PROCEEDINGS

NINETY-FIFTH
ANNUAL MEETING

of the

UNITED STATES ANIMAL
HEALTH ASSOCIATION

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TOWN AND COUNTRY HOTEL
SAN DIEGO, CALIFORNIA
October 26 - November 1, 1991
This book is dedicated in memory to the members of USAHA who passed away in 1991.

Dr. L. W. Hinchman—Past President 1981
Dr. J. F. Badger—Member

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Dr. Michael R. Marshall, Salt Lake City, UT
Dr. Bennie L. Osburn, Davis, CA
Dr. Robert K. Pelant, Little Rock, AR
Dr. R. A. Robinson, St. Paul, MN
Mr. Paul Rodgers, Englewood, CO
Dr. M. D. Salmon, Fort Collins, CO
Dr. Charles M. Scanlan, College Station, TX
Dr. John A. Schmitt, Lincoln, NE
Dr. J. G. Songer, Tucson, AZ
Dr. Jeff L. Stott, Davis, CA
Dr. W. L. Thomas, Reynoldsburg, OH
Dr. Peter H. Timm, Dixon, CA
Mr. O. H. Timm, Dixon, CA
Dr. Percy R. Turner, Water Valley, TX
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COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE – 1992

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COMMITTEE ON TUBERCULOSIS – 1992

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Dr. Mitchell A. Essey, Hyattsville, MD
Mr. Donald P. Fertlka, Helena, MT
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Dr. Michael J. Gilchrist, Sykesville, MD
Dr. Chester A. Gipson, Tampa, FL
Dr. Arthur E. Hall, Indianapolis, IN
Dr. Bob R. Hillman, Boise, ID
Dr. D. Patrick Hoctor, Terre Haute, IN
Dr. D. L. Hunter, Boise, ID
Dr. Sarah B. S. Hurley, Madison, WI
Dr. David A. Jessup, Fair Oaks, CA

Dr. Daryl C. Johnson, Conyers, GA
Dr. Daryl K. Thorpe, Pierre, SD
Ms. Diana L. Whipple, Ames, IA
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COMMITTEE ON ZOOLOGICAL ANIMALS – 1992

Chairman: Dr. B. Amand, Philadelphia, PA
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Dr. David A. Jessup, Fair Oaks, CA
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Dr. S. K. Taylor, Washington, DC
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Dr. E. Tom Thorne, Laramie, WY
Mr. Dave Whittlesey, Steamboat Spr., CO
T.J. HAGERTY

President

J.B. FINLEY

President-Elect

H.W. TOWERS

Second Vice-President

J.C. SHOOK

Secretary-Treasurer

M.R. MARSHALL

First Vice-President

Third Vice-President
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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. Johnston, Springfield, IL</td>
<td>*Mr. D. O. Lively, Fort Worth, TX</td>
</tr>
<tr>
<td>2. Oct. 11-12, 1898</td>
<td>Omaha, NE</td>
<td>*Mr. C. P. Johnston, Springfield, IL</td>
<td>*Mr. Taylor Riddle, KS</td>
</tr>
<tr>
<td>3. Oct. 11-12, 1899†</td>
<td>Chicago, IL</td>
<td>*Mr. C. P. Johnston, Springfield, IL</td>
<td>*Mr. Mortimer Levering, Lafayette, IN</td>
</tr>
<tr>
<td>4. Oct. 2-3, 1900</td>
<td>Louisville, KY</td>
<td>*Mr. C. P. Johnston, Springfield, IL</td>
<td>*Dr. E. T. Eilenman, Louisville, KY</td>
</tr>
<tr>
<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, NY</td>
<td>*Dr. E. P. Niles, VA</td>
<td>*Dr. E. T. Eilenman, Louisville, KY</td>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<td>10. Aug. 15-16, 1906</td>
<td>Springfield, IL</td>
<td>*Mr. M. M. Hanks, Quanah, TX</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>11. Sept. 16-17, 1907</td>
<td>Richmond, VA</td>
<td>*Dr. D. F. Luckey, Columbia, MD</td>
<td>*Dr. C. E. Cotton, St. Paul, MN</td>
</tr>
<tr>
<td>13. Sept. 13-15, 1909†</td>
<td>Chicago, IL</td>
<td>*Dr. W. H. Daileymp, Baton Rouge, LA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</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, Gothen, 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. Ravener, 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. Bahnson, 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. J. Ferguson, Chicago, IL</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>20. Dec. 5-7, 1916</td>
<td>Chicago, IL</td>
<td>*Dr. G. 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>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>23. Dec. 1-3, 1919</td>
<td>Chicago, IL</td>
<td>*Dr. G. W. Dumphy, Lente, MI</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>24. Nov. 29-Dec. 1, 1920</td>
<td>Chicago, IL</td>
<td>*Dr. S. F. Musselman, Frankfort, KY</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>25. Nov. 28-30, 1921</td>
<td>Chicago, IL</td>
<td>*Dr. W. F. Creve, Bismarck, ND</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>26. Dec. 6-8, 1922</td>
<td>Chicago, IL</td>
<td>*Dr. T. E. M Munroe, Harrisburg, PA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>27. Dec. 6-7, 1923</td>
<td>Chicago, IL</td>
<td>*Dr. W. J. Butler, Helena, MT</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>28. Dec. 3-5, 1924</td>
<td>Chicago, IL</td>
<td>*Dr. J. G. Femeyhough, Richmond, VA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>29. Dec. 2-4, 1925</td>
<td>Chicago, IL</td>
<td>*Dr. J. H. McNell, Trenton, NJ</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>30. Dec. 1-3, 1926</td>
<td>Chicago, IL</td>
<td>*Dr. John R. Mohler, Washington, DC</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>31. Nov. 30-Dec. 2, 1927</td>
<td>Chicago, IL</td>
<td>*Dr. L. Van Es, Lincoln, NE</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>32. Dec. 5-7, 1928</td>
<td>Chicago, IL</td>
<td>*Dr. S. A. Cary, Auburn, AL</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>33. Dec. 4-6, 1929</td>
<td>Chicago, IL</td>
<td>*Dr. Chas. O. Lamb, Denver, CO</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</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, 1931</td>
<td>Chicago, IL</td>
<td>*Dr. J. W. Connaway, Columbia, MD</td>
<td>*Dr. O. E. Dyson, Wichita, KS</td>
</tr>
<tr>
<td>36. 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>*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>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>43. Dec. 6–8, 1939</td>
<td>Chicago, IL</td>
<td>*Dr. J. L. Asby, 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. Port, Cheyenne, WY</td>
<td>Dr. Mark Welsh, College Park, MD</td>
</tr>
<tr>
<td>45. Dec. 3–5, 1941</td>
<td>Chicago, IL</td>
<td>*Dr. E. A. Crossman, Boston, MA</td>
<td>Dr. Mark Welsh, College Park, MD</td>
</tr>
<tr>
<td>46. Dec. 2–4, 1942</td>
<td>Chicago, IL</td>
<td>*Dr. I. S. McAdory, Auburn, AL</td>
<td>Dr. Mark Welsh, College Park, MD</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. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>48. Dec. 6–8, 1944</td>
<td>Chicago, IL</td>
<td>Dr. J. M. Sutton, Atlanta, GA</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
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<tr>
<td>49. Dec. 5–7, 1945</td>
<td>Chicago, IL</td>
<td>Dr. C. U. Duckwork, Sacramento, CA</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
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<tr>
<td>50. Dec. 4–6, 1946</td>
<td>Chicago, IL</td>
<td>*Dr. William Moore, Raleigh, NC</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>51. Dec. 3–5, 1947</td>
<td>Chicago, IL</td>
<td>*Dr. Will J. Miller, Topeka, KS</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>52. Oct. 13–15, 1948</td>
<td>Denver, CO</td>
<td>*Dr. Jean V. Knapp, Tallahassee, FL</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>53. Oct. 12–14, 1949</td>
<td>Columbus, OH</td>
<td>*Dr. T. O. Brandenburg, Bismarck, ND</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>54. Nov. 1–3, 1950</td>
<td>Phoenix, AZ</td>
<td>*Dr. C. P. Bishop, Harrisburg, PA</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>55. Nov. 14–16, 1951</td>
<td>Kansas City, KS</td>
<td>*Mr. F. E. Mollin, Denver, CO</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>57. Sept. 23–25, 1953</td>
<td>Atlantic City, NJ</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>58. Nov. 10–12, 1954</td>
<td>Omaha, NE</td>
<td>*Dr. T. C. Green, Charleston, WV</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</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>
</tr>
<tr>
<td>60. Nov. 28–30, 1956</td>
<td>Chicago, IL</td>
<td>Dr. A. L. Bruckner, 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>
</tr>
<tr>
<td>63. Nov. 15–18, 1959</td>
<td>San Francisco, CA</td>
<td>Mr. F. G. Buzzell, Augusta, ME</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>
</tr>
</tbody>
</table>
### RECORD OF PREVIOUS MEETINGS

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<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, NM</td>
<td>*Dr. T. J. Grennan, Jr., Providence, RI</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>70. Oct. 10-14, 1966</td>
<td>Buffalo, NY</td>
<td>Dr. C. L. Campbell, Tallahassee, FL</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>71. Oct. 16-20, 1967</td>
<td>Phoenix, AZ</td>
<td>Dr. Grant S. Kaley, Albany, NY</td>
<td>*Dr. R. A. Hendershott, Trenton, NJ</td>
</tr>
<tr>
<td>72. Oct. 6-11, 1968</td>
<td>New Orleans, IA</td>
<td>Dr. John F. Quinn, Lansing, MI</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
<tr>
<td>73. Oct. 12-19, 1969</td>
<td>Milwaukee, WI</td>
<td>*Dr. John L. O'Hara, Reno, NV</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
<tr>
<td>74. Oct. 18-23, 1970</td>
<td>Philadelphia, PA</td>
<td>*Dr. Frank B. Wheeler, Baton Route, IA</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
<tr>
<td>75. Oct. 24-29, 1971</td>
<td>Oklahoma City, OK</td>
<td>*Dr. M. D. Mitchell, Pierre, SD</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
<tr>
<td>76. Nov. 5-10, 1972</td>
<td>Miami Beach, FL</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
<tr>
<td>77. Oct. 14-19, 1973</td>
<td>St. Louis, MO</td>
<td>*Dr. W. C. Tobin, Denver, CO</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
<tr>
<td>79. Nov. 2-7, 1975</td>
<td>Portland, OR</td>
<td>*Dr. J. E. Andrews, GA</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
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<tr>
<td>80. Nov. 7-12, 1976</td>
<td>Miami Beach, FL</td>
<td>*Dr. H. E. Goldstein, Columbus, OH</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
</tr>
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<td>81. Oct. 16-21, 1977</td>
<td>Minneapolis, MN</td>
<td>*Dr. A. E. Janawicz, Montpelier, VT</td>
<td>*Dr. W. L. Bendix, Richmond, VA</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>*Dr. W. L. Bendix, Richmond, VA</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>
<td>Dr. J. C. Shook, Hyattsville, MD</td>
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<td>84. Nov. 2-7, 1980</td>
<td>Louisvill, KY</td>
<td>Mr. B. W. Hawkins, Ontario, OR</td>
<td>Dr. J. C. Shook, Hyattsville, MD</td>
</tr>
<tr>
<td>85. Oct. 11-16, 1981</td>
<td>St. Louis, MO</td>
<td>*Dr. L. W. Hinchen, Indianapolis, IN</td>
<td>Dr. J. C. Shook, Hyattsville, MD</td>
</tr>
<tr>
<td>86. Nov. 7-12, 1982</td>
<td>Nashville, TN</td>
<td>Dr. G. B. Rea, Salem, OR</td>
<td>Dr. J. C. Shook, Hyattsville, MD</td>
</tr>
<tr>
<td>87. Oct. 16-21, 1983</td>
<td>Las Vegas, NV</td>
<td>Dr. J. R. Ragan, Nashville, TN</td>
<td>Dr. J. C. Shook, Annapolis, MD</td>
</tr>
<tr>
<td>88. Oct. 21-26, 1984</td>
<td>Ft. Worth, TX</td>
<td>Mr. J. O. Pearse, Jr., Okeechobee, FL</td>
<td>Dr. J. C. Shook, Annapolis, MD</td>
</tr>
<tr>
<td>89. Oct. 27-Nov. 1, 1985</td>
<td>Milwaukee, WI</td>
<td>*Dr. David U. Walker, Montpelier, VT</td>
<td>Dr. J. C. Shook, Annapolis, MD</td>
</tr>
<tr>
<td>90. Oct. 19-24, 1986</td>
<td>Louisvill, KY</td>
<td>Dr. N. W. Kruse, Lincoln, NE</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
</tr>
<tr>
<td>91. Oct. 25-30, 1987</td>
<td>Salt Lake City, UT</td>
<td>Dr. J. F. Hudelson, Denver, CO</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
</tr>
<tr>
<td>92. Oct. 16-21, 1988</td>
<td>Little Rock, AR</td>
<td>Dr. J. A. Cobb, Atlanta, GA</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</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>Dr. J. C. Shook, Mechanicsburg, PA</td>
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<tr>
<td>94. Oct. 6-12, 1990</td>
<td>Denver, CO</td>
<td>Dr. M. A. Van Buskirk, Harrisburg, PA</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
</tr>
<tr>
<td>95. Oct. 26-Nov. 1 1991</td>
<td>San Diego, CA</td>
<td>Dr. P. L. Smith, Sacramento, CA</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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+This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION

Michael R. Marshall, DVM
Salt Lake City, Utah

Our Eternal Father in Heaven, we are grateful to meet under such favorable surroundings as members of the USAHA and AAVLD.

We are thankful for this great free land which we enjoy. We humbly asked thy continued blessings upon the nations of the earth that are not free, and live under oppression and dictatorships; that these countries may continue in their quest for freedom and self rule.

Bless the leaders of our nation, that they may guide us toward world cooperation and understanding.

We are thankful to be involved in Agriculture and Animal Health. Bless our eyes and our minds to see and think properly, in order to sincerely know the needs of the animal kingdom and the people of the world.

We recognize and accept the great challenge we have; please help us to measure up to the task.

We thank thee for our families who guide and support us. Please help us to be understanding and patient with our spouses and children.

Dear Lord, we are grateful for the love and kindness, and trust that so many people give unto us. Help us to accept these stewardships with humility and always be willing to be of service.

We pray that you remember and care for those who have departed this life this past year:

Dr. L. W. Hinchman – Indianapolis, IN – President of USAHA 1981 – June 2, 1991

We are grateful to be thy children, and we say this in the name of Jesus Christ, Our Savior,

Amen.
I am very pleased to welcome the United States Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) members to California for their joint 1991 Annual Meetings. The City of San Diego will lend to the enjoyment and success of your meetings.

Usually out-of-staters and Californians themselves do not think of California for its agricultural contributions. We are very proud of our agricultural industry. California's agriculture accounts for one-tenth of the gross State product. It is estimated that agriculture-related activities contributed a total of $73 billion to the State's $730 billion gross product in 1990.

Today, with $18.3 billion in farm production, agriculture continues to play a dynamic role in our State and Nation's economy. California is considered one of the most diversified agricultural regions in the world with over 250 different crops and livestock commodities, with no one crop dominating the State's farm economy.

California's 30 million acres of farmland accounts for only 4% of the Country's farmland, but produces 50% of the Nation's fruits, nuts and vegetables.

Livestock, dairy and poultry receipts totalled $5.5 billion. The leading agricultural product in the State is milk and cream ($2.6 billion) followed in second place by cattle and calves ($1.7 billion). Cash receipts from chicken eggs in 1990 were $433 million, broilers - $338 million and turkeys - $275 million. There is an inventory of over 1 million head of sheep and lambs. We are the number two State in horse population.

In spite of the significant contributions of animal agriculture, it is coming into more public criticism. The future looks rocky. Funding of agriculturally directed programs is diminishing. We will all need to work closely in developing innovative cost-effective disease control programs for both plants and animals. We will need to pool our resources in order to accomplish future disease control objectives.

Monetary and societal constraints will be taken into consideration during the deliberations of the 95th Annual Meeting of USAHA and the 34th Annual Conference of AAVLD. Both of these organizations have had a long and productive history. I know you will have productive meetings in California. I sincerely wish that you enjoy the fine weather and the amenities of San Diego.
RESPONSE TO WELCOME ADDRESS

D. L. NOTTER

Thank you, Mr. President, Director Voss, USAHA, AAVLD, Members and guests.

We are pleased that the sight selection committee chose Louisville for the 1992 convention. This will be the third time Kentucky has been the host state for the USAHA conference. I assure you that we plan to make your visit one that you won't forget.

There are many interesting sights in Louisville and the surrounding area.

Churchill Downs is located here where the Kentucky Derby is run the 1st Saturday in May. Located at Churchill Downs is the Kentucky Derby Museum that is popular and houses many racing and equine artifacts.

Louisville is known for its riverfront development. You may want to visit its' Fine Arts Center which is located close to the Galt House, the convention site.

There are many beautiful horse farms in the area, The Kentucky Horse Park, My Old Kentucky Home, and many other historical sites. And for those of you who aren't used to seeing the season changing into the brilliant colors of fall, you are going to find that Louisville is especially beautiful during this time of the year.

Again, we looking forward to your visit next year and appreciate this opportunity to invite you to Kentucky.

My wife Donna and I, along with my staff and the Department of Agriculture will do our utmost to make your stay most enjoyable.
I want to extend my welcome to the members of the United States Animal Health Association (USAHA), members of the American Association of Veterinary Laboratory Diagnosticians and guests. Having the annual meeting in my home State of California has added to the pleasure of serving as the 1991 President of the USAHA.

During the past year, I have had many pleasant tasks as President. One of the most pleasant is recognizing an individual who has contributed in innumerable ways to USAHA. He has served as president, parliamentarian, helper, fixer and principal resource man. He has always been available to assist USAHA in so many ways, it is impossible to recognize them all. I like to refer to him as Mr. USAHA. In recognition of his long service to the organization, I have proclaimed Monday, October 28, as Dr. Clarence L. Campbell Day. This is a day for all of us to share in recognizing the contributions of Dr. Campbell.

Last year in my President-Elect speech, I used the words from an old song, "Accentuate the Positive", to set the tone of how we should be approaching our work and relationships with each other. We need to "accentuate the positive and eliminate the negative". There are difficult days ahead. Continued limited assets available to devote to livestock welfare and health programs will accentuate the difficulties. As never before, it is time to consolidate and coordinate our collective resources toward resolving animal health issues. We will have our differences in opinion, but we must put emphasis on what we can do and not on what we cannot do. We must put emphasis on what we agree on rather than what we disagree about. So in approaching this 1991 Annual Meeting, let us all accentuate the positive.

Let's have fun at this annual meeting. It is serious business that we are about to engage in, but we can have fun dealing with it. Who ever said you should not have fun working? Let's all work hard here in San Diego and at the same time enjoy ourselves.

