challenged with *B. abortus* type 1 strain 2308 at 106 weeks postvaccination.

<table>
<thead>
<tr>
<th>Weeks post-vaccination</th>
<th>Standard plate&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Rapid card&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Rivanol&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Complement fixation&lt;sup&gt;1&lt;/sup&gt;</th>
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<tr>
<td>0</td>
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<td>0</td>
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<td>11</td>
<td>36</td>
<td>63</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>16</td>
<td>22</td>
<td>75</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>53</td>
<td>3</td>
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<td>0</td>
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<tr>
<td>73</td>
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<td>79</td>
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<td>2</td>
</tr>
<tr>
<td>101</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>106&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>107</td>
<td>129</td>
<td>88</td>
<td>41</td>
<td>90</td>
</tr>
<tr>
<td>125</td>
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<td>100</td>
<td>141</td>
<td>384</td>
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<tr>
<td>128</td>
<td>129</td>
<td>129</td>
<td>129</td>
<td>325</td>
</tr>
</tbody>
</table>

<sup>1</sup>Geometric mean antibody titer, expressed as the reciprocal of a serum dilution.

<sup>2</sup>Expressed as the percent producing a positive reaction.

<sup>3</sup>Blood sample obtained before challenge dose administered in week 106.
Table 4. Serologic response of female elk vaccinated as adults or 8-10 month old calves vaccinated with biobullet delivered reduced dose *Brucella abortus* strain 19 and challenged with *B. abortus* type 1 strain 2308 at 104 weeks postvaccination.

<table>
<thead>
<tr>
<th>Weeks post-vaccination</th>
<th>Number tested</th>
<th>Standard plate</th>
<th>Rapid card</th>
<th>Rivanol</th>
<th>Complement fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cow calf</td>
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<td>cow calf</td>
<td>cow calf</td>
</tr>
<tr>
<td>0</td>
<td>5 3</td>
<td>2 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 5</td>
</tr>
<tr>
<td>5</td>
<td>5 3</td>
<td>115 141</td>
<td>60 100</td>
<td>76 200</td>
<td>44 66</td>
</tr>
<tr>
<td>7</td>
<td>5 3</td>
<td>87 126</td>
<td>80 100</td>
<td>87 158</td>
<td>42 43</td>
</tr>
<tr>
<td>9</td>
<td>5 3</td>
<td>12 100</td>
<td>60 100</td>
<td>11 126</td>
<td>22 36</td>
</tr>
<tr>
<td>11</td>
<td>5 3</td>
<td>5 17</td>
<td>60 100</td>
<td>4 22</td>
<td>15 87</td>
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<td>13</td>
<td>5 3</td>
<td>20 50</td>
<td>60 66</td>
<td>2 9</td>
<td>2 5</td>
</tr>
<tr>
<td>18</td>
<td>5 3</td>
<td>2 3</td>
<td>20 33</td>
<td>2 3</td>
<td>2 0</td>
</tr>
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<td>22</td>
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<td>2 0</td>
<td>20 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>27</td>
<td>5 3</td>
<td>7 0</td>
<td>20 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>36</td>
<td>4 3</td>
<td>2 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>46</td>
<td>4 3</td>
<td>5 0</td>
<td>25 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>57</td>
<td>4 3</td>
<td>16 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>64</td>
<td>4 3</td>
<td>42 5</td>
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<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>74</td>
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<td>25 0</td>
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<td>0 0</td>
<td>0 0</td>
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</tr>
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<td>60 71</td>
<td>50 50</td>
<td>8 10</td>
<td>38 32</td>
</tr>
<tr>
<td>109</td>
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<td>100 100</td>
<td>44 100</td>
<td>166 230</td>
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<tr>
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<td>168 200</td>
<td>100 100</td>
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<td>230 324</td>
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<td>75 100</td>
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<td>141 322</td>
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<td>18 100</td>
<td>75 100</td>
<td>120 200</td>
<td>274 230</td>
</tr>
</tbody>
</table>

<sup>1</sup>Geometric mean antibody titer, expressed as the reciprocal of a serum dilution.

<sup>2</sup>Expressed as the percent producing a positive reaction.

<sup>3</sup>Group included one male through week 64.

<sup>4</sup>Blood sample taken before challenge dose administered in week 104.
### Table 5. Serologic response of adult female elk vaccinated via biobullet with one of two reduced doses of *Brucella abortus* strain 19 and challenged with *B. abortus* strain 2308 at 46 weeks postvaccination.

<table>
<thead>
<tr>
<th>Weeks post-vaccination</th>
<th>Number tested</th>
<th>Standard plate</th>
<th>Rapid card</th>
<th>Complement fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low¹ High² Low High</td>
<td>Low High</td>
<td>Low High</td>
<td>Low High</td>
</tr>
<tr>
<td>0</td>
<td>7  6</td>
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<td>0  0</td>
<td>0  0</td>
</tr>
<tr>
<td>2</td>
<td>7  5</td>
<td>94 200</td>
<td>86 100</td>
<td>77 200</td>
</tr>
<tr>
<td>7</td>
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<td>77 200</td>
<td>86 100</td>
<td>70 200</td>
</tr>
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<td>7  5</td>
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</tr>
<tr>
<td>17</td>
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<td>66 40</td>
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</tr>
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<td>19</td>
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<td>23 11</td>
<td>50 40</td>
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</tr>
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<tr>
<td>48</td>
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<td>4  33</td>
<td>60 80</td>
<td>3  5</td>
</tr>
<tr>
<td>50</td>
<td>5  5</td>
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<td>80 100</td>
<td>37 174</td>
</tr>
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<td>62</td>
<td>5  5</td>
<td>23 87</td>
<td>100 100</td>
<td>152 200</td>
</tr>
</tbody>
</table>

¹Geometric mean antibody titer, expressed as the reciprocal of a serum dilution.

²Expressed as the percent producing a positive reaction.

³Low dose: 3.7 X 10⁷ cfu; High dose 5.6 X 10⁹ cfu.

⁴Blood sample taken before challenge dose administered in week 46.
Table 6. Bacteriologic results and indices of infection at necropsy of reduced dose *Brucella abortus* vaccinated and nonvaccinated female elk challenged with *B. abortus* type 1 strain 2308.

<table>
<thead>
<tr>
<th></th>
<th>Hemoculture Number</th>
<th>Average Weeks post</th>
<th>Number of infected</th>
<th>Tissue infected at necropsy Avg</th>
<th>Tissue infected elk Range</th>
<th>Average infected tissue bacteriologic score</th>
<th>Group index of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number sampled</td>
<td>necropsy</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vaccinate</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>26-42</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>30-33</td>
<td>2</td>
<td>11</td>
<td>1-21</td>
</tr>
<tr>
<td>6 weeks</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vaccinate</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>27-41</td>
<td>7</td>
<td>4.0</td>
<td>1-10</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>27-42</td>
<td>4</td>
<td>7.5</td>
<td>1-14</td>
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<td>104 weeks</td>
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<tr>
<td>Vaccinate</td>
<td>8</td>
<td>4</td>
<td>2.4</td>
<td>28-41</td>
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<td>4.0</td>
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<tr>
<td>Control</td>
<td>4</td>
<td>2</td>
<td>2.0</td>
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<td>7-11</td>
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<td>Vaccinate</td>
<td>4</td>
<td>1</td>
<td>2.8</td>
<td>24-28</td>
<td>1</td>
<td>9</td>
<td>1.9</td>
</tr>
<tr>
<td>Calf</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>24-28</td>
<td>1</td>
<td>3</td>
<td>2.3</td>
</tr>
<tr>
<td>106 weeks</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vaccinate</td>
<td>5</td>
<td>3</td>
<td>9.4</td>
<td>22-29</td>
<td>4</td>
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<td>9.4</td>
<td>23-27</td>
<td>3</td>
<td>2.0</td>
<td>1-6</td>
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<tr>
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<td>1</td>
<td>1</td>
<td>8</td>
<td>22</td>
<td>1</td>
<td>1.0</td>
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**LITERATURE CITED**


VACCINATION OF ELK IN WYOMING


PREPARATION, DOSAGE DELIVERY, AND STABILITY OF A
BRUCELLA ABORTUS STRAIN 19 VACCINE
BALLISTIC IMPLANT

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National Veterinary Services Laboratories
Science and Technology
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Ames, IA 50010

INTRODUCTION

The National Brucellosis Eradication Program has made considerable progress in recent years. Most states of the United States have achieved Certified Brucellosis Free or Class A status for the domestic cattle population. In Wyoming, however, there are foci of Brucella abortus infection in certain free-ranging elk and bison herds. It has been shown that elk respond to B. abortus exposure and infection like cattle. Vaccination of cattle, elk, and bison with strain 19 can be expected to reduce the level of herd infection. If vaccination of free-ranging elk and bison could be accomplished effectively, the exposure potential for both wildlife and domestic livestock would be reduced and the progress toward brucellosis eradication would be preserved.