Being President of USAHA has been one of the outstanding highlights of my professional career. I sincerely thank you for allowing me the opportunity to serve as President.
Dr. J. Lee Alley, President-Elect, USAHA, presents a plaque to outgoing President, Dr. Patton L. Smith for his contributions and outstanding leadership in 1991.

Dr. Smith presenting plaque to Dr. Campbell for Campbell Day.
REMARKS OF THE PRESIDENT OF AAVLD

John J. Andrews, D.V.M., Ph.D.
Ames, IA

US Animal Health Association President Smith, US Animal Health Association President Elect Alley, AAVLD Incoming President Thacker, honored guests, members of USAHA and AAVLD, ladies and gentlemen. As one of my last official acts as outgoing President of AAVLD, I would like to introduce to you, Dr. Leon Thacker, the Director of the Animal Disease Diagnostic Laboratory at Purdue University. Dr. Thacker was installed earlier today as incoming President of the American Association of Veterinary Laboratory Diagnosticians and has now officially begun his service to our organizations in this role. May I present Dr. Thacker. Thank you.

It has been my distinct pleasure to serve AAVLD and USAHA this past year as AAVLD President. Yes, I said serve both AAVLD and USAHA. Just as Dr. Smith and Dr. Alley have served AAVLD in their roles as USAHA officers, so have we served USAHA. We are too closely related to ignore each other and in fact, we do not. We share meeting facilities, we share receptions and joint meetings and we share many of the same goals and concerns related to animal health issues. We are symbiotic, not parasitic nor opportunistic.

Nearly one-third to one-half of the members of many of the USAHA committees are active AAVLD members and many of those individuals are active USAHA committee chairs or co-chairs. Likewise, nearly one-third of the AAVLD members, like myself, are USAHA members. Many of the programs and resolutions of the USAHA committees from the Pseudorabies Committee to the Committees on Infectious Diseases of Sheep and Goats, Cattle and Horses simply cannot be accomplished without the full support and cooperation of diagnosticians and veterinary diagnostic laboratories across the US. I know of few other organizations that share these close ties of membership, issues and interests as does USAHA and AAVLD.

Our organizations could, perhaps, exist separately but what useful purpose would that separation serve? It would not be to the benefit of either group. The officers of both organizations have sought to enhance this USAHA/AAVLD symbiosis. At last year's meeting, Dr. Pat Smith told this group that we must "accent the positive". I believe that he and the other officers of USAHA have indeed sought to do that. We have enjoyed the open lines of communication that have been extended between USAHA and AAVLD. We must continue that positive exchange.

AAVLD is growing in members, activities and quality. In 1991, AAVLD expanded its membership to over 750, it recognized Canada as a fully represented region of the organization, it published its third volume of the Journal of Veterinary Diagnostic Investigation, it assisted 34 laboratories in 30 states to attain or maintain full laboratory accreditation and is helping
many other laboratory systems to improve their facilities, staffing and their capabilities. At this meeting, AAVLD members have presented over 110 scientific posters and papers in the AAVLD sessions and numerous other presentations at USAHA committee meetings. Out membership is strong and it is active. It has been a fantastic group to work with and to work for.

For both the AAVLD and the USAHA to fulfill their missions, we need each other. Let's continue the symbiotic relationship into and beyond the presidencies of Drs. Alley and Thacker. Let's continue to take the positive steps needed to support, enhance and assist both organizations for the ultimate benefit of the livestock producers and the consumers of this great country.
Good evening. Tonight's USAHA's Animal Health Award is being presented to a veterinarian whose seniority and breadth of contribution to State and National animal and public health have earned him the respect of the entire United States veterinary profession and livestock industries. No person yet, and perhaps no one ever will, serve his profession as a State Veterinarian with such a long record of dedication, commitment, and effectiveness.

The high standards set by the 1991 USAHA awardee, in so many different areas of his work, have cemented a fine tradition for all who follow in the years ahead. One cannot help but marvel at the awardee's ability to swim the political and professional tide, -- in one career position -- for almost 40 years. Talk about staying power and a sense of mission!

For those who have not already guessed tonight's recipient, I will give you another hint. He was born in Indiana. However, at a very early age, "Soup" Campbell brought his family southward to settle in Sebring, Florida. Since then he has lived in his "chosen" native State, acquiring there all of his formal education and training with two exceptions: a short stint for his Doctor of Veterinary Medicine Degree at Ohio State University in 1945 and, afterwards, six months of private practice in Kewanee, Illinois.

That same year he returned to Florida as a field veterinarian with the Florida Livestock Sanitary Board (now a part of the Florida Department of Agriculture and Consumer Services). After serving in this capacity for seven years, he was appointed Acting Florida State Veterinarian. A year later, in June 1952, until retiring this past August, he persevered (worked) effectively and tirelessly as the Florida State Veterinarian.

Dr. Campbell has been nationally recognized as the State Veterinarian whose tenure in office is longer than any other in America's history. However, endurance is but one of his admirable traits. He has been responsible for many successful disease eradication programs that have significantly contributed to the health and well-being of the livestock industry throughout Florida and the South. Among the most notable are eradication of the screwworm in Florida and the southeastern States, development of a control program for Equine Infectious Anemia, eradication of hog cholera and tuberculosis from Florida, and his firm leadership in Florida's brucellosis eradication program.

Clarence Campbell's vision has never masked his practicality. Failure is in his vocabulary. With every project or crisis, he first thinks...
through the situation. He remains staunch and prepared, always cognizant of what the cost will be in money and effort, keeping his eye on that important quality called "timing." I have heard it whispered that the secret Campbell weapon is an innate "seventh sense" of when to act. He never pushes beyond what can realistically be accomplished or what his constituencies are ready to accept and support.

Dr. Campbell's accomplishments in brucellosis eradication illustrate this basic instinct for good timing. His personal collegiality and ease in communicating have enhanced his understanding, for instance, of what industry would and would not support. As State Veterinarian, he marked time patiently until the late 70's when the setting finally seemed right. In April, 1979, sensing that industry might be ready to support a full-scale brucellosis program, he advocated their doing so. With his encouragement, legislation was drafted and shortly thereafter was enacted in October of 1980. The impact of his leadership in this momentum is best described by a few statistics. In the fiscal year 1979–80, 28,000 infected animals were removed from Florida ranches after testing one million cattle in the State. 12 years later, in June 30, 1991, 460 reactors were removed from 900,000 tested.

How Dr. Campbell's vision led to his early involvement with screwworm eradication is a pioneering legend. This program was the first to succeed in using biology as a form of eradication tool. Based on the positive results achieved under Dr. Campbell's supervision in Florida, this major undertaking was later moved to Mission, Texas and has now been expanded to its present work-site in Mexico.

Of his many credits, honors, and achievements, I will mention a few of those of which I am told he is most proud. In 1963, Dr. Campbell was named Man of the Year in Service to Florida Agriculture by the PROGRESSIVE FARMER MAGAZINE. In 1985, he was presented the Florida Veterinary Medical Association Award of Veterinarian of the Year and an award from the National Assembly of Chief Livestock Health Officials. In 1987, he received the Florida Department of Agriculture and Consumer Services Distinguished Service Award. And in 1990, the National Association of State Departments of Agriculture honored him with the NASDA 1990 Honor Award for Service.

Dr. Campbell has served USAHA with distinction and dedication. In addition to being President in 1965–66, through the years he has chaired the following USAHA committees: Nationwide Eradication of Hog Cholera, Public Relations, State Federal Relations, Import–Export and Infectious Diseases of Horses. He has also served as President of the National Assembly of State Veterinarians, the Southern Animal Health Association, and the Arabian Horse Association of Florida. The list of his contributions to professional and governmental committees goes on and on.

And so tonight we are proud to salute one of USAHA's own. Clarence, we wish you well in retirement. You have earned the right to choose to pass the gauntlet and to pursue your special hobbies too–long
neglected because of your untiring commitment to a very heavy work schedule.

At last, you can play golf to your heart's content. It is no longer necessary to have to squeeze in a few holes between meetings or during convention recesses. You can, also, finally concentrate on raising and showing your esteemed Arabian horses, and, hopefully, you will be able to sell more than has been possible previously. I must add in this regard that any past lack of sales has resulted not lack of free time or too high a price tag but rather your intense, impeccable screening of prospective buyers. Not every breeder, like you and your wife, have such demanding standards to assure that their animals will continue to be treated and cared for as if their own.

At this time it is my privilege to present Dr. Clarence "Soup" Campbell, with the 1991 USAHA Animal Health Award – recognizing a lifetime of practicing and upholding the highest standards of the veterinarian profession in advancing animal health, care, and well-being.
Dr. Michael R. Marshall, President of the National Assembly of Chief Livestock Health Officials, presents the third National Assembly Award to Dr. Paul B. Doby, Superintendent, Illinois Department of Agriculture. 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 ....................... J. L. Alley, Montgomery, AL.
President-Elect ................... T. J. Hagerty, St. Paul, MN.
First Vice-President ............. J. B. Finley, Jr., Encinal, TX.
Second Vice-President ........... H. W. Towers, Jr., Dover, DE.
Third Vice-President ............. M. R. Marshall, Salt Lake City, UT.
Treasurer ....................... J. C. Shook, Mechanicsburg, PA.

Regional Delegates

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

RESOLUTIONS

United States Animal Health Association
Passed October 26–November 1, 1991
San Diego, California

Resolution No. 1

Source: Committee on Rabies
Subject Matter: CDC Service in Rabies Programs

RESOLUTION

RESOLVED that the United States Animal Health Association encourage CDC to continue maintaining the services it provides in areas of rabies diagnosis, prevention, and control.

Resolution No. 2 (same as 5)

Source: Committee on Wildlife Diseases and Committee on Tuberculosis
Subject Matter: Tuberculosis in cervids, exotic species and other animals

RESOLUTION
RESOLVED that the United States Animal Health Association strongly encourages USDA/APHIS/VS to take all steps necessary to validate appropriate tests, develop authority to regulate the interstate movement of cervids, exotic species and other animals, and develop appropriate tuberculosis control guidelines for these species.

Resolution No. 3

Source: Committee on Parasitic Diseases and Parasiticides
Subject Matter: Products for Exclusion and Eradication of Ticks and Alternative Tick Eradication Strategies for White–Tailed Deer and Exotic Wildlife

RESOLUTION

RESOLVED that USDA, ARS increase their efforts in developing new acaricides and alternative tick eradication strategies including the development of technology for the control of ticks on white–tailed deer and exotic wildlife and that the USDA, APHIS should actively support the registration of highly efficacious products expected to be useful in exclusion and eradication of ticks of significant economic importance to the United States.

Resolution No. 4

Source: Committee on Transmissible Diseases of Swine Committee, United States Animal Health Association
Subject Matter: Swine Infertility and Respiratory Syndrome

RESOLUTION

RESOLVED that USAHA recommend the National Veterinary Services Laboratories and ARS cooperate to develop, as rapidly as scientific technology will allow, a diagnostic test for SIRS suitable for use in diagnosis and testing for movement and that The Swine Diseases Staff should coordinate the design and implementation of studies on SIRS in consultation with NAHMS, NVSL, AASP, NPPC, and university researchers.

Resolution No. 5 (same as 2)

Source: Committee on Wildlife Diseases and Committee on Tuberculosis
Subject Matter: Tuberculosis in cervids, exotic species and other animals

Resolution No. 6

Source: Committee on Brucellosis
Subject Matter: Brucellosis Education

RESOLUTION

RESOLVED that the Brucellosis Committee of the USAHA encourage the re-establishment of funding by APHIS for the continued production and dissemination of educational materials in behalf of the Brucellosis Eradication Program.

Resolution No. 7

Source: Committee on Brucellosis
Subject Matter: Monitoring Brucellosis in Yellowstone Bison

RESOLUTION

RESOLVED that the USAHA support the lethal collection of bison in the greater Yellowstone area to establish the infection rate and serologic correlation with infection as determined by isolation from tissues.

Resolution No. 8

Source: Committee on Animal Disease Surveillance and Animal Health Information Systems
Subject matter: Risk Assessment on the Mexico – U.S. Free Trade Agreement

RESOLUTION

RESOLVED that a qualitative and quantitative risk analysis be conducted on the impact of the impending Bilateral Free Trade Agreement with Mexico on the livestock health and production in the United States.

Resolution No. 9

Source: Committee on Animal Disease Surveillance and Animal Health Information Systems
Subject Matter: Risk Assessment on TB introduction from Mexico and on the TB problem in Cervidae

RESOLUTION

RESOLVED that USAHA request APHIS, VS to conduct two qualitative and quantitative risk analyses: on (1) the importation of TB infected steers from Mexico concurrent with any other activities being implemented to solve this problem of TB introduction, and (2) TB in Cervidae in the U.S.
Resolution No. 10

Source: Committee on Sheep and Goats
Subject Matter: Bluetongue Vaccine

RESOLUTION

RESOLVED that USAHA take the necessary steps to allow the conditional use of the California bluetongue vaccine in areas which can show problems in sheep due to bluetongue virus.

Resolution No. 11

Source: Committee on Sheep and Goats
Subject Matter: Importation of Sheep Genetic Material and Live Animals

RESOLUTION

RESOLVED that USAHA urge and request that a protocol be approved by USDA to permit the controlled importation of sheep genetic material (frozen semen, frozen embryos) and live animals into the United States and that all imports will participate in the Scrapie Certification Program.

Resolution No. 12

Source: Committee on Sheep and Goats
Subject Matter: Immunoblotting Test for Scrapie Diagnosis

RESOLUTION

RESOLVED that USAHA request USDA to support the use of immunoblotting as a supplemental test for diagnosis for scrapie.

Resolution No. 13

Source: Committee on Import and Export
Subject Matter: Importation of Embryo and Germplasm

RESOLUTION

RESOLVED that USAHA strongly urgeAPHIS to proceed immediately to amend Rule 98 to include ovine and caprine embryos, as well as other genetic material, and see that it is ready for public comment, immediately following release of the Scrapie pathology results. This is with the understanding that all genetic imports will go into the Scrapie Certification Program.
Resolution No. 14

Source: Committee on Infectious Disease of Cattle, Bison and Llama
Subject Matter: Protozoal Abortion in Cattle

RESOLUTION

RESOLVED that USAHA recommend that the United States Department of Agriculture support the following types of studies aimed at reducing the impact of the protozoal abortion agent in cattle productivity:

1. Characterization of the agent
2. Development of diagnostic tests
3. Characterization of pathogenesis
4. Characterization of the epidemiology, and approaches to control and/or eradication

Resolution No. 15

Source: Committee on Import/Export
Subject Matter: Proposed USDA/APHIS TB Project

RESOLUTION

RESOLVED that USAHA strongly recommend to USDA/APHIS that they take the leadership role in developing and funding a USDA/APHIS project utilizing the TB infected herds to determine if properly washed bovine embryos collected from TB infected donors can transmit TB to TB negative recipients or resultant offspring.

Resolution No. 16

Source: Committee on Bluetongue and Bovine Retrovirus
Subject Matter: Bluetongue

RESOLUTION

RESOLVED that USAHA recommend the United States Department of Agriculture investigate the feasibility of the following actions in regards to bluetongue in ruminants:

1. Work within the framework of the Office International des Epizooties (OIE) to eliminate bluetongue from List A of reportable diseases.
2. Modify Animal Health Trade requirements of ruminants and their products and germplasm to be consistent with the evidence that the
duration of bluetongue viremia of ruminants is less than 3 months.

3. Consider demonstration of bluetongue virus in the blood rather than semen or other secretious excretions as the definitive test in determining the bluetongue virus status of ruminants. Tests which determine antibody levels in serum should be considered as screening tools.

4. Initiate research to determine virulence of the Central America/Caribbean bluetongue virus serotypes for North American domestic and wild ruminants and to determine vector competence for transmission of these viruses by U. S. Culicoides species.

Resolution No. 17

Source: Committee on Tuberculosis
Subject Matter: Creation of a Task Force committee on Tuberculosis in Mexican Cattle

RESOLUTION

RESOLVED that USAHA strongly urges USDA to create a Task Force Committee to address the need for regulatory changes targeted at preventing the transmission of tuberculosis from cattle imported from Mexico and be it further resolved that USAHA urges the Task Force committee to consider the measures contained in the attached proposed requirements, and that the Committee will include but not be limited to representatives of each of the following groups: importers of Mexican cattle, the dairy industry, cow/calf operations which do not import cattle, rodeos, feedyards, the National Assembly of Chief Livestock Health Officials, the United States Animal Health Association, the United States Department of Agriculture and the American Veterinary Medical Association.

Resolution No. 18

Source: Committee on Tuberculosis
Subject Matter: Importation of cattle from Mexico

RESOLUTION

RESOLVED that USAHA urge USDA/APHIS/VS to require that all imported cattle from Mexico be consigned only to designated feedlots or other approved facilities, for feeding only or release after subsequent negative test, until such time as an adequate testing program for tuberculosis be established at the Mexican – U.S. Port of Entry.

Resolution No. 19
Source: Committee on Tuberculosis
Subject Matter: Depopulation of tuberculosis infected herds

RESOLUTION

RESOLVED that USAHA recommend that USDA/APHIS/VS develop adequate indemnity funding for tuberculosis infected cattle herds and require complete depopulation of all tuberculosis infected cattle herds in the United States.

Resolution No. 20

Source: Committee on Pseudorabies
Subject Matter: Feral Swine

RESOLUTION

RESOLVED that the United States Animal Health Association endorses the concept that states that do not have free roaming feral pigs be encouraged to prohibit their entry.

Resolution No. 21

Source: Committee on Pseudorabies
Subject Matter: Common Gene Deletion for PRV Vaccine

RESOLUTION

RESOLVED that the United States Animal Health Association encourages the manufacturers of PRV vaccine to work toward a common gene deletion pseudorabies vaccine.

Resolution No. 22

Source: Committee on Pseudorabies
Subject Matter: Feral Swine Pilot Studies

RESOLUTION

RESOLVED that USAHA recommend to APHIS, NPPC and the Southeast Wildlife Disease Research Center that pilot studies be undertaken in states of high feral swine populations with the objectives of developing effective, practical methods for prevention of transmission of pseudorabies and swine brucellosis between feral and domestic swine and for control/elimination of infection from feral swine. Suggested for studies are Florida, Georgia, Texas and California.
Resolution No. 23

Source: Committee on Epizootic Attack
Subject Matter: Swine Brucellosis Indemnity

RESOLUTION

RESOLVED that USAHA urge APHIS-VS to increase indemnities for brucellosis infected and exposed swine and require herd depopulation in all infected herds.
REPORT OF THE COMMITTEE ON ANIMAL DISEASE
SURVEILLANCE AND ANIMAL HEALTH INFORMATION
SYSTEMS

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

J.A. Acree, MD; B.L. Akey, VA; J.L. Alley, AL; L.A. Anderson, IA; C.W. Beard, GA; D.L. Berndt, TX; J.J. Bohlender, CO; S.L. Diesch, MN; C.R. Dorn, OH; T.W. Freas, IN; D. Galbreath, MD; M.J. Gilsdorf, MD; M.S. Gosser, MO; F.D. Gregerson, CO; F. Gvillo, CA; B.R. Heron, CA; D.W. Hird, CA; J. Honstead, MD; W.D. Hueston, CO; M.E. Hugh–Jones, LA; L. Hutchinson, PA; N.E. Hutton, OR; L.J. King, MD; D.H. Lein, NY; H. Lloyd, FL; L.D. Mark, VA; E.H. McCauley, MT; T.J. McGinn, NC; R.W. Mead, WA; D. Meeker, IA; W.R. Miller, AL; L.G. Morehouse, MO; J.C. New, TN; J.C. New, TN; W.J. Owen, IA; J.C. Paige, MD; C. Palmer, CA; A.B. Park, MD; P.A. Pickerill, TX; E.I. Piichard, MD; G.C. Poppensiek, NY; J.C. Pruch, MD; P.F. Ross, IA; L.H. Russell, TX; M.D. Salman, CO; V.A. Seaton, IA; R. L. Tharp, MO; W.L. Thomas, OH; W. Utterback, CA.

The committee met at 1:30 p.m., Monday and Tuesday, October 28 and 29, 1991. Fifty–two members and guests were in attendance.

Dr. Will Hueston, Colorado, presented an impressive and informative Qualitative and Quantitative Risk Analysis of Bovine Spongiform Encephalopathy comparing sheep and cattle populations, slaughter, feed, and rendering industries of the U.S. and the United Kingdom. This analysis technique promises to be an important epidemiological tool for assessment of disease threat.

Dr. Mike Chaddock, Michigan, presented a report on the Michigan Equine Monitoring System (MEMS) designed to identify Michigan's major equine diseases and risk factors.

Dr. Bruce Akey, Virginia, presented a Geographical Information System for the state's poultry industry.

Dr. Arch Park, Maryland, outlined the activities of Animal Health Information Systems portion of this committee and will present information on systems available and the application of satellite imaging to disease assessment to the committee next year.

Dr. Charles Palmer, California, presented details on an epidemiological study of Protozoal Abortion Syndrome in California. This project uses a NAHMS–like approach and was initiated by California's Emergency Disease (ADEPT) Program when California's proposed NAHMS add–on was rejected. Over 200 dairy herds and six beef herds have been diagnosed with this syndrome, many of which have suffered abortion storms as described by others at this convention. The project has enrolled 100% of the dairies given the opportunity to enroll.
A section on Dx Monitor, a recently initiated laboratory reporting system, was led by Dr. James Case, California, who gave its history and the progress in refining the report and extending the coverage to other states.

Dr. John Thomson, South Dakota, described their work on improving the use of laboratories in diagnostics, including the development of a users' guide. Dr. Don Lein, New York, discussed the monitoring system and the development of materials to aid in enrolling other state laboratories in the system.

Dr. Lonnie King, Acting Administrator of APHIS, visited and informed the committee of the progress on regionalization of disease reporting and of Veterinary Services' intentions to provide more than 30 new epidemiological officers to that structure.

Dr. Scott Hurd, Colorado, presented an overview of the swine NAHMS project and the results obtained. He announced that a swine disease detailed report should be available in November for those who request it. He also presented preliminary results of the National Dairy Heifer Evaluation Project and the status of preparation for the Beef Monitoring Project.

Two resolutions were passed, both dealing with the use of Qualitative and Quantitative risk analysis. One applied to the risk of tuberculosis introduction via Mexican steers and an assessment of cervidae, the second an analysis of the effect on animal health of the proposed free trade agreement with Mexico.

A recommendation was passed asking the President of USAHA to contact the Administrator of APHIS to recommend continued support of funding of Risk Analysis Services by Veterinary Services, and commending the outstanding Qualitative and Quantitative Risk analysis of Bovine Spongiform Encephalopathy by Dr. Will Hueston, et al.

A discussion of the purposes of the committee was held and a statement prepared as directed by the President of USAHA.
The meeting of the Committee on Animal Welfare was called to order by Chairman Dr. Morton Silberman at 1:30 PM, October 29, 1991. The meeting was attended by 24 members and 42 guests.

The Chairman introduced Acting APHIS Administrator Robert Meland who expressed his appreciation of the role of USAHA in bringing together government and industry in responding to livestock industry challenges. He also praised APHIS-REAC for its work in assuring the welfare of animals in the United States.

The Chairman opened the meeting by citing the PETA advertisement in the Des Moines Register comparing the raising of livestock to the work of an alleged mass murderers as an example of how not to solve animal welfare challenges. He also urged that people involved in agriculture volunteer to serve as public representatives on university Animal Care Committees.

The committee program consisted of six individual speakers and one panel discussion.

Dr. Victor Nettles, University of Georgia, reported on and urged attendance at an AVMA sponsored symposium on animal welfare and wildlife scheduled for November 7, 1991, in Chicago, Illinois.

Dr. Joan Arnoldi, Deputy Administrator, USDA-APHIS-REAC, discussed the organization of REAC and presented a statistical report of REAC's 1990 operations. She said that with increased funding for FY91 and FY92, REAC has significantly increased its ability to achieve its mission.

Dr. Norman Willis, Agriculture Canada, discussed animal welfare regulation in Canada and the Canadian Codes of Practice being developed by Agriculture Canada. He said copies of the Codes may be obtained by contacting Agriculture Canada.

Dr. Carolyn Stull, University of California – Davis, presented a report entitled Interaction of Welfare Parameters in Calf Management Systems. Dr. Stull presented preliminary findings of the project. The Chairman expressed
REPORT OF THE COMMITTEE

the committee's interest in an update of the research in the future.

Ms. Cathy Liss, Animal Welfare Institute, presented concerns about the international trade of wild birds, the poaching of elephants and smuggling of ivory, and the treatment of nonambulatory animals at the South St. Paul, Minnesota, stockyard.

Dr. Richard Crawford, USDA–APHIS–REAC, discussed REAC's developing regulations on the animal welfare aspects of exotic animal auctions.

Chairman Silberman then introduced a panel to discuss animal welfare concerns relating to the commercial raising of dogs and cats in the United States. The panel members included:

1. Dr. Arnoldi, who presented the reasons and justification for organizing REAC's Dog Dealer Task Force.
3. Mr. Robert Baker, Humane Society of the United States, who responded to the task force findings from the perspective of an animal protection organization.
4. Mr. Marshall Meyers, who responded to the task force findings from the perspective of pet dealers.

No resolutions were presented to or approved by the committee during its meeting.

The meeting was adjourned at 5:30 PM.
The meeting was opened by Dr. Robert Goetz, acting Chairman, following attendee introductions. The 1990 Committee Report was reviewed with no objections or comments.

Review of Past Resolution Action

The Committee was presented correspondence in the form of three letters in response to resolutions passed by the Aquaculture Committee. Formal action in the formation of three Joint Subcommittees between the U.S. Department of Agriculture and the U.S. Department of the Interior has occurred. The three JSAs deal with aquatic health management, protective statutes, including the Lacey Act and depredation and research and technology transfer. The lack of aquaculture related funding was addressed in the form of a letter from Dr. John Pitts, Washington State Department of Agriculture.

Dr. V. Nettles, Ga., recommended that the Committee, via the Chairman through President Smith, request the final reports referred to in Dr. James Glosser's, APHIS, letter to Dr. Smith regarding the December 13, 1990 meeting of the work groups on interagency cooperation; and further to request all subsequent reports of the JSA's for distribution to the committee membership. Accepted without discussion.

A second recommendation to request President Smith to invite Dr. Althea Langston, APHIS, and Dr. John Nickum, FWS principles of the JSA's, to provide the 1992 Aquaculture Committee with updates of the JSA action in the form of verbal presentations was made. The recommendation was amended to include an invitation to a member of the FDA/CVM to discuss FDA action on therapeutic agents for aquaculture. Accepted without objection.
REPORT OF THE COMMITTEE

Availability of Therapeutants for Aquaculture

Recent FDA action to restrict the use of chemicals and therapeutic agents to only FDA approved materials was discussed. The lack of adequate therapeutants has and will continue to be of concern to the aquaculture industry, as well as the FWS. Dr. V. Nettles read a statement from a resolution of the International Fish and Wildlife agencies, expressing the need for more therapeutic agents to which they have formed a committee to identify therapeutants, rank them as to need and to address sources of funding for new drug development. A similar letter from the U.S. Trout Farmers Association was also read.

A recommendation was made that the Committee request President Smith to authorize and fund the attendance of the Committee Chairman to a December 5–6, 1991 meeting in Washington, D.C. of the FDA, during which the availability and registration of therapeutants will be discussed. Accepted. As an alternative, or in addition, the findings and report of this meeting should be provided to the USAHA Aquaculture Committee for membership distribution.

Stallings Bill

A bill for funding aquaculture has been written and submitted by Hon. Richard Stallings, Idaho. The bill addresses 6 major points:

1. Designation of U.S. Department of Agriculture as lead agency for private aquaculture.
2. Establish the USDA Office of Aquaculture for policy and development.
3. Provide funding for research and education.
4. Designate Aquaculture is Agriculture.
5. Implement research for therapeutants.
6. Require USDA and USDI–FWS to study application of the Lacey Act to private aquaculture.

Dr. Nettles requested that the Stallings Bill be provided to the Committee membership for review. The membership will be requested to provide response to the chair. If response is negative, no action. If response is positive, Chair to request the President to support the Bill. This was recommended and passed unanimously to the effect that the membership will be provided a copy of the Bill, along with a questionnaire and action taken according to the desire of the membership.
AQUACULTURE

Presentation – Dr. Alfred Montgomery

Alfred Montgomery is a Consumer Safety Officer with the Office of Seafood, FDA. Dr. Montgomery provided an overview of the FDA involvement in aquaculture, including the newly formed Office of Seafood. The office is responsible for the inspection of seafood, fish and fish products for import. The FDA is coordinating with the U.S. Department of Commerce-NOAA on a 'pay for service' inspection system, which will provide seafood a mark (stamp) of approval for meeting inspection standards.

Presentation – Dr. Robert Goetz

Dr. Goetz is co-owner of Keo Fish Farms Inc., Keo, Arkansas. Dr. Goetz gave a slide presentation on hybrid striped bass culture. As an aquaculture industry, hybrid striped bass had its beginnings during the past 10 years. It has grown from a fledgling industry dependent solely on wild brood stock to a maturing industry, which is actively developing a domestic brood stock source. Hybrid striped bass are the result of a cross between striped bass and white bass. Dr. Goetz discussed the development of the husbandry practices for a new industry. The hybrid striped bass industry has grown rapidly and has become an integral part of the aquaculture industry.

Final business concerned the adoption of a purpose statement for the Aquaculture Committee. Thanks in large part to the efforts of Dr. Jerry Walker, D.C., and Thomas Goodrich, WA., a statement was written discussed and adopted by the Committee.

Without further new business, Dr. Goetz closed the meeting.
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; G.A. Anderson, KS; G.M. Buening, MO; M.L. Chapek, NE; G.S. Colgrove, MD; M.L. Crandell, VA; S.R. Ellsworth, NE; D.A. Espeseth, MD; W.H. Fales, MO; J.E. Finnell, IL; R.H. Fulkar, IA; E.P.J. Gibbs, FL; J.S. Gloyd, IL; T.D. Goodrich, WA; J.A. Gourlay, CA; F. Gvillo, CA; K.N. Haffer, NE; D.D. Hancock, WA; S.K. Harris, IA; M. Huff, CO; R.D. Hull, IL; W.L. Kadel, KY; E. Keahey, TX; K.K. Krafka, IA; L.H. Lauerman, Jr., AL; L. Leach, VA; H.M. Lefler, CA; R.L. Levings, IA; D.F. Long, FL; S. McConnell, TX; H.A. McDaniel, MD; R.W. Mead, WA; T.R. Mickel, GA; L.F. Moore, KS; J.B. Payne, TX; D.C. Randall, Jr., IA; J.A. Schmitz, NE; R. Schultz, IA; R.R. Simonson, MN; R.F. Taylor, NJ; O.H. Timm, CA; J.D. Todd, KS; P.R. Turner, TX; H.E. Vanderslice, DE; D.L. Weiss, MN; G.B.E. West, CA; G. Wilder, MO; J.M. Williams, MO; W.H. Wohler, TX.

Forty-four persons attended the meeting on October 30, 1991. Applications for committee membership were invited. Adoption by the Executive Committee of the 1990 committee resolution to extend the exemption status of certain biologics was noted (Resolution No. 38).

Dr. David A. Espeseth, Veterinary Biologics, discussed current Veterinary Biologics licensing issues and summarized the FY 1991 licensing activities. The implementation phase of the 1985 amendment to the Virus Serum-Toxin-Act of 1913 is virtually complete. There are 116 licensed veterinary establishments producing 1984 licensed products. Approximately forty of the licenses are for products resulting from the application of biotechnology. The total also includes 132 diagnostic product licenses and 162 product licenses for further manufacture. During FY 1991, 66 import permits were issued and 95 field trials were approved. The National Veterinary Services Laboratories tested 9.44% of all serials eligible for testing and found 2.17% of these unsatisfactory; slightly higher than the 1.87% found unsatisfactory in FY 1990. Nine proposed, interim and final rules were published and 6 regulations are in the process of adoption. Important current issues include in vitro testing, post-licensing monitoring and international harmonization. Dr. Espeseth announced that the Veterinary Biologics Staff was reorganized, effective October 7, 1991, to increase coordination and effectiveness. Veterinary Biologics is sponsoring a meeting on "Characterization and Quantitation of Immunogens" May 5 and 6, 1992, and a Fourth Public Meeting on Biologics in August 1992.

In an update on state initiatives to restrict the distribution and use of licensed biologicals, Dr. Espeseth stated that APHIS recognizes individual states may impose restrictions on the distribution and use of licensed biological products on a case by case basis relating to local disease conditions. States may also petition APHIS to impose restrictions on a
veterinary product. However, APHIS opposes general restrictions having the
effect of restricting distribution and use of licensed biologicals within a state.
Some state regulations limiting distribution and use have lapsed, some have
been dropped and some have been placed on hold.

Dr. Donald C. Randall, Veterinary Biologics Field Office, reported on
a new program for post-licensing monitoring. Dr. Rick Hill is chairman of the
committee developing the program. The need for this program is related to
1) growth of the biologics industry, 2) increasing consumer expectations, 3)
changing practices in animal health, 4) need for post-licensing detection of
low frequency events which may not appear pre-licensing.

Veterinary Biologics Field Office functions now include pre-licensing
evaluations, inspections, testing, and post-licensing monitoring. An initial
thrust of the office is to collect data and establish baselines.

Dr. Robert Miller, Chief Staff Veterinarian, Veterinary Biologics,
reported on the progress of field trials on a recombinant rabies vaccine
developed by the Wistar Institute. In August of 1990 field trials to orally
vaccinate raccoons and other wildlife were begun on a remote island off the
coast of Virginia. Of 100 bait preparations set, 96 were disturbed. There
was no evidence of any disease caused by the vaccine. Trapped animals
were challenged with rabies. The level of observed protection was
90–100%. The stability of the baits was satisfactory. In June of 1991
another field trial was begun in a remote part of Pennsylvania. It is expected
that a third trial will be started in New Jersey. To date there have been no
safety problems.

Dr. Larry Allen, Staff Veterinarian, California Department of Food and
Agriculture, reported on California’s veterinary biologics program. California
has had a program since 1974. The 1985 amendment to the
Virus–serum–Toxin Act of 1913 requires USDA approval of state programs.
In 1989 USDA gave such approval. Currently California licenses 11
manufacturers and 39 products. The Federal 9 CFR is followed with some
adaptations to accommodate smaller companies. The California Veterinary
Diagnostic Laboratory System serves the state program as the counterpart
to the National Veterinary Services Laboratories in the federal program.
California is divided into 6 sectors with veterinary officers and staff in each.

Dr. George Crenshaw, Consulting Veterinarian, expressed concerns
over the efficacy of several licensed veterinary products as perceived from
the perspective of field use. Some of the combined clostridial bacterins and
inactivated virus vaccines were of most concern. The newly established
post-licensing monitoring program provides the mechanism for investigating
such concerns.

In other business, a request from the Committee on Infectious
Diseases of Horses regarding Venezuelan equine encephalitis vaccination
was considered. The background for this request is that outbreaks of VEE
have not been identified in the U.S. or any other countries in North or South
America in the past 18 years. Further, there are some problems associated with exporting horses with serologic titers to VEE. It is also quite clear that the polyvalent vaccines in use today would not stop a VEE outbreak should one occur. The Committee proposed and passed recommendations in support of ceasing the distribution of polyvalent vaccines that include the VEE fraction. A second recommendation was made to ensure an adequate supply of suitable VEE vaccine should an outbreak occur at some time in the future.

In other discussions, a request for limited interstate distribution of a bluetongue vaccine licensed in California was not acted upon since suitable procedures such as conditional licensing are provided in existing regulations. The draft Statement of Purpose for the Biologics Committee was adopted.

Very strong sentiments were expressed asking that scheduling conflict between the Biologics Committee and Biotechnology Committee be avoided. This scheduling conflict potentially reduces the effectiveness of both committees. Several possible solutions to this were proposed and a recommendation was passed.
REPORT OF THE COMMITTEE ON ENVIRONMENTAL RESIDUES

Chairman: Dr. C.H. Graham, Shawnee Mission, KS
Vice Chairman: Dr. W.T. Hubbert, W. Bowie, MD

J. Adams, VA; P. Basu, WV; W.B. Buck, IL; H.H. Casper, ND; D.R. Cassidy, IA; C.Y. Erbel, NC; R.A. Gesert, MI; H.S. Gosser, MO; R.D. Hull, IL; C.N. Jewett, SC; R.E. John, MO; W.E. Ketter, MD; L.F. Kubena, TX; D. Mallow, VA; M.M. Mamminga, IA; G.D. Osweiler, IA; J.F. Robens, MD; P.F. Ross, IA; L.G. Sullivan, MI; M.A. Thomas, MD; J. Webb, DC.

The Committee met at 1:30 p.m., Tuesday, October 29, 1991, in the De Anza Room. Seven (7) members and 10 guests attended.

Mycotoxin contamination of 1991 Feed Grains Crop:

Wheat – Fusarium (scab) was widespread this year. Wheat produced from eastern Kansas through Indiana and some in the north was severely damaged. There was little to no difference in infection rate between varieties. Rain at the time wheat is blossoming will result in Fusarium infection. Much of the scabby wheat is contaminated with DON (vomitoxin), with levels of 1–5 ppm common, some at 5–10 ppm and a few at 15–20 ppm.

There is little to no zearalenone contamination. Test kit assays values are questionable, it is necessary to get confirmation using proven analytical procedures.

Barley also is commonly infected with scab. No mycotoxin levels reported.

Corn – Aflatoxin contamination spotty, associated with drought areas. White corn reported to be highly contaminated in Texas with yellow corn generally not.

Peanuts – Early crop commonly contaminated with aflatoxin, as harvest progressed, less contaminated.