The development of a ballistic implant delivery system has provided a potential method for vaccinating animals without the use of physical or chemical restraint. This system is now being utilized to vaccinate free-ranging elk and bison for brucellosis.

MATERIALS AND METHODS

Preliminary work involved lyophilization of a dose of strain 19 vaccine directly into the cavity of the ballistic implant and storage under vacuum in a metal foil bag. This procedure did not give the desired vaccine stability, and the lyophilized cake of vaccine sometimes did not adequately withstand the stresses induced by the firing, flight, and impact of the implant. Studies showed that vaccine in a lactose-based pellet should be a suitable preparation. The addition of 2-5% magnesium stearate to the lactose base is necessary for proper die and pellet lubrication during the pellet-forming operation. Increasing the percentage of lyophilized vaccine in the pellet increases the amount of stearate required.

Ballistic Vaccine Stock

Brucella abortus strain 19 lyophilized vaccine was prepared from fermentor-grown organisms as previously outlined. The vaccine bulk liquid was dispensed in 30 ml vials with a fill volume of 10 ml; the vials also contained 6-8 glass beads of 4-5 mm diameter. When lyophilization was
BRUCELLA ABORTUS STRAIN 19 VACCINE

complete, the vials were sealed under nitrogen gas. The vaccine was stored at 4°C, while viability, sterility, lactose dilution, yield, pellet weight, etc. were determined, until it was used to make the implants. The vials were vigorously hand shaken to break the lyophilized cake to a relatively fine powder. Mechanical shaking produced an excessively fine powder that adhered to the glass vial and rubber stopper. Lactose containing 2-3% magnesium stearate was added to each vial in sufficient quantity to dilute the vaccine powder to the desired number of viable organisms and mixed well. The powdered vaccine was then dispensed to a pellet press and formed into pellets 4 mm in diameter and 4-6 mm in length and weighing from 50-85 mg. The pellet length and weight varied according to the amount of lactose required to dilute the vaccine. Each lot of pellets had only small variations of individual weights from the lot mean, and successive serials were very consistent for weights and viability levels. The pellet was inserted into the cavity of a biodegradable ballistic implant, and the cavity edge was crimped to hold the pellet in place. The implants were placed in a plastic rifle clip and held in place with plastic heat seal tape. The clip was placed in a gas-impervious metal foil bag which was first evacuated, then purged with nitrogen gas, and heat sealed while retaining the nitrogen. The bagged vaccine was then maintained at -20°C until used for the vaccination of animals.

Liquid Vaccine

The vaccine was produced on potato infusion agar as outlined and stored at 4°C until used for animal vaccination at the target dose of 1 x 10⁹ viable organisms per 2 ml. The viability was determined both before and after the vaccine was applied to the animals.

Animals

The cattle were about 24-30 months of age and of basically Holstein dairy type; most were 5-6 months pregnant. They were randomly assigned to either the implant group (10 animals) or the liquid vaccine group (6 animals).

Serology

Tests were conducted as outlined for the following tests: standard plate, standard tube, buffered plate, card, rivanol, and complement fixation.

Vaccine Viability Determination

These were conducted as outlined using peptone-saline and tryptose agar plates. Implants were allowed to thoroughly dissolve in the initial dilution bottle of peptone-saline before further dilutions were made. If any delay occurred, the dissolving step was made at 4°C.

Vaccine Stability Evaluation

Aliquots of different vaccine lots were stored at 37°C, 23°C, 4°C, or -20°C, and viability was determined after various time periods appropriate for the expected decline at each temperature.
ANGUS

RESULTS

Animal Vaccination

All six cattle vaccinated with liquid vaccine gave similar serologic responses which peaked at 2-3 weeks and receded to lower levels by 14 weeks post-vaccination. Of the ten animals vaccinated by ballistic implant, seven responded serologically, and these seven animals had very similar serologic responses. This was the first use of a ballistic system gun at the National Veterinary Services Laboratories (NVSL) and the experience of others has shown that gun accuracy is a direct function of familiarity. It was felt that the three non-responding animals were not properly implanted, and they were excluded from the study. The average responses of animals in both groups were almost identical. Detailed serologic data for both groups are given in Table 1 and Graphs 1–6.

DISCUSSION

Delivered Dose

The delivered dose was reasonably even among the animals of the implant group as evidenced by the serologic response to vaccination. The effective delivered implant dose appeared to be about 5% of the total viable organisms originally present in the implant, based upon the relative serologic response between the implant and liquid vaccine groups. This demonstrated that the vaccine dose can be adjusted to reasonably close tolerances, as the preparation of the implant itself is very reproducible (Table 2). Since viability is quite stable over time, especially when stored at low temperatures, the vaccine is very convenient to use under the climatic conditions in Wyoming when elk are present on winter feed grounds and available for vaccinating. Studies are also being conducted on the response of bison to vaccination with B. abortus strain 19 delivered by the ballistic implant method.

Vaccine Implant Stability

Aliquots of different vaccine lots stored at various temperatures showed very good stability; results are presented in Tables 2–4 and Graphs 7 and 8. Exposure to oxygen in the air and the pressures developed during pelleting are probably major causes of viability loss during production. Only 15-20% of the total viable organisms in the original vial of vaccine can be successfully transferred to the completed implant. After formation of the pellet and placement in a nitrogen atmosphere, viability decline is comparable to that which occurs when lyophilized vaccine is stored under vacuum in glass vials (Table 3).

SUMMARY

A system with which a stable B. abortus strain 19 vaccine can be produced in a solid pellet form is described. The vaccine pellet is placed in
BRUCELLA ABORTUS STRAIN 19 VACCINE

a biodegradable ballistic implant that can be injected into an animal from some distance. This system is a feasible method for vaccinating free-ranging animals for brucellosis without individual animal restraint.

REFERENCES


a BallistiVet®, BallistiVet, Inc., White Bear Lake, MN 55127.

b USP Fast Flo Lactose, Foremost Whey Products, Baraboo, WI 53913.

c Magnesium Stearate (Octadecanoic acid magnesium salt) N.F. #MA130 Spectrum Chemical Manufacturing Corporation, Gardena, CA 90248.

d Scotchpak #8, 3M Company, St. Paul, MN 55144.

e Vacuum Impulse Bag Sealer, Model 1273VS, Packaging Aids Corporation, San Francisco, CA 94109.

The use of a particular manufacturer’s product does not constitute an endorsement on behalf of the USDA.
Table 1. Serologic Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implant and Liquid Vaccine

<table>
<thead>
<tr>
<th>Weeks Post Injection (weeks)</th>
<th>Liquid Vaccine (6 animals)</th>
<th>Ballistic Implant (7 animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPA Card Plate Tube Rivanol CF</td>
<td>BPA Card Plate Tube Rivanol CF</td>
</tr>
<tr>
<td></td>
<td>(% positive)</td>
<td>Reaction Values</td>
</tr>
<tr>
<td>0</td>
<td>0 0 0 1.0 I25</td>
<td>0 0 1.0 I25</td>
</tr>
<tr>
<td>2</td>
<td>67 67 6.2 +100+</td>
<td>100 100 7.7 +200-</td>
</tr>
<tr>
<td>3</td>
<td>100 83 8.5 +200+</td>
<td>100 100 9.3 I400+</td>
</tr>
<tr>
<td>4</td>
<td>100 100 8.0 +200</td>
<td>100 100 8.7 I400-</td>
</tr>
<tr>
<td>6</td>
<td>100 100 7.0 I200</td>
<td>100 100 6.9 I200</td>
</tr>
<tr>
<td>9</td>
<td>100 100 6.3 +100+</td>
<td>100 71 5.7</td>
</tr>
<tr>
<td>14</td>
<td>83 67 5.3 I100+</td>
<td>86 43 3.8</td>
</tr>
</tbody>
</table>