Cottonseed – Some aflatoxin contamination reported.

Fumonisins – Frank Ross, NVSL, Ames, Iowa

Fumonisins are produced by all strains of the mold, Fusarium moniliformae. The mold is ubiquitous, found wherever corn is raised. Fumonisins B₁, B₂, and B₃, have been identified. Fumonisin B₁ is produced in greatest quantity. In field, cultures, B₂ will be found at about 32% of B₁ quantity, and B₃ at 12%.

Some fumonisins will be found in most corn, more commonly at 1–5 ppm. Up to 10 ppm is not unusual. Ten (10) ppm and below is a reasonably safe level with 5 ppm and below better. The horse is the most...
sensitive animal to fumonisins. In feeding trials using ponies, 20–50 ppm range caused liver toxicity and ELEM (leucoencephalomalacia). Porcine pulmonary edema has been associated with feeding corn contaminated with 50 ppm and above.

Analytical methodology is continuing to improve. Many laboratories are routinely assaying corn for fumonisins. There are no indications of serious contamination of 1991 corn.

The Committee voted to endorse "Recommendations on Forensic Evidence in Mycotoxicoses" developed by the AAVLD Mycotoxin Committee. These recommendations were developed because of substantial claims being made for alleged mycotoxicoses on inadequate evidence.

AAVLD Mycotoxin Committee Recommendation on Forensic Evidence in Mycotoxicoses
October 27, 1991

Mycotoxins are capable of causing economically important effects to the health of animals. The presence of these naturally occurring toxins in animal feeds is a matter of concern to the animal producer, the veterinarian and the feed supplier. Mycotoxins are produced by molds that are naturally widespread in the environment and on feed commodities. The presence of these molds or minute quantities of detectable mycotoxin need not be considered injurious to animal health. Meaningful levels of mycotoxin in animal feeds, however, should be considered as a serious threat to animal health.

The animal feed industry has a responsibility for marketing safe wholesome products. Similarly the veterinary diagnostic sciences have a responsibility to evaluate animal disease situations and establish criteria for their diagnosis. The feed industry has been subjected to a number of claims of animal loss based on inadequate evidence for mycotoxicoses. These occurrences force hardship on the animal industry by increasing the costs of feed production. Conversely, marketing of animal feeds containing injurious quantities of mycotoxins endangers the health of animals and the prosperity of the animal industry. For these reasons, the Mycotoxin Committee of the American Association of Veterinary Laboratory Diagnosticians makes the following recommendations:

1. Virtually all animal feeds contain viable mold. Cultural identification of a mold, even a potentially toxigenic species, is not in itself acceptable evidence for feed incrimination in mycotoxicosis.

2. Chemical isolation and confirmation of a known mycotoxin from the feed or from the body tissues or body fluids of affected animals is the best evidence for exposure to a
mycotoxin. The mycotoxin, quantitatively demonstrated by chemical testing in the feed or tissue, must be in sufficient quantity to cause the observed illness and/or pathological lesion(s).

3. The presence and quantity of the mycotoxin must be confirmed by a second technique. The use of an official method (AOAC, AOCS, AACC) is strongly encouraged.

4. The feed specimen tested must be representative of the feed consumed by affected animals and must be adequately preserved until the time of testing.

5. In the absence of chemical demonstration of a known toxin, experimental feeding of representative quantities of a suspect feed to healthy animals (e.g. laboratory animals or animals of affected species) to reproduce the observed syndrome may be used for presumptive incrimination of the suspect feed as injurious but not necessarily mycotoxic.

These principles of mycotoxin diagnosis should be used in conjunction with good veterinary medical and diagnostic practice to evaluate other potential etiologic agents.

CLENBUTEROL

There is evidence of considerable usage of the beta agonist, clenbuterol, in show animals and in horses. Canadian visitors at the committee reported improved analytical procedures are being developed.

BSE (BOVINE SPONGIOFORM ENCEPHALOPATHY) DR. DELMAR CASSIDY NVSL, Ames, Iowa

There is no evidence of this condition in cattle in the United States at this time. An intensive surveillance program is in place.
REPORT OF THE COMMITTEE ON EPIZOOTIC ATTACK

Chairman: Dr. S. T. Wilson, Jr., Tuskegee, AL
Vice Chairman: Dr. H. A. McDaniel, Hyattsville, MD

J.B. Anderson, TN; J.L. Blair, VA; W.W. Buisch, NY; J.J. Callis, NY; H.M. Chaddock, MI; T.M. Cook, DC; G.E. Dittbener, CAN; A.K. Eugster, TX; W.D. Felker, IA; J.B. Finley, TX; D.D. Gingerich, IA; P.R. Henry, CO; B.R. Heron, CA; J.P. Huntley, NY; J.L. Hyde, NY; L.P. Jones, TX; J.H. Lang, WI; H.M. Loper, AL; D.W. Luchsinger, VA; E.T. Mallinson, MD; R.H. McCapes, CA; C.A. Mebus, NY; N. Meyer, VA; M.A. Mixson, MD; J.E. Novy, MD; R.E. Omohundro, CO; J.S. Orsborn, CA; B.I. Osbum, 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.

The Committee on Epizootic Attack convened at 1:30 p.m., October 31, 1991, at the Town and Country Hotel, San Diego, California. There were 79 persons in attendance, of which 26 were members of the Committee. Dr. D. L. Thompson, California, served as Vice Chairman in the absence of Dr. H. A. McDaniel.

The Epizootic Attack Committee is charged to provide a forum for state, Federal and industry representatives to cooperatively review, discuss and evaluate plans for responding to animal disease epizootics.

Twelve reports were made to the Committee.

Dr. M. A. Mixson provided current information about Emergency Programs and the N. American Vaccine Bank. He pointed out that the number of emergency disease investigations has ranged from 126 to 260 between 1984 and 1991. The goal is to increase this to 800 per year within the next 2-3 years. There are 353 active Foreign Animal Disease Diagnosticians, with 262 in field positions in the U.S. These 262 include 202 Veterinary Medical Officers (VMO's) in Veterinary Services, 49 state employees, 8 in REAC and 3 in PPQ. There are also 87 Federal VMO's who are in managerial or laboratory positions. This report relieved some of the concerns expressed by the Committee during the 1990 meeting in regards to the number and availability of trained diagnosticians to investigate reports of suspicious cases of exotic diseases.

Dr. Hugh Metcalf described emergency disease guidelines that have been or will be prepared. All guidelines will eventually be in a databank and available for electronic retrieval. An outline was distributed which contained a detailed list of categories of information available for each disease subject to a guideline.

Dr. Bob Miller described the response of USDA to letters to the
Assistant Secretary of Agriculture from the President of USAHA in 1990. These letters recommended that studies be completed to improve methods of carcass disposal during an emergency disease outbreak. Dr. Miller stated that USDA is evaluating several methods of carcass disposal and are close to completing an Environmental Impact Statement (EIS), which is considered to be an important step in making sure that plans are both functionally and legally sound.

Dr. M. A. Mixson responded to three USAHA resolutions passed in 1990. During the last year, APHIS completed a test exercise for an African Horse Sickness epizootic and plans to conduct one in 1992 for hog cholera. He described efforts in progress to establish a core of resources readily available to respond to emergency diseases.

Dr. E. C. Sharman served as moderator of a panel that included a representative of each of the major livestock producer organizations holding allied membership in this association. Each representative described their industry's ability to respond to an epizootic of an emergency disease. Mrs. Amy Mann stated that the horse industry fears African Horse Sickness the most and feels that indemnity would be inadequate. Mr. Don Gingerich described the swine producers' fear of the threat that hog cholera in Mexico poses. He stated that indemnity would probably be inadequate, but that routes of communication to Congress are well established to address concerns during an emergency. Mr. Tom Cook stated that many cattlemen are complacent about exotic diseases, but that the NCA is well prepared to distribute information and support efforts against emergency diseases. Dr. Perry Turner described the concern of sheep producers about screwworm and stated that the sheep industry has no specific response plans. Dr. Arch Park described broad and thorough plans for much of the poultry industry to respond to emergency diseases. These plans are well distributed and documented. In some parts of the country, poultry industries have made available many resources including significant amounts of money and equipment to quickly respond. Dr. Frank Mulhern stated the importance of having many alternative responses available to respond to epizootics.

Dr. Harvey Kryder summarized USDA actions taken in responding to requests from Mexico to ship three agricultural products, eggs, poultry, meat and pork through the United States to other countries. Approval has been given for Mexico to ship eggs through the U.S. under a stringent set of conditions. Poultry meat has not been shipped from Mexico because Mexico asked for conditions which did not fit our bio-security requirements and personnel resources. Discussions are being held regarding the establishment of hog cholera free regions in Mexico. This is being requested as part of Mexico's request to export pork through U.S. ports. Any changes regarding pork are not expected for some time until more study, regulatory changes and public comment are completed.
Free trade negotiations and other trade issues were discussed by Mr. Robert Wicks and Dr. Robert Whiting. Mr. Wicks summarized his presentation to the General Session. The principles upon which the negotiations are conducted were described. These included the U.S. position that there are legitimate health requirements, but they should be based upon science and not be artificial barriers to trade. Mr. Wicks also stated that all parties agree that it is not in the best interests of any trading partner to create conditions that would place health of United States livestock in jeopardy. Such actions jeopardize the agreement. He also stated that all laws and policies are subject to analysis and change as part of the negotiations, but the changes would apply only to a country involved in the agreement. It appears from Mr. Wicks' report that the U.S. trade negotiators are addressing the concerns expressed by this committee in the 1990 resolution #42.

Dr. Don Luchsinger mentioned 11 examples of improved capabilities of Veterinary Services to respond to emergency diseases. These included increased Foreign Animal Disease training for veterinarians and Animal Health Technicians (AHT's), READEO workshops, tremendously increased databank abilities, increased input from an advisory committee, establishment of a reserve corps of ex-employees, computer modeling, greatly improved communication channels with Agriculture Extension personnel, and the establishment of a Foreign Animal Disease Strategic plan which includes resources from 6 agencies in USDA.

The Committee responded positively to the responses of USDA to USAHA Resolutions 43 and 44, which were passed in 1990. However, the Committee also realizes that responses to those two resolutions are not complete. Efforts to establish a basic infrastructure of personnel, facilities, equipment and funds for responding to emergency diseases needs to be completed.

The Committee notes that no action had been taken on a recommendation for APHIS-VS to develop an orientation and briefing for new state veterinarians on state and Federal responsibilities pertaining to emergency disease outbreaks.

There was one resolution passed by this Committee and referred to the Committee on Resolutions. This resolution pertained to increased indemnity for depopulation of swine herds infected with brucellosis. Thirty eight states are now Validated Swine Brucellosis Free. There were only 76 herds under quarantine in the U.S. as of June 30, 1991, most of which are small garbage-feeding herds. It is felt that the elimination of many of these herds would be facilitated by increasing indemnity for depopulation. A Statement of Purpose was approved by the Committee and the meeting was adjourned at 4:45 p.m.
NORTH AMERICAN FREE TRADE AGREEMENT NEGOTIATIONS

Robert J. Wicks
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6621 South Agriculture Building
Washington, DC 20250

The United States is committed to not weaken existing health and safety protection through the NAFTA negotiations. The U.S. Government concurs with the position of the USAHA by recognizing that every nation and the United States has a legitimate right to maintain sanitary, phytosanitary (S&P) and food safety standards for imports. U.S. negotiations have fully adopted the position that health–related measures affecting trade should be administered in a transparent and expeditious manner, not applied arbitrarily or as a disguised barrier to trade.

Existing measures will remain in place so long as they are determined to be necessary to ensure that imported products, commodities, livestock, etc., meet the health and safety requirements deemed necessary to protect human, plant, and animal health.

The U.S. recognizes that even though Mexican veterinary and other standards may be similar to those of the United States, their enforcement may not be as stringent. Consequently, not until the United States is satisfied that Mexico’s inspection systems, certification procedures, and testing procedures can guarantee that U.S. standards are met, will the U.S. consider any modification of border procedures.

The NAFTA will establish a framework through which we can work with our North American trading partners to create a free–trade environment that also uses the best scientific evidence to ensure that the desired level of health protection is maintained.

To this end:

During Congressional debate in May on fast–track negotiating authority, the Administration issued an Action Plan committing the United States not to weaken existing pesticide, energy conservation, toxic waste, or health or safety (including food safety) regulations through NAFTA negotiations on standards.

The NAFTA framework includes a negotiating group on Standards, with three Sub–Groups. The main work of the Sub–Groups will be to establish disciplines for development and application of standards.
and regulations and mechanisms for resolving S&P disputes. Sub-Group A will address the scope of issues included in the GATT Uruguay Round draft text on S&P measures, including animal health. Sub-Group A is co-chaired by USDA and EPA.

U.S. NAFTA negotiators are benefiting from excellent interagency cooperation, drawing on the technical expertise of the Food Safety and Inspection Service and the Animal and Plant Health Inspection Service of USDA, as well as the Environmental Protection Agency and the Food and Drug Administration.

The following slides were presented and discussed during the presentation.
RESOLUTION 42

-- RESOLVE that animal health regulations between the U.S. and Mexico be based on scientific justification.

-- RESOLVE that the FTA negotiators will not compromise animal health for the sole sake of expanding trade.

-- RESOLVE that animal health status be considered a priority with the FTA negotiations between the U.S. and Mexico and all future trade negotiations.

MECHANISMS TO ADDRESS SANITARY AND PHYTOSANITARY CONCERNS

-- Congressional Fast-Track Authority Approved May
  * Commitment to not weaken health requirements

-- Agricultural Trade Advisory Committees
  * Industry Input Solicited
  * Resolutions

NAFTA AGRICULTURAL ISSUES

-- Timing/Phaseout of Tariffs and Non-Tariff Barriers

-- Food Safety Concerns

-- Rules of Origin

-- Sanitary and Phytosanitary Regulations

STANDARDS NEGOTIATING GROUP

-- Will not address specific market access issues for Mexico
-- Will establish disciplines for development of standards and regulations
-- Will establish mechanics for resolving sanitary and phytosanitary
-- I comprised of three sub-groups
* Subgroup A Standards and Regulations issues—USDA/EPA
* Subgroup B Health and Environmental issues — EPA/FDA
* Subgroup C Consumer Protection/Safety issues — USDOC
### SUBGROUP A RESPONSIBILITIES

- All health related regulations impacting agricultural trade
  - * Food safety issues
  - * Plant health issues
  - * Animal health issues
- Environmental issues linked to sanitary and phytosanitary measures

### GUIDING PRINCIPLES FOR SUBGROUP A

- Are the same as those used for GATT
- Were developed by USTR, EPA, FDA, USDA (APHIS & FSIS)
- Scientific basis for sanitary and phytosanitary requirements
- Sanitary and phytosanitary requirements non-arbitrary

### U.S. NEGOTIATING POSITION RECOGNIZES

- There are legitimate sanitary and phytosanitary requirements
- Sanitary and phytosanitary requirements must be transparent
- Sanitary and phytosanitary requirements must not be simply a disguised barrier to trade

### EQUAL STANDARDS BUT LESS STRINGENT ENFORCEMENT

- U.S, recognizes Mexico’s difficulties with enforcement
- U.S, will continue border measures
- Only if satisfied with enforcement will we consider modification of our border procedures
## PRINCIPLE OF EQUIVALENCY

-- U.S. recognizes that different regulatory methods can achieve same levels of protection

-- U.S. has adopted a "same results" criteria for health measures
  * consistent with our position in GATT and Uruguay Round
  * support equivalency -- rather than "same as" requirements

## U.S. POSITION ON SCIENTIFIC BASIS FOR SANITARY AND PHYTOSANITARY REGULATIONS

-- If requirements differ from international standards

* importing country to demonstrate scientific foundation for requirements

* For questions of equivalency, burden of proof on exporter

## AGRICULTURE NEGOTIATING GROUP & STANDARDS NEGOTIATION GROUP

-- Will use Uruguay Round sanitary and phytosanitary text as a starting point for NAFTA negotiations

## WILL HARMONIZATION UNDERMINE PROTECTION

-- The U.S. will not compromise our sanitary and phytosanitary standards

-- Each country makes its own risk assessment

-- Each country determines its appropriate level of protection
<table>
<thead>
<tr>
<th>NAFTA PARTNERS AS A PLATFORM FOR UNRESTRICTED ENTRY BY OTHERS</th>
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<tr>
<td>-- Rules of origin requirements (based on GATT)</td>
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<tr>
<td>* Canadian/U.S. Trade Agreement contains same provisions</td>
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<tr>
<td>* Benefits of NAFTA limited to NAFTA</td>
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<th>MEXICAN COMMODITY POSTS VIA CANADA</th>
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<tr>
<td>-- Canada legitimately accepts some products not meeting U.S. standards</td>
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<tr>
<td>-- Tropical products pose no threat to Canada</td>
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<tr>
<td>-- Like GATT or the CTA, rules of origin included in NAFTA</td>
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<tr>
<td>-- Special provisions to cover cases where we have different requirements than Canada</td>
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The Government Relations Committee met with representatives of the Washington based Allied Industry, the United States Department of Agriculture and the Food and Drug Administration–Center for Veterinary Medicine. These persons interacted with the committee relative to their responsibilities and programs and discussed key issues of mutual concern.

Committee members strongly believe that disease prevention and eradication, food, safety and animal diseases research can best be solved through cooperative state–federal efforts with industry involvement and support.

The Committee is appreciative of the opportunity to meet with Assistant Secretary of Agriculture, Jo Ann Smith and we especially appreciate her understanding of animal health issues. We are also very appreciative of Dr. James Glosser, APHIS Administrator and Dr. Lonnie King, VS Deputy Administrator in helping to develop a more effective working relationship between USDA, APHIS and the USAHA.

AGRICULTURAL RESEARCH SERVICE (ARS)

R. D. Plowman, Bob Oltjen, Gary Colgrove, and Ralph Graham of ARS reviewed current initiatives in animal health research. Projects underway are planned to: (1) study the pathogenesis and develop better diagnostics for the spongiform encephalopathies, (2) develop better acaricides and alternative control methods (such as the release of sterile male hybrids) for use in tick control or eradication programs, and (3) test a genetically engineered "vaccine" to prevent cattle grubs.

FOOD SAFETY INSPECTION SERVICE (FSIS)

Dr. Lester Crawford, FSIS Administrator, described FSIS's commitment to "areas of Emphasis" which include: (1) Hazard Analysis and Critical Point Program (HACCP), (2) the elimination of chemical residues, (3) Food Labelling Reform, and (4) fostering expanded research to improve methodology for increasing food safety. HACCP relies on the identification and analysis of "critical points" within food production and handling.
REPORT OF THE COMMITTEE

processes followed by the development of model procedures designed to eliminate or reduce the risk of contamination at these critical points. Dr. Crawford urged USAHA to become involved in the CODEX (the U.N.'s Food Safety and Standards Organization) Committees on Food Hygiene and Veterinary Drugs.

FOOD AND DRUG ADMINISTRATION (FDA)

Dr. Gerald Guest of the FDA's Center for Veterinary Medicine discussed the FDA's plans for monitoring and reducing Salmonella contamination of rendered meat and poultry by-products. These plans include; (1) increasing the level of sampling, and (2) the development and implementation (in cooperation with the rendering industry) of "Good Manufacturing and Handling Processes" for these products.

The Committee encouraged the FDA to actively enforce compliance on any producer or dealer who might repeatedly sell livestock with violative drug residues.

NATIONAL VETERINARY SERVICES LABORATORY (NVSL)

It became apparent to the Committee that current funding is not adequate for NVSL to fulfill its mission.

We were advised that non-program user fees have been mandated. NVSL is the national animal disease reference laboratory. User fees for reference assistance would be detrimental to the effectiveness of national diseases surveillance. The Committee recommends that user fees for reference assistance not be assessed.

VETERINARY ACCREDITATION

Dr. Joan Arnoldi, Associate Administrator for REAC, met with the Committee to discuss animal welfare and veterinary accreditation. She noted that the 1985 "Farm Bill" addressed only the "enrichment" of primates, psychologically and physically, and that the 1990 "Farm Bill" has as a part of it the "Pet Theft Act" to prevent someone's pet from being confiscated and delivered to research institutions. She stated that the "Veal Calf Protection Act", which has been introduced and defeated in the past, will be introduced again this year.

Dr. Arnoldi reported that a "Task Force" has been meeting to develop a revised accreditation program. Their recommendations include structuring accreditation on a national basis, replacing the accreditation exam with an orientation program, and they have determined that the self-study modules and computer examination are not appropriate for the accreditation program.
GOVERNMENT RELATIONS

Dr. Arnoldi advised the Committee that Dr. Ken Meyer, of Purdue University, has agreed to join APHIS, for a year on a sabbatical leave to develop a manual for accreditation training and implementation of a uniform curriculum.

The Committee is concerned with the philosophy that accreditation is only a USDA function. Accreditation must be a cooperative effort with joint responsibilities between USDA, states and the accredited veterinarian.

USAHA AND USDA INTERACTIONS

USAHA and USDA continue to review their interaction to more effectively interact. Each group through surveys, discussions and committee activities are making sincere efforts to understand each others concerns and feelings for more effective interaction. The dialogue started with the team building exercise last year caused both groups to realize that a second team building session would be of benefit.

USAHA RESOLUTIONS

Responses to the 1990 resolutions were reviewed. We appreciate the responses and hope the USAHA effort to prioritize the resolutions was helpful in developing their responses. USAHA is still concerned and realizes a need for an improved system of resolution development and approval by the Association.

ALLIED INDUSTRY

The Committee met with the Washington based allied industry representatives. Frank discussions occurred regarding USAHA's role in national animal health programs and suggested methods of strengthening these roles. Specific topics such as NAHMS, brucellosis, pseudorabies, core funding, and preparedness of state and federal animal health agencies to respond to a foreign animal disease outbreak were reviewed. This group emphasized the need to strengthen the relationship between industry, state agencies, federal agencies and USAHA. This was a most productive session and we appreciate very much industry's efforts to participate. We feel that USAHA should actively cultivate a closer working relationship with allied groups to strengthen USAHA's role in national animal health efforts.

VETERINARY SERVICES

B.S.E.

This Committee commends VS and ARS in the manner in which they
responded to the BSE threat. The research and information developed should continue to receive broad dissemination. Adequate funding must be obtained to continue the research and to further develop plans that would be implemented if BSE is diagnosed in the U.S.

SCRAPIE

We commend VS for their leadership in working with the sheep industry and establishing the negotiated rule making process. We encourage VS to proceed in publishing the findings of the negotiated rule making committee in the CFR. We encourage continued research on scrapie and the development of new diagnostic tests that will allow the diagnosis of this disease in live animals.

BRUCELLOSIS

We are concerned that all portions of the rapid completion plan are not being properly addressed including adjacent herd testing, timely retests of infected herds and funding to encourage depopulation of infected herds.

SALMONELLA ENTERITIDIS (S.E.)

The Committee was briefed by the S.E. Task Force Director, Dr. John Mason, on VS involvement in control of this disease. We were pleased with changes incorporated in the final CFR rule. The Committee commends VS for its leadership in initiating communications on future control alternatives with the states and industry. The Committee recommends that the negotiated rule making process be considered as the method for designing future SE activities. The development of an integrated risk reduction program based on the HACCP concept is encouraged. The Committee strongly urges the need for extension of responsibility for risk reduction beyond the poultry breeder, or egg producer to the distributors, food handlers, and consumers.

CORE FUNDING

The Committee wholeheartedly supports the concept of core funding and commends VS for their innovative approach to provide continued funding for a basic, experienced and well trained force of animal disease professionals capable of protecting our nation's animal and poultry industries.
FREE TRADE AGREEMENT

The USAHA, by a resolution, expressed concern about the lowering of disease import safeguards as a result of Free Trade Agreements. The Committee emphasizes that disease import requirements must continue to be based on sound scientific criteria.

NATIONAL ANIMAL HEALTH MONITORING SYSTEM (NAHMS)

The Committee has expressed concern about the direction and productivity of the NAHMS program in its previous reports. VS has responded to the criticisms in an effort to better coordinate the program at the state level. The concern has been that the program has been diverted from a national disease surveillance system to that of primarily gathering information on environmental issues and animal husbandry practices. Veterinary Services should investigate more cost effective ways of accumulating this type of information.

It should be recognized that VS has a national constituency to satisfy with the NAHMS program while cooperating states need to explore projects that have regional value so that state funds can continue to be used to augment the federal program. This concern has been addressed in the past by the addition of so called "add ons" for specific state interests. The adoption is greatly appreciated and makes the program more acceptable at the state level. The NAHMS staff is to be commended in initiating contacts with state agencies in program design. More effort is needed to bring cooperating states into the process of program design and goal setting. Further the Committee feels that programs should be designed to increase participating veterinarians' diagnostic and epidemiological skills. Recent NAHMS surveys have been information accumulation projects which could be accomplished by well trained lay personnel.

Testing of laboratory samples collected under this program should be financed from the NAHMS budget.

The NAHMS accumulative report on the diagnostic findings of state veterinary laboratory systems is an area of recognized benefit and its continuation and expansion is recommended. The Committee reviewed two reports prepared by NAHMS on the qualitative analysis of BSE risk factors in the U.S. These reports are excellent examples of what NAHMS can accomplish.
Chairman: Dr. John R. Cole, Tifton, GA
Vice Chairman: Mr. John E. Finnell, White Hall, IL

M.F. Baker, CAN; R. W. Behan, IA; C.A. Bolin, IA; R.M. Bunte, IL; D.W. Chladek, MO; S.L. Diesch, MN; J.M. Donahue, KY; J.C. Frantz, NE; J.W. Glosser, DC; L.E. Hanson, IL; R.L. Morter, IN; R.M. Nervig, IA; H.L. Rubin, FL; M.H. Smith, ND; J.G. Songer, AZ; J. R. Taylor, GA; A. B. Thiermann DC; D.N. Tripathy, IL; H.E. Vanderslice, DE; D.L. Weiss, MN.

The USAHA Committee on Leptospirosis met on Tuesday, October 29, 1991, with 16 members and guests in attendance.

Dr. Dave Miller of the Diagnostic Section (APHIS-NVSL) of the National Reference Center for Leptospirosis reported on the 1990-1991 activities of the section. The number of microscopic agglutination tests (MAT) performed this year (768) decreased significantly from last year (10,289). Thirty-two submissions containing 43 specimens were received for leptospira isolations from 8 states. These submissions were from cattle (18), swine (2), horses (2), rodents (8), dogs (1) and a giraffe (1). Cultures for serotyping were received from 5 states and included the following species: cattle, Leptospira interrogans serovar kennewicki (1), swine, serovar ballum (1) horses, serovar kennewicki (7) and serovar grippotyphosa (2) dogs, serovar grippotyposa (1); and deer, serovar hardjo (2). Reagents for diagnosis of leptospirosis continue to be available. Four publications from this section are in press.

Dr. Carole A. Bolin of the Research Section (ARS-NADC) of the Center provided a report on areas of research conducted during the past year. These are 1) Studies concerning the epidemiology and clinical signs of Leptospira interrogans serovar bratislava infection in midwestern swine herds was completed and published. The most commonly observed clinical signs included stillbirths, birth of weak pigs, late term abortions, and delayed returns to heat or infertility. Various techniques were used to detect infection with serovar bratislava in these herds. A combination of techniques including sow and fetal serology and fluorescent antibody testing were found to be the most useful techniques for establishing the diagnosis; 2) Swine kidneys were collected from a local slaughterhouse and cultured to detect the presence of leptospires. Isolates were obtained from 11% of the kidneys from 153 adult swine. Eighteen isolates were obtained from 17 kidneys with two serovars isolated from one kidney. Fifteen of 18 isolates were serovar bratislava and three isolates were serovar hardio type hardjo–bovis. This is further supportive evidence that serovar bratislava is now the most common leptospire in adult swine in the Midwest. These are also the first isolates of serovar hardio from pigs in the U.S., although serovar hardio has been
isolated sporadically from pigs in N. Ireland; 3) Restriction endonuclease analysis of leptospires isolated from pigs with reproductive failure and from tissues collected at the slaughterhouse was completed. Fifty-two isolates were typed using this method. Forty-nine isolates were serovar bratislava and 3 isolates were serovar hardjo type hardjo-bovis. Further analysis of the serovar bratislava isolates revealed that the genotype distribution of serovar bratislava is quite different than that seen in Northern Ireland as reported by Ellis. Furthermore there was no correlation of genotype with clinical syndrome as seen in N. Ireland. Our results are as follows: 10 of 50 serovar bratislava isolates were genotype BI, 18 isolates were genotype B2a, and 21 isolates were genotype B2b; 4) Analysis of the outer membrane proteins of Leptospira interrogans serovar pomona type kennewicki was completed and published. A summary of the findings includes: A 31 kDa protein was found to be the major outer membrane protein. Five surface-exposed proteins were also identified in the outer membrane. The 31 kDa protein was found to be heat-modifiable and to be a potent antigen in pigs infected with serovar pomona. A 60 kDa protein was secreted from serovar pomona and was identified as an hemolysin; 5) The structure and composition of the periplasmic flagella of serovar pomona type kennewicki was determined. The proteins which compose the flagella were analyzed and their structural location in the flagella identified by electron microscopy. The flagella was composed of a 12 nm core, surrounded by two sheaths. Cloning of the genes coding for the flagellar sheath proteins is underway; and 6) Leptospirosis was identified as a significant cause of a hemolytic anemia syndrome seen in black rhinoceroses in captivity in North America. The hemolytic anemia syndrome has a high mortality rate and is the leading cause of death among captive black rhinoceroses. Leptospiral vaccination programs were begun and since institution of vaccination there has been a marked reduction in the incidence of this condition.

Dr. Mike Donahue, University of Kentucky, reported on the results of a 3-year study on leptospirosis infections in Kentucky horses. During this period, they have found leptospiral abortions in 60 mares on 52 different farms. Serovars thought to be responsible for these abortions, based on culture and microscopic agglutination test results, are kennewicki (41), grippotyphosa (7), bratislava (1), hardjo (1), and unknown (10).

Drs. Lyle Hanson and Deoki Tripathy, University of Illinois reported on a leptospiral outbreak which occurred in seven boys this past July in Central Illinois. The boys had been swimming in either a pond adjacent to or in the Vermillion river. Tests of paired sera indicated development of antibodies to several leptospiral antigens. Leptospires were isolated from the urine of one boy. Preliminary studies indicate the isolate is grippotyphosa. The study involved a cooperative effort of CDC, the Public Health Department of the State of Illinois and LaSalle County, and the
LEPTOSPIROSIS

College of Veterinary Medicine of the University of Illinois. In addition, they have made isolations of serovar *grippotyphosa* from 1 raccoon, 1 pig, and 1 dog. Both the pig and the dog had contact with raccoons.

Concern was expressed by members of this committee regarding the fee for leptospiral antigen of $124 per antigen to be charged by the National Veterinary Services Laboratories. This fee was thought to be excessive and would discourage requests for quality reagents which must be used by diagnostic facilities.
REPORT OF THE COMMITTEE ON MASTITIS

Chairman: Dr. Thomas J. Fuhrmann, Tempe, AZ
Vice Chairman: Mr. Norman J. Corlett, Strongsville, OH

Mr. John Adams, Arlington, VA; Dr. Brij N. Bhargava, Fort Washington, MD; Dr. Thomas F. Conner, Indianapolis, IN; Dr. Max L. Crandall, Barhamsville, VA; Dr. Frank Dickinson, Columbus, OH; Dr. David F. Dineen, Augusta, ME; Mr. Philip A. Dukas, Columbus, OH; Dr. Nancy E. East, Davis, CA; Dr. B. J. Edmundson, Olympia, WA; Dr. W. H. Fales, Columbia, MO; Dr. Thomas W. Freas, Indianapolis, IN; Dr. Donald A. Gable, FairfHx, VA; Mr. Francis D. Gregerson, Longmont, CO; Dr. Dale D. Hancock, Pullman, WA; Dr. Fred S. Idtse, Madison, WI; Dr. Wade L. Kadel, Hopkinsville, KY; Dr. Eldred Keahey, College Station, TX; Dr. John D. Kopec, Hyattsville, MD; Mr. John H. Lang, Madison, WI; Dr. Donald H. Lein, Ithaca, NY; Mr. J. C. Lemmermen, Okeechobee, FL; Dr. E. Travis Littledike, Hastings, NE; Dr. Tatsuo Matsushita, Sterling, VA; Brig. Gen. T. G. Mumane, Ft. Worth, TX; Ms. Mabel A. Owen, Boston, MA; Dr. Duane N. Rice, Lincoln, NE; Dr. F. James Schoenfeld, Roy, UT; Dr. Mohammad Sharar, Gaithersburg, MD; Mr. Walter E. Stemler, Waterloo, IL; Dr. Keith E. Sterner, Ionia, MI; Dr. John M. Vetterling, Fort Collins, CO.

This committee did not meet in San Diego, California.
This committee met at 1:30 P.M. on Monday, October 28, 1991, in the Sheffield Court Room at the Town and Country Hotel, San Diego, California.

The meeting was called to order by Dr. Scroggs, Chairman. Eighteen persons were in attendance, 10 of which were committee members.

Dr. Victor Nettles, Director, Southeastern Cooperative Wildlife Disease Study, The University of Georgia, reported on the wildlife study being conducted on the islands of Antigua and Guadeloupe in the West Indies, to determine the ability of various wildlife to serve as hosts for the tropical bont tick. Mongooses were trapped exhibiting an incidence rate of 16%. Only larvae and nymphs were recovered; no adult ticks. Cattle egrets were marked with a dye to determine migration from island to island. Migration did occur but few if any, harbored any ticks. Therefore, a limited factor would be assigned to cattle egret in the spread of ticks from island to island.

Dr. Bob Bokma, USDA, APHIS, VS gave an update on the Puerto Rico Tick Eradication Program. The program is under the control of APHIS at this time. Out of a total population of 370,000 head of cattle, representing 24,000 premises, 167,000 head are considered free of Boophilus. Amitraz has been used as a spray every 21 days with no resistance reported. A budget of $14 million for fiscal year 1992 is in place. It appears that the program is showing progress assuming that another "Hugo" doesn't occur.

Dr. Phil Pickerill, USDA, APHIS, VS reported on the cattle fever tick program in Texas. A tick buffer zone of 9 infested premises has been established for 1991. There are 2 options for the producer. The first being leave cattle on the premise and treat with coumaphos every 14 days or option 2, vacate the premise for a specified period of time. Five of these premises are vacant at this time. The problem with vacating the premise is that wildlife harvest the ticks and are not treatable. There is a project underway in cooperation with the research branch at Kerrville to develop an ivermectin bait for the infested deer.

Dr. Pickerill also reported that resistant cattle fever ticks were found
REPORT OF THE COMMITTEE

in Mexico in December, 1990. These animals had been dipped twice with coumaphos.

A question on the importation of ratites was asked. Dr. Pickerill stated that ratites 36 inches or shorter were allowed into the United States but entry is through the quarantine station in New York only. Hatching eggs are allowed to be shipped in and quarantined at other facilities. The exporting facilities are inspected by USDA personnel.

Dr. John George, entomologist, USDA, ARS, reported that pesticide resistance is being given a great deal of attention. A study is underway to determine if DNA patterns of insects would give a quicker indication of possible insecticide resistance than the more conventional methods of rearing the pests and then submitting to treatment. Resistance to organophosphates seems to be wide spread in Mexico and the potential for resistance to pyrethroids is increasing. As mentioned above, the avermectins are being studied for use if and when, organophosphates and pyrethroids are totally ineffective.

Dr. George reported that a tick vaccine being developed in Australia may be several years away from commercial use. He also mentioned that there is commercial interest in developing a vaccine against swine mange mites.

The screw worm situation in Libya has been controlled. Dr. George stated that sterile flies are being released in Central America as the program progresses southward.

There is a growing concern about the development of new pesticides and the reregistration of currently available pesticides with the Environmental Protection Agency. Dr. Bill Waggoner, Biochemistry Manager, Research and Development, Mobay Corporation gave a very enlightening presentation on the problem of reregistration of currently available pesticides. It appears that personnel in EPA are very familiar with crop production but lack knowledge of animal production methods. Support through the USAHA and the Animal Health Institute is solicited to request EPA to better familiarize their agency personnel with animal product usage.

The Chief, Reregistration Branch, for pesticides of the EPA was invited to present information on the reregistration program but was unable to attend due to budgetary restraints.

Last year, Dr. Patricia Conrad, parasitologist, University of California, made a presentation on a protozoan disease causing abortion in cattle, especially dairy cattle. Dr. Bradd Barr, pathologist, University of California, gave an update on the situation known as Bovine Protozoa Abortion. Abortion rates up to 20% have been attributed to this protozoan parasite in drylot dairies in California. It appears to be related to Neospora caninum but this has not been proven. A couple of isolates have been made and will be given to pregnant cows for further study.
Dr. George Hausman, technical service, Syntex reported the intraruminal injector for use with Synanthic, an anthelmintic, has been well accepted with no apparent problems. Use of Synanthic with the intraruminal injector is a prescription product.

Dr. M. G. Scroggs, technical service, AGVET division of Merck & Co., Inc. reported that Ivomec Pour-On was approved by FDA in December, 1990. The product does not have a claim for scabies but has claims for controlling horn flies up to 28 days post treatment, including pyrethroid resistant flies, and chewing or biting lice.

Two resolutions were presented. The first resolution states that USDA, APHIS should actively support the registration of highly efficacious products expected to be useful in exclusion and eradication of ticks of significant economic importance to the United States. The resolution was passed and is referred to the Resolutions Committee.

The second resolution urges USDA, ARS to increase their efforts in developing new acaricides and alternative tick eradication strategies including the development of technology for the control of ticks on white-tailed deer and exotic wildlife. This resolution was passed and is referred to the Resolutions Committee.