ND = Not Done
Reaction Values = Numerical value assigned to various titer levels (i.e. + 50 and 4 + 10 titer = 4.0, + 200 and 4 + 20 titer = 8.0 etc.)
BRUCELLA ABORTUS STRAIN 19 VACCINE

Table 2. *Brucella abortus* Strain 19 Implant Preparation – Reproduction of Successive Lots and Effect of Dilution with Lactose

<table>
<thead>
<tr>
<th>Lyophilized Vaccine (Lot)</th>
<th>Lactose Added/Vial (mg)</th>
<th>Pellet Weight (mg)</th>
<th>Viability Count (x10⁹)</th>
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<tbody>
<tr>
<td>1</td>
<td>1200</td>
<td>76</td>
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<td>80</td>
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<td></td>
<td>76</td>
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<td></td>
<td></td>
<td>83</td>
<td>5.56</td>
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<tr>
<td></td>
<td>average</td>
<td>78.75</td>
<td>5.94</td>
</tr>
<tr>
<td>2</td>
<td>1500</td>
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<td>6.02</td>
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<tr>
<td></td>
<td></td>
<td>83</td>
<td>8.57</td>
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<td></td>
<td>81</td>
<td>4.11</td>
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<td></td>
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<td>1200</td>
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<tr>
<td></td>
<td>1500</td>
<td>64</td>
<td>3.22</td>
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</table>

Table 3. Stability of Lyophilized *B. abortus* Strain 19 Reduced Dose Vaccine at 4°C Storage

<table>
<thead>
<tr>
<th>Serial</th>
<th>Viability Count (x10⁹)</th>
<th>Range (5 vials)</th>
<th>Storage Time (days)</th>
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<tbody>
<tr>
<td>1</td>
<td>3.76</td>
<td>3.56 – 4.12</td>
<td>48</td>
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<tr>
<td></td>
<td>3.82</td>
<td>3.35 – 4.59</td>
<td>232</td>
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<tr>
<td></td>
<td>2.75</td>
<td>2.43 – 3.14</td>
<td>1580</td>
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<tr>
<td></td>
<td>3.25</td>
<td>3.01 – 3.56*</td>
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</tr>
<tr>
<td>2</td>
<td>6.20</td>
<td>6.11 – 6.31</td>
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<tr>
<td></td>
<td>4.71</td>
<td>4.10 – 5.16</td>
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<tr>
<td></td>
<td>4.43</td>
<td>4.02 – 5.22</td>
<td>912</td>
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<td></td>
<td>4.25</td>
<td>3.96 – 4.49</td>
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*for 3 vials*
Table 4. Stability of *B. abortus* Strain 19 Vaccine Implants when Stored at 25°C, 4°C, and -20°C

<table>
<thead>
<tr>
<th>Storage Temperature (°C)</th>
<th>Viability Count (x10⁹)</th>
<th>Range (3 pellets)</th>
<th>Storage Time (days)</th>
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<td>25</td>
<td>6.28</td>
<td>5.67 - 6.70*</td>
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<td></td>
<td>3.81</td>
<td>3.47 - 4.17</td>
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</tr>
<tr>
<td></td>
<td>8.0 x 10⁸</td>
<td>**</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6.06 x 10⁸</td>
<td>5.1 - 6.8**</td>
<td>4</td>
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<tr>
<td></td>
<td>7.79 x 10⁸</td>
<td>7.37 - 8.50</td>
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<tr>
<td></td>
<td>6.46 x 10⁸</td>
<td>5.60 - 7.00</td>
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<tr>
<td></td>
<td>5.65 x 10⁸</td>
<td>4.56 - 6.24</td>
<td>18</td>
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<tr>
<td></td>
<td>1.80</td>
<td>1.66 - 1.89</td>
<td>25</td>
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<td></td>
<td>1.40</td>
<td>1.37 - 1.44</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>5.2 x 10⁸</td>
<td>4.06 - 5.76</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>2.96</td>
<td>2.48 - 3.28</td>
<td>7</td>
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<td>1.89</td>
<td>1.40 - 2.34</td>
<td>11</td>
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<td>1.71</td>
<td>1.63 - 1.77</td>
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<td>5.60</td>
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<td>5.32</td>
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<td></td>
<td>3.25</td>
<td>2.77 - 3.65</td>
<td>1199</td>
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*5 pellets for initial viability

**The dilution was too low and an accurate count was hard to determine.
BRUCELLA ABORTUS STRAIN 19 VACCINE

Graph 1. Buffered Plate Test Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implants and Liquid Vaccine

Graph 2. Card Test Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implants and Liquid Vaccine
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Graph 3. Plate Test Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implants and Liquid Vaccine

Graph 4. Tube Test Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implants and Liquid Vaccine
Graph 5. Rivanol Test Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implants and Liquid Vaccine

Graph 6. Complement Fixation Test Results Following Vaccination of Adult Cattle with Strain 19 by Ballistic Implants and Liquid Vaccine
Graph 7. Stability of Lyophilized *B. abortus* Strain 19 Reduced Dose Vaccine at 4°C Storage

Graph 8. Stability of *B. abortus* Strain 19 Vaccine Implants when Stored at 25°C, 4°C, and -20°C
The Committee on Wildlife Diseases met at 1:30 PM on Monday, October 30, 1989. Twelve members and approximately 55 guests were present. The Committee's work for the past year was reviewed and new activities were planned for several items on the agenda. Summary statements of reports and the Committee's recommended actions are cited as follows:

1. Feral Swine, USAHA Ad Hoc Subcommittee

This ad hoc committee was comprised of seven USAHA members who represented various USAHA Committees such as Transmissible Diseases of Swine, Wildlife Diseases, Pseudorabies, Subcommittee on Swine Brucellosis, Infectious Diseases of Cattle, and Foreign Animal Diseases. Consultation also was obtained from the Livestock Conservation Institute, National Pork Producers Council, and Veterinary Services, USDA.

The Wildlife Diseases Committee was presented with a memorandum from Dr. George Beran, Chairman of the Feral Swine ad hoc Committee that summarized their work. The memorandum provided a definition for feral swine and suggested changes for the Uniform Methods and Rules for Brucellosis Eradication and the Program Standards for Pseudorabies Eradication. Also included are recommendations for epidemiologic and experimental studies.

Dr. Victor Nettles, who had represented the Wildlife Diseases Committee on the Feral Swine ad hoc Committee, requested that the Wildlife Diseases Committee endorse the recommendations in the memorandum.

Recommended Action:

The Wildlife Diseases Committee voted to recommend acceptance of the Subcommittee's suggested changes for the Uniform Methods and Rules For Brucellosis Eradication and the Program Standards for Pseudorabies Eradication.

2. Relationship between Domestic Livestock, Bighorn Sheep and Diseases
Recent large bighorn die-offs in many of the Western states have caused speculation that domestic sheep were carriers of diseases fatal to bighorns, even with casual contact. Available evidence has not confirmed this disease relationship. As state wildlife agencies have continued their active transplant programs, livestock interests have expressed a concern over disease threats to their animals as well as policies formulated by public land agencies in their efforts to segregate bighorns from domestic sheep. This has led to increased polarization between wildlife and livestock interests.

On June 20-21, 1989, a Symposium on Bighorn/Domestic Sheep Diseases was held in Reno, Nevada to identify and bring into focus the causes of the conflict between bighorn and domestic sheep interests, gain an understanding of the problems and devise a cooperative effort to resolve the problems.

The symposium was co-chaired by Dr. David Hunter, California Fish and Game Department; Dr. George West, California Department of Food and Agriculture; and Mr. George Tsukamoto, Nevada Department of Wildlife. Representation at the meeting came from governmental agencies, private industry, livestock producers and other citizens groups. Dr. Bob Hillman, Idaho Bureau of Animal Health, reported to the committee on the symposium.

The introductory portion of the symposium gave a historical perspective and reviewed current policies and problems experienced by states. Dr. William Foreyt, Washington State University, presented the bighorn disease perspective by reviewing his work on *Pasteurella hemolytica* and sharing his conclusions and recommendations. Dr. Bob Hillman, Idaho Bureau of Animal Health, presented the agricultural perspective of the bighorn sheep issue. At the conclusion of these presentations, a general discussion was held in an attempt to more narrowly define the problems and recommend solutions.

The following problems were identified:

1. Bighorn sheep are susceptible to a variety of diseases and populations once infected may be adversely affected for a considerable period of time.
2. Some diseases in bighorns may result from contact with domestic sheep.
3. Land management decisions may be influenced by concerns over potential disease transmission between domestic and bighorn sheep and these decisions may affect domestic sheep grazing on public lands.
4. Conflicts between wildlife interest groups and livestock interest groups have resulted in legal challenges and suits.
WILDLIFE DISEASES

5. Relocations and enhancements of bighorn populations are currently threatened by the conflicts mentioned above.

6. *Pasteurella hemolytica* and *P. multocida* have been the pathogenic organisms most frequently isolated from pneumonic bighorn sheep. Current serotyping and biotyping methodologies are not adequate to accurately characterize the isolates from bighorn sheep, nor are they adequate to pinpoint the origin of the bacteria.

7. A lack of standardized procedures for collecting data and coordinating efforts among states and agencies is a major problem. There is an urgent need to identify laboratory facilities where the best expertise is available to identify and characterize disease causing organisms.

8. There is a need to evaluate the merits of forming a Western States Wildlife Disease Cooperative.

Committees on *Pasteurella*, Standardization and the Western States Cooperative concept were formed to discuss various aspects of the problems and recommend solutions.

*Pasteurella Committee Report:* Factors were evaluated and identified that are known about pasteurellosis in sheep. Bighorn and domestic sheep are susceptible to this disease. The organism is frequently isolated from normal healthy bighorn and domestic sheep. Pasteurella organisms are frequently isolated from bighorns, domestic sheep, and cattle that do not fit into the current serotyping and biotyping schemes. Using the current characterization mechanisms, one cannot accurately determine the source of these organisms or their relative pathogenicity. The committee recommended that an accurate system of identification and characterization be developed and that a specific serological test be developed.

*Standardization Committee Report:* The committee recommended that a set of training materials consisting of an indexed procedures manual, visual aid training film, videos or slides for formal presentation of an In-Service Training Workshop on data and sample collection be developed. The committee also recommended that laboratories and their capabilities in analysis of samples from wildlife species be identified.

*Western States Cooperative Study Committee Report:* The committee recommended the concept of a western wildlife study group as a consortium of laboratories and personal expertise as opposed to a core laboratory system. The group would have a director and support personnel who would serve as a clearing house and provide assistance in studies of wildlife disease or issues affecting wildlife. The committee also recommended formation of a steering committee that could identify funding sources, serve as a coordinating
group, and be comprised of the state veterinarians and the directors of fish and wildlife agencies within the respective public land states of the west.

The following recommendations were made by symposium participants:

1. Fund a research project to develop a fingerprinting technique for *Pasteurella hemolytica* isolates from bighorn sheep.
   
   *Action Taken:* A proposal for the project was developed, has been funded, and is underway.

2. Develop field manuals and training aids for field biologists to aid in standardization of sample collection and develop a listing of potential laboratory facilities for wildlife diagnostics.
   
   *Action Taken:* Some work has begun on this project.

3. Take the concept of a Western States Wildlife Disease Cooperative to the Western Association of Fish and Game Agencies and solicit their support for the concept.
   
   *Action Taken:* Mr. Tsukamoto has made a report to the Western Association and the Association supported the concept of a Western States Wildlife Disease Cooperative. It appears that the next step is to develop a steering committee to bring the concept to fruition.

4. Plan to have an annual meeting or symposium until the bighorn issue is resolved.
   
   *Action Taken:* A meeting is planned for Spring of 1990.

**Recommended Action:**

The Wildlife Diseases Committee endorsed the Symposium on Bighorn/Domestic Sheep Diseases and the recommendations generated by the Symposium. Furthermore, the Committee recommended the agencies and organizations responsible for management of bighorn and domestic sheep in the west work together to solve common problems without contributing further to the polarization between the livestock industry and wildlife interests.

3. **Possibilities of Importing Red Deer from New Zealand.**

   Mr. Peter Floyd and Mr. James Innes reported on the red deer deer-farming industry in New Zealand. Red deer have been farmed successfully in New Zealand under intensive rotational grazed management systems for the past 20 years.

   The world market place is paying excellent prices for red deer products. Venison has similar nutritive values to that of poultry and fish. Antler velvet is utilized, particularly in oriental medicine, as a growth stimulant and general tonic to aid bloodflow and reduce stress. Western medical researchers and practitioners are becoming more involved in
prescribing this product. Deer hides are well recognized as quality leather used in fashion clothing as well as coats, hats, shoes and the like. Other byproducts — tails, pizzles, sinews, blood, etc. have extensive markets in the oriental community and bring good returns to the deer farmer. Hard antlers and eye teeth have a good market in the accessory and jewelry trade. Hooves are used extensively by Native Americans to produce Indian rattles and instruments.

The success with farming red deer has been significant due to a number of factors. They have a superior viability to other breeds and have adapted better to domestication. They are now farmed under a wide range of climates and environments. Their temperament is superior and, therefore, they do not require elaborate, specialized handling facilities and equipment and their husbandry is now similar to sheep and cattle. They are a large deer, mature bucks will weigh 500 to 600 pounds — smaller than the North American Elk but larger than most other species.

Health care is an integral part of farming. New Zealand stock is routinely tested for tuberculosis and brucellosis. Farms are certified and maintained free of these diseases. Vaccination programs appropriate to the threat of infection are actively pursued. Parasites are closely monitored and drenching or injection programs pursued as necessary to control them.

Mr. Floyd and Mr. Innes stated that venison consumption in the United States will continue to increase. World production cannot increase rapidly enough to keep up with the potential demand through the 1990s and into the 21st century. The U.S., already importing well over 80% of its commercial venison, will continue in this vein unless domestic production increases. The lack of red deer is limiting the rate of increase of domestic production. The imported share of domestic consumption will continue to grow unless breed stock is imported to spur production.

There are problems with red deer importation from New Zealand into the U.S. at the federal and state levels. Federal quarantine facilities in Hawaii, New York, and Florida are expensive, geared to small numbers and were principally established to support zoos. Protocols need to be established to allow private importation and quarantine under federal supervision of large numbers of deer, i.e. several hundred at a time, if foreign supplementation of domestic breed stock in the U.S. is to be economically feasible. The precedent for this exists in that large numbers of sheep were successfully imported through private quarantine facilities. Deer are routinely, privately quarantined and imported into Canada. Mr. Floyd and Mr. Innes felt the states need to recognize deer farming as a legitimate agriculture endeavor. The mindset that all deer are wildlife has to be overcome in the face of their belief that farmed
deer are domestic livestock subject to the same rules and regulations that govern the husbandry of more traditional ruminants such as sheep, goats, and cattle. The profitability of deer farming even on relatively small acreage offers alternatives to the steady erosion of the U.S. family run farm base.

The Wildlife Diseases Committee has received several inquiries from persons in Canada and the U.S. regarding concern about accidental introduction of the parasite *Elaphostrongylus cervi* through the importation of red deer from New Zealand. Dr. Victor Nettles was called upon to explain the concerns about this parasite.

The major concerns with introduction of *E. cervi* into the United States seem to be for the health of native wild ungulates and the animals involved in the deer farming industry. That *E. cervi* is pathogenic to red deer and reindeer has been documented fairly well. Two separate problems occur . . . neurologic disease in young animals and subclinical eosinophilic abscesses in the meat. Taxonomically, the North American elk (wapiti) is closely related to red deer and caribou is conspecific with reindeer. Therefore, infection of *E. cervi* in native elk and caribou would have the same undesirable effects as described elsewhere. Moose also are susceptible. The pathogenicity of *E. cervi* to other native ungulates, viz., white-tailed deer, mule deer, black-tailed deer, pronghorn antelope, bighorn sheep, mountain goat, etc., is unknown.

**Recommended Action:**

The Wildlife Diseases Committee does not oppose importation of red deer from New Zealand, but it does encourage the U.S. department of Agriculture to use extreme caution in allowing potentially *E. cervi* infected red deer to enter the U.S. Long quarantines in facilities where there are no gastropods, coupled with multiple fecal examinations should be mandated; infected red deer should be destroyed. Treatment of animals with anthelmintics should be prohibited because the medications tend to mask infection by temporarily stopping larval shedding in the feces.