This committee adjourned at approximately 5:00 P.M. after a very informative meeting.
REPORT OF THE COMMITTEE ON PROFESSIONAL OVERSIGHT

Chairman: Dr. John R. Ragan, Nashville, TN
Vice Chairman: Dr. J.A. Cobb, Atlanta, GA

P.E. Bradshaw, IL; C.L. Campbell, FL; P.B. Doby, IL; L.J. King, MD; L.D. Konyha, FL; N.W. Kruse, CO; J.O. Pearce, FL; P.L. Smith, CA; S.T. Wilson, Jr., AL.

The Professional Oversight Committee met on Thursday, October 31, 1991.

A draft Statement of Purpose was reviewed and adopted.

The previous year's report was reviewed.

The draft manual for accredited veterinarians was discussed and the continuing need for brevity and simplicity in accreditation materials was noted.

A proposal to develop student chapters of USAHA at Colleges of Veterinary Medicine was considered. The Committee feels that the idea may have some merit, but should likely be considered by a broader-based group within the Association. It was concluded that a sense of the Board of Directors' feelings on the proposal should be obtained prior to further action.

No additional items were referred to the Committee and no resolutions were presented.
REPORT OF THE COMMITTEE ON RABIES

Chairman: Dr. Leon H. Russell, College Station, TX
Vice Chairman: Dr. Nancy A. Frank, Lansing, MI

D.J. Briggs, KS; H.M. Chaddock, MI; D.S. Davis, TX; T.J. Galvin, TX; E.P.J. Gibbs, FL; K.N. Haffer, NE; S.K. Harris, IA; R.E. Hill, Jr., IA; O. James, MT; F.D. McCasland, TX; R.B. Miller, MD; J.C. New, TN; C.E. Rupprecht, PA; L.A. Sawyer, MD; J.C. Wright, AL.


Program:

2. Update on Field Trials of Recombinant Rabies Vaccine – Dr. Charles E. Rupprecht, Wistar Institute, Philadelphia, PA.
3. Update of Wolf Rabies Vaccination Study – Dr. Larry J. Swango, Auburn University, Auburn, AL.
4. Post-Exposure Prophylaxis with Rabies Vaccine, Adsorbed (Rhesus) – Dr. Larry J. Swango.
5. Longevity of Antibody Titers in Recipients of Human Diploid Rabies Vaccines – Dr. Deborah Briggs, Kansas State University, Manhattan, KS.

There was no old business.

Business Meeting:

Under new business, the Committee discussed a statement of purpose as requested by President Pat Smith. A statement was proposed and accepted by the Committee by unanimous vote. The statement will be presented to President Smith.

Also under new business, concern was expressed about change in personnel in the Rabies Section of the Center for Disease Control (CDC). Dr. George Baer has retired and Dr. Dan Fishbein has been moved to a new assignment next week. The expertise of these people will be missed. It was suggested that the Committee propose a resolution to address this issue. Wording of the resolution was discussed. Final wording was approved by unanimous vote of the committee after Chairman Russell discussed the
REPORT OF THE COMMITTEE

resolution process and was assured that no one was uncomfortable with the resolution. Chairman Russell will propose that the resolution be directed to the Director of the Infectious Diseases Division at CDC.

Dr. Russell announced that he is stepping down as Chairman of the Rabies Committee having served in this capacity for 10 years. He will suggest that Dr. Frank, current Vice-Chairman, assume the position of Chairman. A Vice-Chairman is needed. Committee members thanked Dr. Russell for his valuable leadership of the Rabies Committee.

The meeting was adjourned at approximately 5:30 p.m.
The Transmissible Diseases of Swine Committee met at the Town and Country Hotel, San Diego, California, at 1:30 p.m., Monday, October 28, 1991. Fourteen committee members and 42 guests were present.

Dr. Robert Glock representing the Enteric Diseases of Swine Committee, AAVLD, informed us of his committee’s intent to update its monograph on swine dysentery and to develop similar publications on colibacillosis and clostridial diseases over the next year. The following year they hope to publish monographs for an additional two diseases. Monographs will include a review of current knowledge on epidemiology, pathogenesis, tests, availability of reagents, etc. This concept of publishing up-to-date information on important swine diseases with the hope of standardization of testing had been requested by Dr. W. Kadel in a letter to our committee. Discussion followed on the value of such information to diagnostic laboratories for enhancing disease surveillance and disease prevention programs. Dr. Glock advised the committee that his committee would consider a resolution on swine dysentery and that if passed the resolution would come to our committee in 1992. The resolution states a concern for lack of sensitive and specific tests to detect carriers of swine dysentery and urges USDA to implement research to develop and apply such tests for routine diagnosis and determination of disease incidence.

Dr. Tom Hagerty, as Acting Chair of the Joint USAHA, LCI, NPPC, AAVLD and AASP subcommittee on Swine Dysentery, presented a report from the subcommittee. He noted that a cost study had been undertaken in Minnesota. Preliminary results indicate a $50/sow cost of medication in infected herds. The subcommittee hopes to present a comprehensive report at next year’s meeting.
REPORT OF THE COMMITTEE

Dr. Carlos B. J. Pijoan, University of Minnesota, presented a report on the current status of respiratory diseases within the U.S. and abroad. He noted that during the past 20 years, little progress had been made in reducing chronic respiratory disease in swine. He also noted that respiratory diseases account for greater production losses than had been previously estimated and that even in the best managed herds close to 50% of slaughter swine have pneumonic lungs. Dr. Pijoan then presented a review of current knowledge of the respiratory diseases caused by *Pasteurella multocida* and *Actinobacillus pleuropneumonia*.

Dr. James E. Collins, University of Minnesota, presented a report on his work on Swine Infertility and Respiratory Syndrome (Mystery Swine Disease). He described the disease and noted that a similar emerging disease has been reported in Europe. The disease has spread rapidly in Europe and he reported that serologically the European and U.S. agents appear similar. Dr. Collins presented data confirming experimental reproduction of respiratory and reproductive forms of the disease with an agent isolated from infected tissue and grown in cell culture. He also indicated that a serum neutralization test had been developed for serological diagnosis. He reported preliminary seroepidemiologic data on a number of herds. Six out of seven SIRS herds had a high prevalence of antibody as compared to two out of seven control herds. Properties of the SIRS (Minnesota) virus are similar to the PRRS (European) virus isolates and studies are underway to further characterize and compare the isolates.

Dr. Patrick G. Halbur, Iowa State University, reported experimental production of Swine Infertility and Respiratory Syndrome pneumonia in three-day old gnotobiotic pigs with intranasal inoculation of tissue filtrates from a field case. Pigs died by seven days post inoculation. Lung lesions were characterized by a severe diffuse interstitial pneumonia with infiltration of alveolar septa by mixed mononuclear cells and accumulation of exudate and necrotic cell debris in alveolar spaces. One pig developed lymphohistiocytic encephalitis with vasculitis similar to that described in Europe with the Lelystad agent. An enveloped virus-like particle (80–100nm) with surface spicules has been observed in cell culture. A pig inoculated with small doses of tissue filtrate produced serum antibody to the Lelystad agent. Current investigations include attempts to reproduce the syndrome via inoculation of pregnant gilts.

Dr. Halbur also reported research on endemic proliferative interstitial pneumonia in nursery age pigs. Lesions of necrotizing and proliferative interstitial pneumonia and lymphoplasmacytic myocarditis were observed in field cases. The disease has been experimentally produced in gnotobiotic and conventional pigs. Pigs recover from clinical signs by 15 days but lung lesions persist through 35 days PI. Lung lesions were characterized by pronounced Type II pneumocyte proliferation, syncytial cell formation and accumulation of mixed inflammatory cells and necrotic cell debris in alveolar
spaces. Myocarditis similar to field cases was also present. Lesions described with influenza viruses were absent and no known swine viruses were isolated from experimental pigs. Two virus-like particles have been observed in cell culture. Characterization of these agents is underway.

Dr. Ronald D. Wesley, National Animal Disease Center, presented his results on a study which indicated that immunization of pregnant gilts with Porcine Respiratory Coronavirus induced partial resistance to challenge with TGE virus. He noted that Porcine Respiratory Coronavirus (ISU 1 strain) has been recognized in the U. S. for three years and is a deletion mutant of TGE virus. He stated that the PRCV in the U. S. is not identical to the PRCV isolated in Europe. He presented experimental results that indicated PRCV vaccinated pigs have substantial immunity to challenge with TGE virus.

The final presentation was given by Dr. Patrick Halbur on the pathogenicity of a porcine respiratory coronavirus isolate (AR 310) in gnotobiotic and conventional pigs. This virus was isolated from the intestines of a pig from a herd with endemic TGE. The isolate produces lesions characterized by bronchiolar necrosis, metaplasia, and proliferation and mononuclear cell infiltration of alveolar septa with accumulation of inflammatory cells and necrotic cell debris in alveolar spaces. No lesions were present in the intestines. The lung lesions resolved by 10–15 days PI. These findings are similar to previous reports in Europe, but the first report of a pathogenic PRCV in the U. S.

In the business section of the meeting, Dr. Roy Schultz introduced a resolution relating to the Swine Infertility and Respiratory Syndrome. The resolution was unanimously passed.

The meeting adjourned at 4 p.m.
REPORT OF THE COMMITTEE ON ZOOLOGICAL ANIMALS

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

P.M. Eppele, SD; L.H. Glenn, CO; A. Gonnerman, MO; W.P. Heuschele, CA; P.D. Hoctor, IN; D.L. Hunter, ID; D.J. Kelley, NC; J. Lubroth, CT; C.W.S. Lum, HI; C.J. Mikel, OK; G.P. Pierson, MD; R.L. Rissler, MD; C. Rives, TX; M.S. Silberman, GA; R.M.S. Temple, OH; C.O. Thoen, IA; E.T. Thorne, WY; D. Whittlesey, CO.

Committee Members Present:

W.P. Heuschele, CA; P.D. Hoctor, IN; D.L. Hunter, ID; C.J. Mikel, OK; C. Rives, TX; M.S. Silberman, GA; R.M.S. Temple, OH; C.O. Thoen, IA; E.T. Thorne, WY; D. Whittlesey, CO.

Meeting Minutes:

The Zoological Animals Committee met at 1:30 P.M. Wednesday, October 30, 1991. The Chair reported that no resolutions were offered.

In accordance with President Smith's directive, a draft committee statement of purpose was developed and presented to the committee members present for review and comment. Such comments were to be provided to the Chair prior to noon Thursday, October 31, after which time the final draft will be prepared and submitted to the USAHA Executive Committee.

Dr. Mort Silberman expressed concern about the confusion created when using the terms "zoo" and "zoological" within the context of proposed regulations or recommendations affecting a variety of facilities housing non-domestic species. After brief discussion the Chair proposed to appoint a subcommittee comprised of members of both the Zoological Animals Committee and the Wildlife Diseases Committee. This subcommittee will develop appropriate definitions and report back their findings to both the Wildlife Disease and the Zoological Animals Committee at or prior to the next annual USAHA meeting.

The Chair called the participants attention to the International Conference on Bovine Tuberculosis in Cervidae which took place in Denver, Colorado, July 16-17, 1991. Specific attention was given to the following recommendations which appear in the report from the Working Group on TB in Zoo Ungulates:

1. We support the concept of tuberculosis surveillance and control.
2. We believe that currently there is no viable test or testing program that will definitively identify antemortem tuberculosis in an individual animal, across the wide range of zoo ungulates.
3. It is our recommendation that in the absence of a reliable test, a
cervical skin test should be performed on ungulates prior to being shipped, and that the results of the test should be interpreted on the basis of herd history, pathology data and supplemental tests. The positive response of one animal to a single cervical test may not necessarily warrant a full-scale testing of a herd.

4. We recommend that as an alternative to euthanasia for tuberculosis test responders that are making a unique species contribution, consideration be given for treatment, in accordance with a protocol worked out through the cooperation of regulatory agencies and the zoo.

5. We recommend that zoos, researchers and regulatory agencies work together, financially and philosophically, to develop and evaluate reliable testing procedures that will be sensitive to the uniqueness of zoo ungulates.

6. We acknowledge that different tiers of institutions are referred to as zoos. Rule makers should take into account the internal, self-imposed health controls within the institutions.

7. We urge development of a spirit of cooperation and interaction between the zoo community and regulatory agencies. The American Association of Zoo Veterinarians (AAZV) stands ready to assist in the development of protocols or plans in cooperation with any government agency. AAZV will also inaugurate a program to establish an in-house tuberculosis surveillance protocol for use by zoo veterinarians.

Following a brief discussion of the above Mr. C. Rives made the following recommendation:

"That the Zoological Animals Committee endorses the resolution adopted by the Wildlife Disease Committee regarding tuberculosis in cervids, exotic species and other animals. This resolution strongly encourages USDA/APHIS/VS to take all steps necessary to validate appropriate tests; develop authority to regulate the interstate movement of cervids, exotic species, and other animals; and develop appropriate tuberculosis control guidelines for these species."

A discussion was held after which the requested endorsement was approved by the members present.

A brief summary of the REAC training program was given by the Vice Chair. Specific reference was made to the recently developed memorandum of understanding between APHIS and individual accredited AAZPA institutions in regard to the training of veterinary medical officers and animal care inspectors. A brief discussion ensued and the committee expressed support for continued REAC training and requested that appropriate funding be provided to facilitate expansion of this program. This training should include not only AAZPA accredited institutions but as well facilities within the non–domestic wildlife industry.

The Chair reviewed the final rule regarding the importation of ostriches and other ratites that became effective 12 August 1991. It was
ZOLOGICAL ANIMALS

noted that except for ratites intended for importation as zoological birds, all ratites and hatching eggs must be produced by and maintained as pen-raised birds. Also, except for zoological birds, a health certificate will be required after inspection of the entire flock of origin. During quarantine, all ratites will be tested for the routine viral diseases of poultry and will be treated with 5% carbaryl.

The Chair reported the fact that under the bird conservation act of 1991, two bills were introduced into the United States House of Representatives (H.R. 2540 and 2541) and two companion bills were introduced into the United States Senate (S.1218 and 1219). Five key differences between the bills were outlined:

1. Rate of phase out of imports of wild birds for sale as exotic pets.
2. Mandatory marking of birds.
3. Limitations on continued imports after the phase out of pet imports.
5. Preemption of state laws.

It was noted that H.R. 2541 and S. 1218 are being supported by the Cooperative Working Group On Bird Trade which includes: The American Association of Zoological Parks and Aquariums, the American Federation of Aviculture, the American Pheasant and Waterfowl Society, the Association of Avian Veterinarians, the International Council for Bird Preservation, the National Audubon Society, the Pet Industry Joint Advisory Council, TRAFFIC USA, and the World Wildlife Fund.

Bills H.R. 2540 and S. 1219 are being supported by the Humane Society of the United States, the Animal Welfare Institute, and the Defenders of Wildlife.

The Committee supports the intent of the legislation but varies on its support for its various bills. During discussion of the above matter it was noted that as a consequence of a letter writing campaign by the supporters of bills H.R. 2540 and S. 1219 over 25 airlines have halted all transportation of wild caught birds. In addition there have been reports of refusal by domestic airlines to transport captive bred wild birds. The Committee will explore this latter allegation.

The Chair called the groups attention to the concerns being raised over the potential for disease to be introduced into wild populations by the reintroduction of captive bred animals - The legitimacy of these concerns will be explored via a meeting being organized by the Captive Breeding Specialists Group of IUCN. This working group meeting is scheduled to convene in Oakland, California in November of 1992.

The Chair concluded his remarks by calling attention to the development of minimum quarantine standards for zoos and aquariums as a prerequisite for AAZPA accreditation; the preparation of a final draft of elephant handling guidelines for AAZPA accredited zoos; and the
REPORT OF THE COMMITTEE

collaborative effort between zoos holding wild bovids and the USDA to
develop semen importation protocols.

The meeting was adjourned at 3:34 P.M.
INTRODUCTION

To maintain competitiveness in U.S. and world markets, the U.S. livestock industry must have the latest technologies to improve production efficiency, increase leaniness, maintain product quality, and assure a safe product.

Advances in biotechnology during the last decade provide the means to develop new technologies for animal production and protection at a rapid rate. These new molecular biology methodologies provide unique opportunities to understand basic biological processes and to regulate key rate limiting steps in biological systems. These advances have already aided scientists in the development of genetically engineered vaccines, diagnostic probes, transgenic livestock, and pollutant eating microbes.

We have only scratched the surface in the use of these new technologies for agriculture. The public supported sectors of the agricultural research community have moved much too slowly in the use of molecular biology techniques for agriculture. We are too hesitant to integrate the biological and physical sciences into a comprehensive multidisciplinary team approach. Agricultural research and the commercial livestock industry have made tremendous progress in improving moderately and highly heritable traits such as milk production, growth rate, and proportion of edible lean meat and in the development and use of vaccines for preventative herd health programs. But we have made very little progress toward improving conception per service and disease resistance.

The reason for this slow rate of change from a conventional agricultural research approach to a new, more aggressive team approach for solving agricultural problems is not simply due to a lack of funding by the Congress and state legislators, but rather a desire at all levels—researchers, administrators, policy makers, and industry leaders—to continue with business as usual. We are changing, but it is clear the rate of change needs to be accelerated substantially to incorporate biological and physical scientists with whole animal biologists into problem solving multidisciplinary teams to develop specific information and technologies for animal agriculture.
GENOME MAPPING AND GENETIC CONTROL

POTENTIAL BENEFITS OF GENE EXPRESSION

A new program getting under way at the U.S. Meat Animal Research Center (MARC) is genome mapping of cattle, swine, and sheep. Some potential benefits of genome mapping and gene expression in livestock are: (1) use of genetic markers in a marker-assisted selection index program, (2) isolation of genes and gene insertion to produce transgenic animals with specific desired traits, (3) movement of genes from otherwise non-desirable to desirable animals to improve specific traits such as disease resistance and ovulation rate, (4) ability to more freely move germ plasm among all countries of the world, and (5) sustain biodiversity in endangered species. The potential benefits of marker-assisted selection to the livestock producer exceed the likely benefits from production of transgenic animals. Technology resulting in improved animals should have a proportional benefit to all livestock producers.

The availability and benefits of genome mapping and gene expression to the general public have been questioned because of patent rights and the cost of developing much of the technology in this area. Large commercial companies are more likely to be interested in producing diagnostic probes and products of biotechnology such as vaccines than in producing improved animals. Production of improved animals per se is too long-term, requires too much investment, and provides too low a return on investment for large-scale commercialization by agribusiness companies. But gene mapping of livestock will be highly beneficial to agribusiness companies in their development of diagnostic probes, vaccines, and other products.

Livestock producers, agribusiness, and, ultimately, consumers will benefit from the production and marketing of these safer, more effective, and potentially cheaper products for the livestock producer.