4. Rabbit Viral Hemorrhagic Disease

Drs. Juan Lubroth, International Services and Doug Greg, Science and Technology, F.A.D.D.L. reported on the ongoing outbreak of an emerging disease of domestic and wild rabbits. A highly pathogenic viral disease of rabbits recently erupted in Mexico. Investigations had revealed over 2,000 cases with infected animals in 12 states of Mexico. The disease is termed Viral Hemorrhagic Disease (VHD), Viral Hemorrhagic Septicemia, Necrotic Hepatitis, etc. and is characterized by a 1 to 2-day incubation period, sudden onset, nasal hemorrhages, respiratory difficulties, and high mortality rate. The primary victim is the domestic rabbit, *Oryctolagus cuniculus*; however, the possibility that
other rabbits (*Sylvilagus* spp.) and hares (*Lepus* spp.) are susceptible cannot be dismissed. Other animals and humans have not been affected.

The history of VHD of rabbits has been relatively short but dramatic. The disease was first reported in China in 1984 where it was linked to rabbits imported from Germany. More recently, VHD has been diagnosed in Czechoslovakia, France, East and West Germany, Italy, Korea, Spain, and, now, Mexico. Millions of rabbits have died in the wake of this pathogen; a loss of 32 million domestic rabbits was reported from Italy alone. The virus appears to be transmissible by live animals, rabbit meat, or recently contaminated objects such as cages, feeders, clothing, etc. In Mexico, over 6.5 million rabbits have died and another 100,000 destroyed. At this time the Mexican government is conducting a disease eradication program that includes quarantine of infected farms and destruction of diseased animals.

The threat of VHD to domestic and wild rabbits in the U.S. is being evaluated by a Risk Assessment Committee within APHIS, USDA. Commercial rabbits and rabbit products entering the U.S. have not been subjected to inspection for diseases except for basic screening for wholesomeness by the Food and Drug Administration. Considering the high morbidity and mortality rates of VHD, tighter restrictions on rabbit importation may be in order.

Information on the occurrence of VHD in wildlife is not definitive, although a disease syndrome affecting brown hares in Europe has alarmingly similar characteristics. Since rabbits and hares of the U.S. are a major food source for mammalian and avian predator species as well as being important game mammals for hunting, wildlife biologists should be alerted to VHD and be prepared to investigate any sudden loss of rabbits.

**Recommended Action:**

The Wildlife Diseases Committee felt the Secretariat of Agriculture and Water Resources of the Government of Mexico should be commended for its work and effort in the eradication campaign against viral hemorrhagic diseases of rabbits. Furthermore, the Committee recommends that APHIS, USDA, along with the Food and Drug Administration, through their various agencies that are responsible for importation, become active in granting permits that assure that only healthy rabbits and rabbit by-products are imported into the U.S.

5. **Field Testing of Wild Turkeys for Mycoplasma**

The Western Association of Fish and Wildlife Agencies has passed a resolution that indicates a preference for field testing of wild turkeys intended for relocation by the rapid plate agglutination test. Their resolution was actually a request of the Wildlife Disease Association (WDA)
asking them to revise their “Recommendations or Guidelines for the Interstate Movement of Wild Turkeys” by adding a sentence as follows: “If the respective State Department of Agriculture requires serologic tests before importing wild turkeys, properly trained personnel may make the initial rapid plate agglutination tests to screen suspect turkeys in the field prior to transport.” Wildlife management officials want this provision so that wild turkeys can be relocated without delays associated with laboratory submission of samples. Rapid translocation of wild turkeys without excess holding time is extremely important in the survivability of the birds and ultimate success of the restoration effort.

Turkey biologists and wildlife veterinarians have performed the field test as directed by state agriculture officials in several states with good success, and the desire of conservationists is to have the field testing procedure accepted nationwide. The field testing of wild turkeys provides an excellent opportunity for state animal health and wildlife officials to work together on a well-received, highly visible wildlife restoration effort. Therefore, the Committee could see that field testing by properly trained personnel was desirable as long as the State Veterinarian was kept totally informed of the results and maintained the final decision on the importation of seropositive birds. It was further suggested that testing be conducted in the state of origin at the trap site, when possible.

Recommended Action:

The Wildlife Diseases Committee believes the suggested revision is acceptable and recommends the Wildlife Disease Association favorably consider changing its Guideline for the Interstate Movement of Wild Turkeys.

6. Brucellosis and Bison in Yellowstone National Park and Montana

Dr. D. P. Ferlicka, Montana, reported on the 1988-1989 experience in Montana when nearly every animal in the approximately 900 head northern bison herd emigrated out of Yellowstone National Park into Montana between December 12, 1988, and March 20, 1989. Because of concern about transmission of brucellosis from bison to cattle, it was necessary to harvest 570 bison using Warden-Supervised hunters to maintain separation of bison from cattle within the “buffer zone” of Southern Park County, Montana. The concept of boundary control using hunters was never intended to be applied to these vast numbers. In spite of controversy and criticisms, the necessary harvest was successful in preventing the introduction of Brucella into Montana cattle. Eight hundred ten cattle in eighteen herds in the area shared habitat and associated with bison during the winter. They were tested for brucellosis April 7 and June 6, 1989 and found sero-negative to the
standard battery of *Brucella* tests. Although separation was maintained, it was necessary to consider that undetected, prolonged contact may have occurred or that possibly an aborted fetus, uterine discharge, or infectious viscera established contact with domestic cattle or their feed supply. It was concluded that none of these events occurred, and Montana's reputation for brucellosis free cattle remained secure.

According to Dr. Ferlicka, the 1988-1989 experience was unacceptable from the standpoint of: 1) brucellosis infected bison entering Montana; 2) a tarnished image of Montana's livestock industry through exposure to brucellosis and their chosen plan of action; 3) pressures on state officials and state agencies; and 4) no prospect that the current dependence on boundary control harvests or hunting will control emigration of brucellosis infected and contagious animals.

Recommended Action:

The Wildlife Diseases Committee views the situation with concern and regrets the controversy and polarization the conflicting policies of the National Park Service and the Montana Department of Livestock have created.

7. Evaluation of Strain 19 Calfhood Vaccination in Bison

Dr. Don Davis, Texas A&M University, reported on research trials evaluating *Brucella abortus* Strain 19 as a calfhood vaccine for bison. Bison appear to respond to brucellosis in a similar way to that of cattle, except that they experience a higher abortion rate. Approximately 5 percent of the bison vaccinated as calves maintained vaccine induced titers, which was not considered significant. However, protection against abortion and infection after challenge by artificial infection was no better in vaccinates than in nonvaccinated controls. Dr. Davis concluded that, at the doses examined, calfhood vaccination of bison with Strain 19 is not effective.

8. Report on Strain 19 Ballistic Delivery Trials

Dr. Dale Angus, National Veterinary Services Laboratories, reported on development of a ballistic implant system that has provided a technique that is now being used to vaccinate free ranging elk for brucellosis in Wyoming. The preparation procedures for the vaccine have been developed so that a product can be produced that has stable storage characteristics quite comparable to regular lyophilized *Brucella* vaccines. The delivered dosage can be adjusted to meet requirements that may be needed to vaccinate different species and/or ages of animals, and animal response to ballistically delivered Strain 19 vaccine is very comparable to that of animals hand vaccinated with liquid vaccine. The product is convenient to use under the prevailing winter conditions in Wyoming.

Dr. Tom Thorne, Co-chairman of the Technical Committee on Brucellosis in the Wildlife of the Greater Yellowstone Area reported on the Committee's third meeting, which was held October 29, 1989, in Las Vegas. The Technical Committee heard reports on a forthcoming Staff-level meeting between National Park Service and U.S. Department of Agriculture, APHIS, and on deliberations of Canada's Environmental Assessment Review Panel on the Wood Buffalo National Park diseased hybrid bison situation. The U.S. National Environmental Policy process was also explained as it pertains to Environmental Assessments and Environmental Impact Statements; these processes will likely be necessary for any attempt to manage brucellosis in a National Park. The numbers of bison in Yellowstone National Park, which have declined somewhat from previous years, were discussed; and there was considerable discussion regarding the origin of Brucella abortus in bison of Yellowstone National Park. A trial was recently conducted in Yellowstone National Park that demonstrated that it may be feasible to ballistically vaccinate free-ranging bison with the BallistiVet biobullet system. In addition, Wyoming's Interim Bison Management Plan, which allows for sport hunting of excess bison was discussed. There was extensive discussion of the use of Strain 19 to vaccinate bison, and it was the consensus of the Technical Committee that, based upon current knowledge, there is no justification for vaccination of bison with Strain 19. Recognition by the Technical Committee that conflicting goals and missions of federal and state agencies responsible for brucellosis eradiation, public health protection, National Park policy, and wildlife management preclude development of sound brucellosis control or eradication recommendations for the Greater Yellowstone Area prompted the committee to prepare a draft resolution for the Wildlife Diseases and Brucellosis Committees to consider for submission to USAHA.

Recommended Action:

The Wildlife Diseases Committee commends the technical dialogue and information exchange established by the Technical Committee. Furthermore, the Wildlife Diseases Committee adopted a resolution encouraging federal and state agencies to identify the major constraints to controlling or eradicating brucellosis and resolve conflicting goals and missions regarding this important issue.

10. Diseased Hybrid Bison in Wood Buffalo National Park, Canada

Dr. Bulmer, Agriculture Canada, reported to the Committee on hybrid bison in Canada's Wood Buffalo National Park that are infected
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with tuberculosis and brucellosis. The situation was created in the 1920s when plains bison infected with tuberculosis and brucellosis were translocated from southern Canada to Wood Buffalo National Park. Once there, the plains bison hybridized with native wood bison to create the current problem by which diseased hybrid bison threaten other populations of wood bison, which are considered endangered, and livestock. Canada is going through a Federal Environmental Assessment Review process to evaluate and make recommendations on a Task Force Report that provided a series of options for managing the problem. If the diseased hybrid bison are not dealt with soon, they will eventually commingle with the expanding wood bison population in the nearby Makinzie Wood Bison Sanctuary, which will compound the scope of the problem and render it insolvable. A preferred option seems to be repopulation of Wood Buffalo National Park with disease-free wood bison after depopulation of the diseased hybrid bison.

Recommended Action:

The Wildlife Diseases Committee adopted a resolution encouraging depopulation of the diseased hybrid bison after recognizing that the Wood Buffalo National Park situation should not be compared with Yellowstone National Park because of the presence of hybrid bison, presence of tuberculosis, and threat to an endangered species in Canada.
CONSTITUTION AND BYLAWS
OF THE
UNITED STATES ANIMAL HEALTH ASSOCIATION

ARTICLE I — NAME
The name of this Association shall be "The United States Animal
Health Association," a non-profit association.

ARTICLE II — PURPOSE
The purpose of this Association shall be the study of animal health
science, milk and meat hygiene, and the dissemination of information
relating thereto, the unification so far as possible of laws, regulations,
policies, and methods pertaining to milk and meat hygiene, and to the
prevention, control, and eradication of transmissible animal diseases;
to maintain coordination among the various animal health regulatory
organizations, and to serve as the animal health science clearing
house between this Association and the following: The livestock
owner, the animal health scientist, the milk and meat hygienist, the
veterinary practitioner, the transportation and stockyard companies,
the milk and meat producing and distributing companies, and various
other interested agencies. The word "animal" as herein used shall be
understood to include poultry.

ARTICLE III — MEMBERSHIP
There shall be five kinds of members: Official, allied organization,
individual, elected regional delegates, and nonvoting juniors.

OFFICIAL MEMBERSHIP
The animal health departments of each state, also the United
States, and the Canadian, and Mexican governments, Puerto Rico,
the Virgin Islands, and Los Angeles County, California, and of such
other governmental units as the Executive Committee may by a two-
thirds vote approve, shall be eligible to official membership in this As-
sociation and be represented on the Executive Committee by the
animal health executive official.

ALLIED ORGANIZATION MEMBERSHIP
Any nonprofit organization approved by the Executive Committee
that is national in scope and actively and directly concerned with the
interests and objectives of this Association as outlined in Article II —
Purpose, may be elected to allied organization membership and be
represented on the Executive Committee by a duly authorized mem-
ber of the organization. Such organizations applying for membership
shall have and shall continue to maintain no less than 50 (fifty) individual members of the U.S. Animal Health Association to qualify.

INDIVIDUAL MEMBERSHIP

Any person engaged in animal health work for Federal, provincial, state, county, or municipal governments, and any other person interested in animal health science or milk and meat hygiene, may be elected to individual membership.

Any individual member who has maintained membership in this Association for 35 years, or if such member is at the point of retirement, for 25 years, may be elected to life membership by the Executive Committee. Such life membership shall carry with it all the rights and privileges of regular individual membership, including receipt of the Annual Proceedings of this Association. Such life membership shall be exempt from the payment of dues or any other assessments.

All past presidents shall automatically become life members.

Members of the Executive Committee will be eligible for such life membership; but for such member, the requirements for maintaining individual membership will be waived. But the period of time for such membership will be as herein provided.

The Executive Committee may, at its discretion, confer honorary individual memberships. Such memberships shall be exempt from the payment of dues and other assessments and may be withdrawn at the discretion of the Executive Committee.

ELECTED REGIONAL DELEGATE MEMBERSHIP

Such elected regional delegates as provided for in Article V — Executive Committee shall by virtue of such election automatically become members of this organization for such term or terms as may be decided by the Executive Committee and shall pay such dues as the Executive Committee may decide.

NONVOTING JUNIOR MEMBERSHIP

Students in agriculture, medicine, veterinary medicine, vocational agriculture, or any 4-H Club member, as well as future farmers under 21 years of age are eligible to election as nonvoting junior membership.

ARTICLE IV — MEETINGS

The meetings of this Association shall be annual and special.
ARTICLE V — OFFICERS
The officers of this Association shall be: President, President-Elect, First Vice-President, Second Vice-President, Third Vice-President, Secretary, Treasurer, Board of Directors, and an Executive Committee.

BOARD OF DIRECTORS
The Board of Directors shall consist of the officers, including the immediate Past President with the exception of the Executive Committee. It shall handle the financial, administrative, and internal affairs of the Association during such time as the Association and/or the Executive Committee is not in session. It shall handle all other duties and responsibilities as may be assigned to it by the Executive Committee or as may be provided in the Constitution. The Board of Directors shall meet immediately after the adjournment of each annual meeting of this Association and at the same place. The purpose of such meeting is to review plans for the administrative functions of the Secretary for the coming year, to give administrative guidance to the Secretary, and to approve the operations of the office of the Secretary including, upon consultation with him, the employment of an Executive Director and such other employees as may be required which are not otherwise in conflict with the Constitution and Bylaws. The Board of Directors may meet at such other times and places as it, by a majority vote, deems necessary. The Secretary shall keep minutes of all meetings of the Board of Directors, and after approval of such minutes by the president, they shall be presented to the Executive Committee at the next annual meeting of this Association.

EXECUTIVE COMMITTEE
The Executive Committee shall be composed of the executive officer representing the animal health departments of the various states, the principal animal health officer of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive animal health officer of Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, and of such other governmental units as may be approved for official membership by the Executive Committee,

*For the purpose of having proper credentials, the name of the Executive Committee representative or substitute, if applicable, shall be provided to the Association Secretary by the executive officer of those entities named herein.
the elective officers of this Association, not more than eight (8) 
delegates at large representing the livestock industry, including 
poultry, and allied organization members. All past presidents in 
attendance not included in any other section shall be ex-officio 
members.*

There shall be five districts. Said districts shall be known as (1) The 
Northeast: consisting of the states of Connecticut, Delaware, Maine, 
Maryland, Massachusetts, New Hampshire, New Jersey, New York, 
Pennsylvania, Rhode Island, and Vermont; (2) The North Central: 
consisting of the states of Illinois, Indiana, Iowa, Kansas, Michigan, 
Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, 
and Wisconsin; (3) The Southern: comprising the states of Alabama, 
Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North 
Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, 
West Virginia, Puerto Rico, and the Virgin Islands; (4) The Western 
district: consisting of the states of Alaska, Arizona, California, Colo-
rado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, 
Washington and Wyoming; (5) the District-at-Large: consisting of 
Allied Organization Members and all Elected Regional Delegate 
Members.