NEED FOR A GENOME MAP OF LIVESTOCK

Genetic markers have been and are being identified and mapped in the mouse, human, and, to a limited extent, in livestock. The development of maps for the genome of the different livestock species per se will not provide technology for improvement of livestock. These maps are necessary for the effective and efficient development and exploitation of gene expression in livestock. In other words, a map for each economically important livestock species is a necessary starting point, not an ending point, for technology development of gene regulation in livestock.

Progress made in the last decade in molecular biology and implementation of the Human Genome Project have brought us to the point where we can realistically undertake an effort to map the genome of cattle, swine, and other farm animals. Without the spinoff of technology and information from the Human Genome Project, mapping the genome of
livestock would likely be too expensive and too long-term for the agricultural research system.

**GENOME MAPPING**

A limited, but very significant, amount of information has been developed on genome maps in livestock by Dr. J. E. Womack and associates at Texas A&M University, College Station; Dr. R. Fries and associates, Swiss Federal Institute of Technology, Zurich; and Dr. D. J. S. Hetzel and associates, Commonwealth Scientific and Industrial Research Organization, Rockhampton, Australia. If substantial additional resources are committed to this area by several institutions, if a central focus is maintained to develop genome maps, and if data and technology from the Human Genome Project continue to be available at the present or a faster rate, I believe a limited but adequate map of the genome for cattle and swine can be developed within a relatively short period of time.

The Agricultural Research Service and, to my knowledge, others mapping or planning to map the genome of cattle, swine, and sheep, have different objectives and approaches than those of the Human Genome Project. The objective in farm animals is to develop 150 to 250 markers at approximately equi-distance along the genome of each species; not to sequence the entire genome, but to define the complexity and relative importance of genes involved in specific quantitative trait loci. In the disease area of farm animals, we will focus on identifying the resistant animal; whereas in human studies, the focus is on the susceptible individual. As important quantitative trait loci and specific genes associated with economically important traits are identified, fine mapping and sequencing of that section of the genome can be done and appropriate action taken to regulate the expression of specific genes.

Much progress can be made in livestock by the identification and use of genetic markers for quantitative trait loci associated with economically important traits. There is also a need to identify specific genes or groups of genes that regulate rate limiting steps in important biological processes such as marbling, ovulation, and resistance to a specific disease organism. Long-term progress in livestock improvement will be limited by the ability of multidisciplinary teams to focus on a specific area and to understand and identify rate limiting steps in important biological processes of the whole animal. Industry leaders and public funded institutions must work together to identify a limited number of important areas and establish priorities because funds will not be available to adequately research everything.
A primary objective of the Agricultural Research Service's program at the U.S. Meat Animal Research Center is to identify genetic markers linked with traits of biological and economic importance and to develop information needed to use these genetic markers in a marker-assisted selection index to improve livestock for desired traits.

Initially, a team of eight scientists, plus three postdoctoral fellows and support staff, will work exclusively on mapping the genome of cattle and swine. These scientists will focus over the next two years on developing a linkage and physical map with 150 to 250 markers at approximately equi-distance along the genome. The exact length of time will depend on progress by this and other groups in the U.S. and other countries. Progress will also depend on effective collaboration and exchange of information and material. After about two years, three or four MARC scientists will continue mapping the genome of cattle and swine and also focus on sheep. Other scientists will focus on genes or quantitative trait loci related to specific traits.

Specific objectives of the genome mapping project at Clay Center are:

1. Develop a linkage and physical map of the cattle, swine, and sheep genome
2. Develop reference populations and permanent storage of DNA from each animal
3. Coordinate the use of this DNA from MARC populations to accumulate linkage data
4. Develop, exchange, and use probes to map the populations
5. Maintain a probe bank

As maps are developed, the objectives will be expanded to include:

1. Find genetic markers linked with traits of economic importance
2. Characterize certain genes at the phenotypic and molecular levels
3. Use genetic markers for marker-assisted selection

**MARC REFERENCE AND RESOURCE POPULATIONS**

Establishment of cattle reference populations was initiated at MARC in 1990. The objective is to establish several three-generation reference families with 20 to 30 full-sibs per family. The reference families involve breeding F1 Longhorn-Hereford, Longhorn-Angus, Nellore-Hereford, Piedmontese-Hereford, and Piedmontese-Angus cows to a F1 Gelbvieh-Simmental bull. We are maintaining all paternal and maternal granddams, and we have stored semen on all paternal and maternal grandsires. In addition, we are establishing several reference families by backcrossing F1 Brahman-Hereford bulls to Hereford cows with DNA from all paternal and maternal grandsires and granddams. Development of reference populations for swine will begin in late 1991.
LASTER

Due to other program requirements, MARC maintains several unique resource populations. These resource populations include three breeds of swine obtained from the People's Republic of China, high and low backfat lines of swine, Booroola Merino sheep, a cow herd with a twinning frequency in young cows over 25 percent, and other populations with relatively wide genetic diversity.

OTHER

As a national federal research center, MARC's scientific staff and other unique resources provide an excellent opportunity to implement and sustain a multidisciplinary program involving molecular and population genetics. Numerous problems and knowledge gaps involving a relationship between genetics and meats, reproduction, nutrition, and animal health can be uniquely addressed at MARC. Scientists at MARC will participate in a national genome initiative and cooperate with scientists in universities, private industry, other federal laboratories, and other countries.
A NON-POCK FORMING VACCINIA VIRUS DOUBLE RECOMBINANT EXPRESSING F AND H GENES PROTECTS CATTLE AGAINST RINDERPEST

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Running Title: A VACCINIA VIRUS DOUBLE RECOMBINANT VACCINE FOR RINDERPEST

ABSTRACT

Rinderpest, a highly contagious viral disease of ruminants in Africa and Asia, causes greater than 95% morbidity and mortality. We have developed a vaccinia virus (Wyeth strain) recombinant that expresses both the fusion and the hemagglutinin genes of rinderpest virus. A single vaccination by intradermal inoculation with the recombinant virus provided cattle 100% protection from challenge inoculation with greater than 1000 times the lethal dose of the rinderpest virus. No pock lesions were detected in vaccinated animals, and no transmission of recombinant vaccinia virus could be demonstrated from vaccinated to contact animals. We believe that the insertional inactivation of both the thymidine kinase and the hemagglutinin genes of vaccinia virus led to increased attenuation of the virus which was manifested by the lack of the development of pock lesions in vaccinated animals. Unlike the tissue culture vaccine for rinderpest, the lyophilized form of vaccinia virus is thermostable and allows circumvention of the logistical problems associated with the distribution and administration of vaccines in the arid and hot regions of Asia and Africa. The development of an effective vaccinia virus recombinant which does not cause pock lesion may have wide application in the development of safe and efficacious recombinant vaccines for humans and animals. This becomes quite relevant with the concern of the use of vaccinia virus in a population with a high incidence of the human immunodeficiency virus.

Rinderpest, a highly contagious viral disease of cattle, buffalo, and other ruminants in Africa and Asia, is characterized by high fever, profuse bloody diarrhea, and greater than 95% morbidity and mortality. The history of rinderpest's effects on cattle populations has been documented since 376 A.D. and, until recently, it has accounted for a loss of over two million cattle
VACCINE FOR RINDERPEST

and buffalo per year. The rinderpest virus (RPV), is a member of the family Paramyxoviridae and the genus Morbilliviruses. Other antigenically-related members of the group include peste-des-petits-ruminants (PPR) virus of small ruminants (sheep and goats), canine distemper virus of dogs, measles virus of humans, and the morbillivirus of seals. RPV has a single-stranded RNA genome with a minus polarity. We have characterized at least 6 proteins of the virus, including the large (L), phosphoprotein (P), the hemagglutinin (H), the nucleoprotein (N), the fusion (F), and the membrane (M) proteins.

There is an effective cell culture adapted vaccine for rinderpest (TCRV) developed by the Muguga modification of the virulent Kabete O strain of RPV; however, its thermolability and high cost of production limits its use in the hot and arid regions of nomadic Africa and Asia. In preparation for the development of inexpensive and thermostable vaccinia virus (VV) recombinant vaccines for rinderpest, we made cDNA copies of the H and F genes of RPV and constructed VV single recombinants expressing either of these genes. Cattle vaccinated with a recombinant expressing either the F or the H gene were completely protected when challenge inoculated with greater than 1000 times the lethal dose of RPV. However, cattle vaccinated with a mixture of both recombinants (H+ F) had a sterilizing immunity (no detectable anamnestic response) to RPV after challenge inoculation, indicating a complete lack of replication of the challenge virus. The use of the cocktail in the field for vaccination against rinderpest is cumbersome and expensive, particularly taxing for developing countries. Since viral infection and spread are mediated by receptor binding (H) and membrane fusion (F), a vaccine preparation containing both antigens is superior to one containing either the H or the F glycoprotein alone. We report here the development of a highly attenuated vaccinia virus double recombinant that expresses both the F and the H genes (vRVFH) of RPV and causes no pock lesions in vaccinated cattle. Cattle vaccinated with the recombinant were protected 100% against a heavy challenge with RPV.

MATERIALS AND METHODS

We propagated Vero, human 143 TK-, and CV-1 cells in Dulbecco's modified Eagle's medium (DMEM) supplemented with heat-inactivated fetal bovine serum. DMEM was supplemented with 5-bromodeoxyuridine (BrdUrd) at 25 ug/ml for the propagation of TK- cells. The Wyeth (the New York City Board of Health) strain of vaccinia virus (clone B-3-1), obtained from Flow Laboratories (McLean, VA), was used exclusively for the generation of recombinants. The highly pathogenic Kabete "O" strain of RPV was propagated in Vero cells and used in all studies. All VV recombinants and viruses were propagated in Vero cells according to the guidelines of the Animal and Plant Health Inspection Service of the United States Department
We utilized plasmid vectors pVY6\textsuperscript{11} and pGS53\textsuperscript{12} for the construction of vRVFH, the vaccinia virus double recombinant expressing both the F and the H genes of RPV. Plasmid vectors pVY6 and pGS53 direct the cloning of genes in the HA and the TK regions of the vaccinia virus genome, respectively. First, we constructed vRVF, the single recombinant expressing the F gene from the HA region of the vaccinia virus genome. Briefly, the F cDNA of RPV was excised from plasmid pVRVF\textsuperscript{6} by digestion with EcoR1, filled in with Klenow polymerase, and then cloned in the Sma1 site of plasmid vector pVY6 to generate pVYRVF. The F gene was then cloned in the HA region of vaccinia virus by homologous recombination between pVYRVF and the Wyeth strain of vaccinia virus in CV-1 cells. Recombinants expressing the F gene (vRVF) were selected by their blue phenotype in the presence of X-gal. To construct vRVFH (double recombinant), we first excised the H gene of RPV from plasmid pRVH6 by digestion with EcoR1\textsuperscript{7}. The fragment was then blunted with Klenow polymerase and cloned in the Sma1 site of the vaccinia virus shuttle vector pGS53 to generate pGSRVH. The double recombinant (vRVFH) was generated by homologous recombination between pGSRVH and vRPVF in CV-1 cells\textsuperscript{10}. TK\textsuperscript{-} vaccinia viruses were picked by BrdUrd selection, and double recombinants were distinguished from contaminating, spontaneous TK\textsuperscript{-} mutants by plaque hybridization with H cDNA of RPV.

Proteins expressed by VV recombinants were characterized by radioimmunoprecipitation as previously described\textsuperscript{5}. Rabbit anti-measles H was used for specific immunoprecipitation of the H protein of RPV. Rabbit antiserum directed against the carboxy terminus of measles virus F protein was used for the immunoprecipitation of the F protein of RPV. Except for the first amino acid, the 18 amino acid long peptide used for generation of the antisera was completely conserved between measles virus and RPV\textsuperscript{13,14}. The expression of authentic F and H proteins of RPV by vRVFH, F by vRVF, and H by vRVH was demonstrated by specific immunoprecipitation. Fluorographs revealed that cells infected with vRVFH expressed both the F and the H proteins of RPV of the expected size. Similarly, the F protein expressed by vRVF and the H by vRVH were of the correct size suggesting that the extent of glycosylation is similar to that occurring in cells infected with RPV\textsuperscript{5}.

Protective immune response studies were conducted in yearling cattle in the high containment facility at the Plum Island Animal Disease Center (USDA–APHIS) according to proper institutional guidelines\textsuperscript{14}. Cattle used in these experiments were shown to be seronegative to RPV and vaccinia virus by serum neutralization (SN) and plaque reduction assays, respectively. Cattle were vaccinated with \(10^8\) pfu of VV recombinants by intradermal inoculation in the neck region. In addition, contact animals were housed with vaccinates in order to test for transmission of VV recombinants.
from vaccinated to nonvaccinated groups of animals. For the determination of protective immunity, all cattle were challenge inoculated with $10^3$ TCID$_{50}$ of RPV one month postvaccination. We have demonstrated previously, in a study using 19 animals, that as little as one TCID$_{50}$ administered subcutaneously in the prescapular lymph node region induced clinical rinderpest with 100% mortality.

Two groups of cattle were vaccinated with VV recombinants in separate isolation rooms. In the first group, five animals were vaccinated with vRVFH (Table 1). In the second group, four animals were vaccinated with a cocktail of vRVF and vRVH (Table 2). In addition, two unvaccinated animals were included in each group in order to assess the transmissibility of VV recombinants from vaccinated to contact animals. Pock lesions developed as early as four days in cattle vaccinated with the cocktail. The lesions were limited to the site of inoculation and were healed completely by two weeks postvaccination. In contrast, animals vaccinated with vRVFH developed no detectable pock lesions. A thorough examination failed to demonstrate pock lesions in the contact animals in both groups. Further, serum samples taken from contact animals on the days of vaccination (day 0) and challenge (day 28) were negative to vaccinia virus by SN and plaque reduction assays (data not shown). All cattle vaccinated with the recombinants produced SN antibody to RPV as early as 8 days after vaccination. However, all contact and control animals lacked detectable SN antibody to RPV during the course of the experiment (Tables 1 & 2).

One month post vaccination, all animals in both groups were challenge inoculated with $10^3$ TCID$_{50}$ of the pathogenic Kabete "O" strain of RPV. Cattle vaccinated with VV recombinants (both groups) were completely protected from rinderpest, exhibiting no detectable illness, and a normal temperature of 38°C. The four unvaccinated contacts developed high fever (42°C) by day two and died by day six after challenge. They also developed lesions typical of severe rinderpest, characterized by sloughing and erosion of the epithelial lining of the gastrointestinal tract and bloody diarrhea. After daily monitoring for two weeks and a lack of detectable clinical disease in vaccinated animals, the experiment was terminated.

DISCUSSION

In our previous communication, we reported the construction of VV recombinants which expressed the F or the H glycoproteins of RPV. Further, we demonstrated that cattle vaccinated with either recombinant (F or H) or a cocktail of both recombinants (F + H) were completely protected against a challenge inoculation greater than 1000 times the lethal dose of RPV. Although the single recombinants (F or H) alone provided 100% protection against rinderpest, humoral immune response of vaccinated cattle indicated the superiority of the cocktail over the single recombinants. Unlike
the single recombinants, the cocktail vaccine provides protection against both the initial infection (H-mediated) and the secondary spread (F-mediated). In addition, inclusion of several immunologically relevant proteins in a single vaccine increases the number of available epitopes and thus the chances for protective immunity in an outbred population. Consequently, it is essential to employ a vaccine that provides both the F and the H immunogens for field use. However, the use of a cocktail preparation in the field is cumbersome and more expensive than use of a single VV recombinant. A cocktail containing equivalent doses of vRVF and vRVH would be very difficult to produce by the scarification method in a calf and would require cell culture facilities for the quantification of infectious virus.

In this communication, we report the construction of a vaccinia virus double recombinant (vRVFH) which expresses both the F and the H glycoproteins of RPV. Further, we demonstrate that cattle vaccinated with the recombinant are completely protected against a challenge inoculation greater than 1000 times the lethal dose of the virus. Indicating the enormity of the virus load, no contact or control animals survived beyond six days post–challenge inoculation (Table 1). Usually animals can last 12 days or longer after exposure to the virus by natural contact.

We recognize the potential safety problems with widespread use of vaccinia virus. However, we have constructed the double recombinant using the Wyeth strain of vaccinia virus used worldwide in the successful eradication of smallpox. It has been demonstrated that insertional inactivation of the TK gene further attenuates the virus. In the double recombinant, the TK region has been inactivated by insertion of the H gene of RPV. In addition, the insertional inactivation of the HA region by the F gene of RPV has led to further attenuation of the recombinant virus. The absence of detectable pock lesions at the site of vaccination by the double recombinant is an indication of its greater attenuation. This did not affect its immunogenicity, however, since it provided cattle with 100% protection against a heavy challenge of RPV. Further, in comparing results of groups vaccinated with vRVFH and the cocktail, there was no significant difference in SN titers. No significant anamnestic response was observed after challenge indicating solid protection (Table 1 & 2). In addition, the safety of both the double and the single recombinants was confirmed by lack of transmission of vaccinia virus from vaccinated to contact animals. No pock lesions or antibody to vaccinia virus, as measured by SN and plaque reduction assays, could be demonstrated in contact animals. We believe that these outstanding safety features of the double recombinant make it highly suitable for field use. We also have the potential to further attenuate the recombinant virus by additional insertion of lymphokine genes such as interferon–gamma or interleukin–2.
VACCINE FOR RINDERPEST

Many of the reasons for recrudescence of rinderpest in Africa are related to problems in vaccine production, preservation, and delivery. The continuous movement of nomadic herdsmen and their animals also makes it difficult to assemble groups for vaccination. We have developed an effective and safe vaccinia virus double recombinant vaccine that protects cattle against a severe challenge of RPV. Unlike TCRV, the production and use of a vaccinia vector vaccine does not require refrigeration, trained personnel, or expensive cell culture facilities. The vaccinia virus recombinant vaccine can be produced with the same simple methods used during the WHO smallpox eradication campaign; in this campaign, smallpox vaccine was produced by extensively scarifying the skin of a calf and seeding the wounds with vaccinia virus.

In large scale vaccination program for rinderpest with TCRV, vaccination had to be repeated yearly for at least two years since calves under the age of six month could not be vaccinated. Inactivation of the vaccine virus by colostral antibody to RPV is a major problem in young calves. In contrast, calves can be vaccinated with the recombinant vaccine at any age, even in the presence of high levels of anti-RPV antibody, since anti-vaccinia virus antibody is not normally found in cattle.

Rinderpest is an excellent candidate for eradication using the vaccinia virus recombinant vaccine. There is only one serotype of RPV, although there are different strains manifesting different degrees of pathogenicity in the field. A vaccine against one strain will immunize against all, including peste-des-petits-ruminants of sheep and goats. The potential to develop a polyvalent vaccine with a single vaccinia virus recombinant expressing a number of heterologous genes has great significance, especially for nomadic regions of the Africa and Asia.

The absence of pock lesions in animals vaccinated with the double recombinant, and the lack of transmission of the virus from vaccinated to contact animals, may have important application in the development of safe and efficacious recombinant vaccines for humans and animals. This becomes quite relevant with the concern of the use of vaccinia virus in a population with a high incidence of the human immunodeficiency virus (HIV).