Each district, as provided above, shall on a rotating basis, annually 
submit to the Nominating Committee, nominees for vacancies that 
shall occur in the following offices: President; President-Elect; First 
Vice-President; Second Vice-President; Third Vice-President. The 
order of rotation shall be as follows: Northeastern; Western, South-
ern; Region-at-Large; North Central. In the event that an elected 
officer is unable to complete an elected term, the District that origi-
nally submitted the nominee shall have the opportunity to resubmit 
a nominee to fill the vacancy; or, the provisions of Article VII — Duties 
of Officers shall apply.

The elected officers shall have the authority to place before the 
Executive Committee applications for allied organization mem-
bership. Not more than five (5) such applications shall be presented to the 
Executive Committee for consideration at any annual meeting of the 
United States Animal Health Association.

The Executive Committee shall constitute the administrative body 
of this Association and shall determine its activities and policies.

All recommendations and reports of officers and committees shall 
be referred for consideration to the Executive Committee.

The President-Elect shall be ex-officio chairman of the Executive 
Committee.

The Executive Committee shall elect yearly a Secretary for the As-
association. The Secretary shall receive such salary and allowance as 
may be fixed by the Executive Committee.
The Executive Committee shall cause to be audited annually, or oftener if deemed necessary, the receipts and disbursements of the Secretary and of the Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall accept or reject applications for individual and for allied organization membership properly placed before it. Three negative votes shall disqualify for either such membership.

That, with the exception of a change in the name of this Association, upon the dissolution of this corporation nor the termination of activities thereof, all remaining assets thereof shall be contributed for utilization in the advancement of research of diseases of animals, and no part of the net assets shall inure to any person or group of persons for private gain.

ARTICLE VI — PROGRAM COMMITTEE

The President, the Chairman of the Executive Committee, the Secretary, the Treasurer, and the Chairmen of the respective committees shall constitute the Program Committee. It shall be the duty of the members of the Program Committee to make the necessary arrangements and provide the program for the annual and special meetings.

ARTICLE VII — DUTIES OF OFFICERS

1. President: It shall be the duty of the President to preside at all meetings of this Association and of the Board of Directors; to appoint all committees excepting the Executive and officer faction of the Program Committee; to call special meetings of the Association whenever he considers the holding of such meetings necessary for the good of the livestock industry or upon written request of five members of the Executive Committee. The President shall be an ex-officio member of all committees.

The President shall officially represent this Association in such places and at such meetings as he, with the concurrence of a majority of the Board of Directors, deems desirable or necessary in the best interests of this Association. He may at his discretion designate a member of the Executive Committee to substitute for him. A report of such attendance shall be made annually to the membership, and all actual expenses incidental thereto shall be paid by this Association.

2. President-Elect: The President-Elect shall be chairman of the Executive Committee. In the absence of the President, he shall preside at the meetings of the Association. In the event of the absence, disability, or resignation of the President, he shall perform all duties of the President. He shall be an ex-officio member of the Executive and Program Committees and of the Board of Directors.
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3. First Vice-President: The First Vice-President shall assume the duties of the President in the event of the absence, disability, or resignation of the President and President-Elect. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability, or resignation of President-Elect. He shall be an ex-officio member of the Executive Committee and the Board of Directors.

4. Second Vice-President: The Second Vice-President shall assume the duties of the President in the event of the absence, disability, or resignation of the President, President-Elect, and First Vice-President. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability, or resignation of the President-Elect and First Vice-President. He shall be an ex-officio member of the Executive Committee and of the Board of Directors.

5. Third Vice-President: The Third Vice-President shall assume the duties of the President in the event of the absence, disability, or resignation of the President, President-Elect, First Vice-President, and Second Vice-President. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability, or resignation of the President-Elect, First Vice-President, Second Vice-President. He shall be an ex-officio member of the Executive Committee and of the Board of Directors.

6. Secretary: The Secretary shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee, he shall publish said proceedings and distribute them to the members of the Association. The Secretary shall also keep an accurate record of the proceedings of the Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association.

He shall keep an accurate account of all Association moneys received and disbursed. All moneys due this Association received by the Secretary shall be promptly turned over to the Treasurer, accompanied by transmittal information identifying the amount, the source, and such other information as the Treasurer and the Board of Directors may require. He shall draw on the Treasurer, on proper warrants, over his signature and that of the Executive Director, such sums as may be necessary to discharge the financial obligations of this Association, provided however that for the payment of incidental expenses of his office, the Secretary may draw on the Treasurer from time to time sums not to exceed one hundred dollars ($100) at any one time on his own authority over the sole signature on warrants signed by the Executive Director. The President shall be furnished at the end of each month, for his validation, a list of financial obligations satisfied during the preceding period. He shall also present to the chairman of the Executive Committee a list giving the name, occupa-
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237 tion, and address of each applicant for individual membership for the
238 approval of the Executive Committee. He shall prepare forms for
239 applicants for allied organization membership and shall notify each
240 of the elected officers upon receipt of such completed application. He
241 shall perform such other duties as may be authorized and prescribed
242 by the Executive Committee. He shall be ex-officio secretary of the
243 Executive Committee, ex-officio secretary of the Board of Directors,
244 and an ex-officio member and secretary of the Program Committee.
245 He shall be bonded for not less than ten thousand dollars ($10,000).
246 7. Treasurer: The Treasurer shall keep an accurate account of all
247 Association moneys received and disbursed. He shall receive from the
248 Secretary all moneys of the Association paid directly to the Secretary
249 along with proper identification of such moneys. By and with the
250 approval of the Board of Directors, he shall deposit the funds of this
251 Association in such types of accounts as may be approved by the Board
252 of Directors, and he shall invest the funds of the Association or
253 liquidate Association investments in such manner as may be ap-
254 proved by the Executive Committee upon recommendation of the
255 Board of Directors. He shall honor warrants for the proper expendi-
256 ture of Association funds furnished him by the Secretary over his
257 signature and that of the Executive Director. He shall honor warrants
258 from the Secretary on the Secretary's own authority for incidental
259 expenses of the Secretary's office in sums not to exceed one hundred
260 dollars ($100) for any given expenditure over the sole signature on
261 warrants signed by the Executive Director. He shall be given guidance
262 and general administrative supervision by the Board of Directors, and
263 he shall furnish the Executive Committee with a financial statement
264 of the Association's funds annually. He shall be bonded for not less
265 than ten thousand dollars ($10,000), and he shall receive such salary
266 as the Executive Committee may from time to time determine.

267 ARTICLE VIII — AMENDMENTS

268 The Constitution and Bylaws of this Association may be amended
269 by a two-thirds vote of the members of the Association present and
270 voting at an annual meeting, provided that the specific amendment to
271 be acted upon shall have been presented in writing at a previous
272 annual meeting, printed in the annual proceedings, and further
273 provided that the amendment has received the approval of a majority
274 of the Executive Committee members present and voting.

275 ARTICLE IX — ELECTION OF OFFICERS AND ELECTED
276 REGIONAL DELEGATES

277 The Nominating Committee shall annually report to the member-
278 ship of this Association at the first morning general session. Their rec-
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ommendations for the offices of President, President-Elect, First Vice-
President, Second Vice-President, Third Vice-President, and Treas-
urer, and Elected Regional Delegates shall constitute their report.
Nominations shall not originate within this committee but shall be
submitted by the appropriate district. Said recommendations shall be
posted on the registration bulletin board immediately following their
presentation. Any member may propose amendments to the slate
presented by the Nominating Committee prior to, or at the second
morning general session.** The report shall be presented to the
Executive Committee for consideration. The Acceptance of the report
or amendment shall constitute election.

BYLAWS

ARTICLE I — ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary
Report of Treasurer.
President-Elect’s Address.
Reading of Papers.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nominations and Election of Officers and eight members to Execu-
tive Committee.
Adjournment.
A suspension of the Bylaws may be made by a two-thirds majority
for the purpose of changing the order of business or to facilitate
important business.

** The phrase “at the second morning general session” shall be deemed to mean at a time
certain specified in the program the Report of the Action of the Nominating Committee during
that session; provided that if a paper is being presented at that specified time, its presentation
will be completed, immediately after which the Nominating Committee Report will be read.
Provided further, if the program is ahead of schedule for that session, a recess will be taken
until the time certain established in the program for the Report of the Action of the Nominating
Committee.
ARTICLE II — APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary. The application shall give the name, occupation, and address of the applicant and shall be accompanied by a fee of thirty dollars ($30) which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary, who shall in turn submit them to the Executive Committee.

Applications for allied organization membership shall be made in writing to the Secretary on an appropriate form prepared by him. In turn, notice of receipt of such application shall be provided each of the elected officers.

An individual or allied organization member may be expelled for cause by the Executive committee. A majority vote by the members of the Executive Committee present and voting shall be required in order to expel any such member.

ARTICLE III — MEETINGS

The annual meetings shall be held in a location selected at a previous annual meeting by a majority of the members of the Executive Committee. The annual meetings shall be held in a location selected at a meeting of the geographical districts as outlined in Article V, Executive Committee, on a rotating basis as follows: North Central, Northeast, Western, Southern, and in concurrence with the executive officer of the animal health department of the state in which the meeting is proposed.

Each meeting site in the selected location shall be determined by the secretary with the approval of the Board of Directors, and in consultation with the executive officer representing the animal health department of the state in which the meeting is to be held. The Executive Committee shall be advised of said selecting at least five (5) years in advance of any annual meeting.

The annual meetings shall begin in the month of October.

The Board of Directors is authorized to select an alternate location and a site in the event that the previous selections, because of any unforeseen circumstance, become unavailable and/or unacceptable.

The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.
ARTICLE IV — QUORUM

Twenty-five members of the Association shall constitute a quorum. Thirty members of the Executive Committee shall constitute a quorum, providing at least two-thirds of this number are executive officers representing the animal health departments of their respective states.

ARTICLE V — DUES

The dues for individual membership in this Association shall be thirty dollars ($30) per annum, payable in advance (on or before January 1st of each year) to the Secretary of the Association. The dues for nonvoting junior members shall be three dollars ($3) per annum, payable (on or before January 1st of each year) to the Secretary of this Association. The dues for official and allied organization memberships shall be two hundred dollars ($200) each per annum, payable in advance (on or before January 1st each year) to the Secretary of this Association.

The interpretation of the constitution as reflected in the explanatory footnotes was concurred in by the Executive Committee on October 26, 1984 at the 88th Annual Meeting in Fort Worth, Texas.
STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR PERIOD JANUARY 1, 1989 THROUGH DECEMBER 31, 1989

CASH BALANCE – DECEMBER 31, 1988:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signet Bank/Virginia</td>
<td></td>
</tr>
<tr>
<td>Richmond, Virginia</td>
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</tr>
<tr>
<td>Cash on Hand</td>
<td>$2,850.31</td>
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<tr>
<td>Checking Account</td>
<td>3,134.11</td>
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<tr>
<td>Money Market Savings</td>
<td>52,903.76</td>
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<tr>
<td>Certificate of Deposits:</td>
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<tr>
<td>#0021944020</td>
<td>14,232.24</td>
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<tr>
<td>#0011563889</td>
<td>10,000.00</td>
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<tr>
<td>#0021944038</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>$93,120.42</strong></td>
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RECEIPTS:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Individual Dues – 1989 and 1990</td>
<td>31,794.00</td>
</tr>
<tr>
<td>Official Dues – 1989 and 1990</td>
<td>19,800.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>5,895.16</td>
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<tr>
<td>Foreign Animal Books</td>
<td>305.93</td>
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<tr>
<td>Reprints</td>
<td>3,924.18</td>
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<tr>
<td>Registrations</td>
<td>97,571.00</td>
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<tr>
<td>Tours and Wednesday Evening Dinner</td>
<td>14,827.87</td>
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<tr>
<td>Miscellaneous</td>
<td>1,058.83</td>
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<tr>
<td>Interest</td>
<td>3,551.48</td>
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<tr>
<td>Change Fund</td>
<td>700.00</td>
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<tr>
<td>AAVLD's Share of the Annual Meeting</td>
<td>3,368.19</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>$182,796.64</strong></td>
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TOTAL BEGINNING BALANCE AND RECEIPTS                   | **$275,917.06** |
**STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS**

**DISBURSEMENTS:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Annual Meeting</td>
<td>$13,371.04</td>
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<tr>
<td>Printing</td>
<td>35,608.55</td>
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<tr>
<td>Office Supplies</td>
<td>2,934.15</td>
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<tr>
<td>Copier (Service Agreement) &amp; Supplies</td>
<td>632.31</td>
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<tr>
<td>Union Central Retirement Blanton &amp; Ragland 1989</td>
<td>2,148.00</td>
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<tr>
<td>Union Central Retirement Blanton &amp; Ragland 1990</td>
<td>2,268.82</td>
</tr>
<tr>
<td>Salaries</td>
<td>44,495.61</td>
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<tr>
<td>Wages</td>
<td>6,347.67</td>
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<tr>
<td>Social Security Tax</td>
<td>3,810.58</td>
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<tr>
<td>Directors Insurance</td>
<td>654.00</td>
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<tr>
<td>Hospitalization Insurance</td>
<td>5,301.46</td>
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<tr>
<td>Computer Expenses</td>
<td>1,819.00</td>
</tr>
<tr>
<td>Public Relations</td>
<td>1,513.30</td>
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<tr>
<td>Exhibit</td>
<td>181.26</td>
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<tr>
<td>Communications (Postage &amp; Telephone)</td>
<td>13,624.85</td>
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<tr>
<td>Rent (Office Space)</td>
<td>9,610.00</td>
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<tr>
<td>Virginia Unemployment Tax</td>
<td>18.57</td>
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<tr>
<td>P.O. Box Rental</td>
<td>73.00</td>
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<tr>
<td>Membership Dues — Price Club</td>
<td>45.00</td>
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<tr>
<td>Henrico County License</td>
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<td>Personal Property Tax</td>
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<td>AAVLD Contract</td>
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<td>Shipping Books</td>
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<td>IRS</td>
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<td>Travel and Expenses</td>
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<tr>
<td>Mr. Philip E. Bradshaw</td>
<td>4,248.58</td>
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<tr>
<td>Dr. J. C. Shook</td>
<td>2,785.01</td>
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<tr>
<td>Ella R. Blanton</td>
<td>2,150.72</td>
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<tr>
<td>Mr. J. O. Pearce</td>
<td>1,445.02</td>
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<tr>
<td>Dr. J. E. Slauter</td>
<td>18.10</td>
</tr>
<tr>
<td>Dr. C. L. Campbell</td>
<td>237.40</td>
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<tr>
<td>Linda B. Ragland</td>
<td>378.00</td>
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<tr>
<td>Miscellaneous Expenses</td>
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<td>State Federal Expenses</td>
<td>889.58</td>
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<td>Delaware Franchise Tax</td>
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<tr>
<td>Surety Bond</td>
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<td>Safe Deposit Box</td>
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<td>Bank Service Charges</td>
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<tr>
<td>Reimbursement of Reg. &amp; Dues</td>
<td>2,034.45</td>
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<tr>
<td><strong>Total</strong></td>
<td>$184,447.93</td>
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</tbody>
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STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS

SUMMARY OF OPERATIONS
FOR PERIOD JANUARY 1, 1989 THROUGH DECEMBER 31, 1989

<table>
<thead>
<tr>
<th><strong>REVENUE:</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total Cash Receipts</td>
<td>$182,796.64</td>
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<tr>
<td>Less Expenditures</td>
<td>184,447.93</td>
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<tr>
<td>Excess of Expenditures over Receipts</td>
<td>$1,651.29</td>
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</table>

<table>
<thead>
<tr>
<th><strong>CASH BALANCE – DECEMBER 31, 1989:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Signet Bank/Virginia</td>
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<tr>
<td>Richmond, Virginia</td>
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<td>Cash on Hand</td>
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<td>Money Market Savings</td>
</tr>
<tr>
<td>Certificate of Deposits:</td>
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<td>#0021944020</td>
</tr>
<tr>
<td>#0029061108</td>
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<tr>
<td></td>
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</tbody>
</table>

Henry H. Budd, Accountant
94th ANNUAL MEETING
October 6–12, 1990
RADISSON HOTEL, DOWNTOWN
Denver, Colorado

95th ANNUAL MEETING
October 26–November 1, 1991
TOWN & COUNTRY HOTEL
San Diego, California

96th ANNUAL MEETING
October 31–November 6, 1992
THE GALT HOUSE HOTEL
Louisville, Kentucky