ACKNOWLEDGEMENTS

We thank Drs. J. Bittle, B. Osburn, C. House, and J. House for assistance and support, and S. Owens for critical review of the manuscript. We are grateful to Drs. B. Moss for providing vaccinia virus and plasmid vectors, E. Norrby for antisera to the measles H protein, and C. Richardson for measles F antisera. We acknowledge the excellent technical assistance of M. Beminger. The development of the double recombinant vaccine was supported by grants from the Food and Agriculture Organization of the United Nations (891487). The development of the single recombinant
vaccines and testing in cattle was supported by Cooperative Agreement DAN–4178–A–00–6040–00 from the United States Agency for International Development of the State Department. L. J. is a recipient of NIGMS Graduate Training Program in Biotechnology (GMO8343–01A1)

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14. The experiment was conducted with the approval of the USDA–APHIS (permits15648 and 16261). The current Administrator of the USDA–APHIS is on the Project's Advisory Panel selected by the USAID to ensure no infraction of regulations occur. The vaccine is now approved by the USDA–APHIS for field trial in Africa and Asia. When the field study is initiated, the head of the veterinary service of the host country will serve as a co–principal investigator on the project.

56
Table 1.
SN titers of cattle vaccinated with the double recombinant (vRVFH) expressing the F and H genes of RPV.

<table>
<thead>
<tr>
<th>Cow #</th>
<th>Day 0</th>
<th>Day 8</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 42</th>
<th>Day 57</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>2</td>
<td>64</td>
<td>16</td>
<td>64</td>
<td>48</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>32</td>
<td>24</td>
<td>48</td>
<td>24</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>126</td>
<td>0</td>
<td>128</td>
<td>48</td>
<td>64</td>
<td>24</td>
<td>&lt;100</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>0</td>
<td>48</td>
<td>64</td>
<td>64</td>
<td>96</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>0</td>
<td>12</td>
<td>64</td>
<td>32</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

Cow #102 was a control animal that was included in the group on the day of challenge (day 28). Animal #122 were unvaccinated, contact control. The rest of the animals were vaccinated on day 0 with \(10^8\) pfu of vRVFH, and serum samples were taken weekly during the course of the
experiment. SN titers were determined by the prevention of the cytopathic effects of 100 TCID₅₀ of RPV in vero cells. On day 28, all animals were challenged with 1000 TCID₅₀ of the pathogenic strain of Kabete "O" RPV.

Table 2.

SN titers of cattle vaccinated with a cocktail of vaccinia virus single recombinants expressing the F (vRVF) and H (vRVH) genes of RPV.

<table>
<thead>
<tr>
<th>Cow #</th>
<th>Day 0</th>
<th>Day 8</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Dead</td>
</tr>
<tr>
<td>133</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>Dead</td>
</tr>
<tr>
<td>101</td>
<td>0</td>
<td>24</td>
<td>24</td>
<td>96</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>118</td>
<td>0</td>
<td>128</td>
<td>64</td>
<td>24</td>
<td>12</td>
<td>64</td>
</tr>
<tr>
<td>124</td>
<td>0</td>
<td>24</td>
<td>32</td>
<td>64</td>
<td>96</td>
<td>64</td>
</tr>
<tr>
<td>131</td>
<td>0</td>
<td>96</td>
<td>384</td>
<td>32</td>
<td>128</td>
<td>64</td>
</tr>
</tbody>
</table>

Animals #121 and #133 were unvaccinated, contact controls. The rest of the animals were vaccinated on day 0 with 10⁶ pfu of vRVF+vRVH, and serum samples were taken weekly during the course of the experiment. SN titers were determined by the prevention of the cytopathic effects of 100 TCID₅₀ of RPV in vero cells. On day 28, all animals were challenged with 1000 TCID₅₀ of the pathogenic strain of Kabete "O" RPV.
The biotechnology industry in animal health and production is still very much in its early stages. It is, and will continue, to change many things as occurs with any revolution. The pace of these changes appear to have hastened, and have been generally beneficial, but in most instances have required adjustments and in all cases they clearly have costs attached to them.

While there are still many concerns about biotechnology, in general the public appears to be accepting it more readily each day, although there are still many trouble spots on the horizon. How quickly the public accepts these changes will be paramount not only in obtaining regulatory approvals, but also decisive in rate of investment of public funds in developing new products and technologies.

In the committee this year, we heard that USDA has licensed several products produced through biotechnology methods. Six licenses have been canceled due to replacement by other products. More than half of these licenses, or 28, concerned approval of diagnostic kits using products of biotechnology and one involved use of the polymerase chain reaction (P.C.R.), clearly a test born during the recent biotechnology revolution, which has taken place this past decade. We heard about 7 live gene deleted products which are in use and it was also explained that vaccinia vectored rabies had been tested with success and no untoward developments on an island and that this same product is under test at a site in Pennsylvania, where raccoon rabies abounds. A third test site in New Jersey is in the planning stages.

Another investigator presented information to the committee on the use of vaccinia virus for vectoring the genes of rinderpest virus encoded for the 2 proteins which are immunogenic. This product has been safety and potency tested in biocontainment facilities and the formalities required for its test in Africa where the disease exists are being planned.
REPORT OF THE COMMITTEE

Significantly, the investigator reported that the vaccinia virus had been so weakened in the process that it no longer produces a lesion at the site of inoculation, yet livestock are fully protected. Tests in primates to evaluate infectivity are soon to begin.

The speaker elaborated on a project underway at a large multidisciplinary research facility for genome mapping and genetic control of important traits in livestock. Such work is expected to assist in maintaining competitiveness of U.S. livestock in world markets, as well as improve production efficiency, increase leanness of meat, maintain product quality and assure a safe product. As in vaccines, advances in biotechnology provide the means to develop new technologies for animal production at a rapid rate.

Along the same line, we heard about studies underway on the recently imported Chinese pigs and, true to predictions, they are indeed turning out to be more resistant to entero-toxigenic E. Coli than are our conventional swine. These studies are expected to be expanded to develop methods for phenotyping animals. Through biotechnology, E. coli resistance and other desirable traits are expected to be transferred to other swine.

Biotechnology is being applied to all phases of the animal industry, including zoological collections such as in zoos and aviaries. It is being used to monitor population genetics so as to properly manage certain species and P.C.R. technology is extremely useful for detecting animals which harbor low levels of certain microbes which might endanger the species.

As mentioned earlier, biotechnology has given a boost to new, more sensitive and specific diagnostic tests. At least three new methods for diagnosing Johne's disease have been developed and these include a DNA probe coupled with P.C.R., absorbed antibody ELISA and the bovine gamma interferon test. These are all in lieu of the conventional inoculation of Johnin into the caudal fold. There is the hope these methods will prove useful for other Mycobacterium infections in other species where significant diagnostic problems have arisen, such as in deer and llama.

As mentioned above, P.C.R. offers new methods for diagnosis and with speed, accuracy and sensitivity not heretofore available. There were 5 papers on application of this technology, one a description of the test system, another describing its use for detecting pseudorabies virus carriers and the third for detection of foot and mouth disease virus in meat. Other enzyme methods for replication of specific pieces of DNA are also being developed and, of these, the enzyme Q–Beta replicase is equally exciting.

Biotechnology is still relatively new and developments are expected to continue. The effects of this technology will be felt in the structure and operation of research and development processes and in the further organization and development of agriculture production.
THE EPIDEMIOLOGY OF BLUETONGUE –TOWARDS 2000
Implications for Regulatory Control.

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I. SUMMARY

In the last decade, there have been significant advances in our knowledge of bluetongue (BT) and the causal virus. Insights into the pathogenesis and molecular structure of the virus provide the impetus for comparing and contrasting the epidemiology of BT in different parts of the world. This review outlines current research on the epidemiology of BT and the implications for disease control.

II. INTRODUCTION

It is axiomatic that research equates to better prevention and control of disease. This paper is a review of advances in our knowledge of the epidemiology and pathogenesis of bluetongue (BT), worldwide, and their relevance to international trade in animals and germ plasm. The review is presented as a series of questions and answers.

III. THE EPIDEMIOLOGY OF BLUETONGUE VIRUS: THE QUESTIONS

For assessing risk, prior to re-examining the appropriateness of current import/export policy, regulatory veterinary authorities, such as the Animal and Plant Health Inspection Service (APHIS) of the USDA, invariably ask questions. Relative to our present knowledge of the epidemiology of BT, it is possible to define 5 major questions and to frame them within the context of "patterns".

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a This paper is based on a review presented at the 2nd International Symposium on Bluetongue and Related Orbiviruses held at the Office International des Epizooties, Paris, in June of 1991. I am grateful to the organizers of this symposium for their permission to present this paper.

b For simplicity, most of the references cited are reviews from which the reader may determine the strict chronology of events leading to the concept under discussion.
EPIDEMIOLOGY OF BLUE TONGUE

1. Is there a pattern of stable ecosystems supporting the arthropod transmission of BT viruses in different climatic zones?

2. Do trading patterns in live animals and germplasm significantly contribute to the geographical distribution of BT viruses?

3. Is there a pattern of arthropod vectors of BT virus being transported over long distances and initiating infection/disease in livestock?

4. What factors influence the predominant pattern of distribution of disease (compared to infection) in the different climatic zones?

5. Is global warming of significance and, if so, will a pattern emerge and affect the above?

A. QUESTION 1. Is there a pattern of stable ecosystems supporting the arthropod transmission of BT viruses in different climatic zones?

1. Perspective.

The discovery of several orbiviruses, including BT viruses, in Australia and the subsequent molecular comparison with isolates from South Africa led Gorman and his colleagues to conclude that the differences detected were consistent with the evolution of the Australian BT viruses in continental isolation. It was also proposed that related viruses are perpetuated in separate geographical regions by different vector species. Parallel studies on the genome of BT viruses emphasized that since a) a minority of gene segments determine the serotype of a virus and b) that reassortment of the genome segments between viruses can occur, "molecular epidemiology" has greater relevance than serological comparisons of isolates when defining ecosystems.

Support for the pioneering work in Australia has come from studies in the New World. While acknowledging the limitations of serotypes as an indicator of ecological zones, studies through the 1980's on the epidemiology of BT virus in Florida, several Caribbean countries, Northern countries of South America, and most of the countries of Central America revealed that the range of serotypes of BT viruses in this region was different from those in North America. Gould and his colleagues have extended Gorman's approach by comparing the coding sequence of the highly conserved gene segment 3 of BT viruses from different regions of the world, including North America. The data support the concept that viruses from at least 5 geographic areas can be differentiated, irrespective of serotype. A dendrogram of the percentage nucleotide difference of the viruses that have been examined will soon be published.
2. Answer.

Although the geographical partitioning of populations of BT viruses by the criterion of vector species and competence remains to be vigorously tested, the concept that BT virus is maintained in several separate ecosystems is gaining acceptance. Molecular comparisons of BT viruses from different regions of the world indicate that it is now unlikely that bluetongue "emerged" from Africa in this century as previously thought.

While several of the ecosystems appear to be relatively stable, a caveat needs to be inserted here. Despite the evidence that the Australian isolates of BT viruses appear to have evolved in isolation on that continent, the faunal ecology of Australia has been "disturbed" by the comparatively recent introduction of domestic ruminants and associated Culicoides species to the continent. The epidemiology of bluetongue in Australia may therefore be more dynamic and take a different course from other areas of the world especially if the predictions of global warming prove correct (see below).

B. QUESTION 2. Do trading patterns in live animals and germplasm significantly contribute to the geographical distribution of BT viruses?

1. Perspective.

Until the mid-1980's, there was lingering concern that cattle could be persistently infected with BT virus and further, that the virus could be secreted in semen. The relevance of earlier work on persistence of the virus in cattle was questioned at the First Symposium on Bluetongue in California in 1984. Subsequent studies were reviewed by Maclachlan and colleagues who concluded that "[restrictive trade] policies are in large part based upon the unsubstantiated assumption that persistent BT infection of cattle is significant to the epizootiology of BT infection". Other concerns over the possible international transfer of the virus in embryos were similarly dispelled in a series of detailed experiments that resulted in the publication in 1985 of international protocols for the safe transfer of germplasm. The sensitivity and specificity of the currently available diagnostic techniques, combined in the case of live animals with appropriate quarantine, now make it unlikely that BT viruses will be transmitted between continents through legal trade in live animals and germplasm. Between neighboring countries, however, the relaxation of regulations associated with common trade areas may lead to inadvertent importation of infected animals. Nevertheless, unregulated trade as it applies to cattle and BT has existed for many years between some countries, such as the USA and Mexico, without incident.
3. Answer.

The realization that persistent infection and transmission of the virus in germplasm are now of comparatively little importance in the epidemiology of the virus, together with the emerging information on the evolution of viruses within ecosystems as discussed above, indicate that trading patterns, particularly in recent years, have probably contributed comparatively little to the geographical distribution of BT viruses.

C. QUESTION 3. **Is there a pattern of arthropod vectors of BT virus being transported over long distances and initiating infection/disease in livestock?**

1. Perspective.

  If we accept that a) international, particularly intercontinental, trade contributes little to the introduction of BT viruses and b) the viruses are perpetuated in several defined ecosystems, it is pertinent to ask how do epidemics of disease occur? Several factors contribute to an epidemic, the susceptibility of livestock being one of the most important as will be discussed under question 4.

  The concept of windborne transmission of BT virus, first advanced in the 1970's, remains attractive. Outbreaks of BT and the related orbivirus infection of epizootic hemorrhagic disease in deer in Canada in 1987 may have resulted from the northerly movement of vectors from the USA. Similarly, it is conceivable that BT serotype 2 may have been introduced to Florida from the Caribbean, or elsewhere, by prevailing winds. Two observations made during the epidemiological study of BT in the Caribbean and Central American Region also add weight to the concept. In 1987, BT serotype 3 first appeared as a sub-clinical infection in sentinel herds in the Eastern Caribbean, before isolation on more westerly islands and in Central America. In 1988, a massive introduction of the desert locust (Schistocerca gregaria) extended from Guyana in South America to Jamaica in the Western Caribbean. The introduction was traced to tropical disturbances in the wake of Hurricane Joan. It was estimated that the locusts crossed the Atlantic in approximately 6 days in a low level jet stream. While this does not prove that less robust insects such as Culicoides could also have made the trans-Atlantic crossing, it at least suggests the need to compare Caribbean and West African strains of BT virus.

3. Answer.

  While there have not been any outbreaks of BT, for example on islands with no recent history of animal trade, that can provide definitive
GIBBS

proof that windborne introduction of Culicoides species can lead to epidemics of BT, there is now considerable circumstantial evidence supporting the concept. The power of the molecular approaches to epidemiology, used by Gould and colleagues\(^3\) as discussed above, will be invaluable for future analyses.

D. QUESTION 4. What factors influence the predominant pattern of distribution of disease (compared to infection) in the different climatic zones?

1. Perspective.

The obsessional fear of BT that characterized the period from the epidemic in the Iberian Peninsula to the mid 1970’s was based on the premise that the introduction of virus into a country would lead to dramatic and extensive outbreaks of disease. The introduction of simple serological tests led to the discovery that ruminant livestock in probably all countries in the tropics and sub-tropics is endemically infected with BT virus, yet clinical disease is rarely seen. An adequate explanation why the indigenous ruminants in these endemic areas rarely, if ever, develop clinical disease has never been established. In contrast, there are many examples of outbreaks of BT occurring in flocks of susceptible European breeds exported to such endemic areas on the African continent and Indian sub-continent, thus confirming that the circulating viruses are pathogenic.

One theory to explain the above phenomenon was based upon the concept that in endemic areas ruminants are sub-clinically infected while still protected by passive immunity acquired from the dam. While this may be true, one study, in an endemic area for BT virus, has shown that there are years when virus activity is minimal and animals may be one year old before sub-clinical infection occurs.\(^{11}\) Recent work in Australia, in which sheep of various ages were experimentally infected with BT viruses of different serotypes, indicates that the severity of the disease increases with age.\(^{12}\)

2. Answer.

Although one can postulate that in the temperate areas of the world, outbreaks of disease may be more severe and extensive than in endemic areas because of a) breed susceptibility, b) no prior passive immunity, and c) heightened susceptibility of older animals (>4 years), we still have comparatively little understanding of the mechanisms of viral pathogenicity in different regions of the world.

E. QUESTION 5. Is global warming of significance and, if so, will a pattern emerge and affect the above?
EPIDEMIOLOGY OF BLUETONGUE

1. Perspective.

If the forecasts of the global warming trend—or "greenhouse effect" as it is often called—are correct, there could be profound changes in the geographical distribution of arthropod-borne diseases. Accurate prediction, however, of how the distribution of a disease might be affected in a specific area has proved to be difficult.

Stem and his colleagues studied the possible consequences of the greenhouse effect on the distribution of BT virus in the USA and concluded that by 2050 livestock in a further 15 states will be endemically infected. The corollary is that the greenhouse effect will lead to an encroachment of a subtropical climate into the Nearctic, with the implication that the range of serotypes of BT virus may also increase. Similar concerns have been expressed in Australia, where it is feared that the increased temperature may allow more effective Culicoides vector species to spread into the southern sheep-rearing areas of the continent.14

2. Answer.

The implications of global climatic changes are difficult to define, but there is general agreement that, as temperatures rise and rainfall patterns adjust, the area of BT activity will expand.

IV. CONCLUSIONS

It is axiomatic that epidemiological knowledge provides the base for risk assessment which, in turn, translates into disease control. Analysis of the epidemiology of BT has traditionally focused on the prevalence of serotypes in the various regions of the world and their ability to cause disease. This is both understandable and pragmatic because it reflects the history of research on the virus and an empirical approach to vaccination. In countries, such as South Africa, where disease occurs regularly, control by vaccination is testament to the effectiveness of this philosophy. Viewed globally, however, risk assessment by simply focussing on the serotypes of BT raises the question "what is the cost-benefit in terms of money to track them down, restrain them, and keep them from spreading from place to place?"0

In this review, I have attempted to outline some alternative approaches for exploring the epidemiology and thereby the control of BT on a global scale. Unquestionably, the advent of molecular epidemiology, has provided us with sophisticated tools for the analysis of geographical variants in BT viruses and their relationship to ecosystems and arthropod vectors. The results are already providing us with data upon which more accurate assessments of risk can be based. In the parallel field of vaccine
development, recombinant DNA technology is providing a similarly exciting toolkit with the prospect of novel vaccines for control of BT that may be generic rather than type specific. The mind-set whereby the epidemiology of BT is largely seen from the perspective of serotypes is broken!

And what of the future for import/export policy? All indicators point to the decade of the '90s providing rapid advances in our ability a) to detect the virus in infected animals and b) to protect animals through vaccination. Provided with such tools and with a better understanding of the epidemiology of the virus, veterinarians and farmers can look forward to 2000; they can anticipate simplified regulations regarding BT and the international movement of animals and their products.

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CONTROL OF BOVINE LEUKEMIA VIRUS INFECTION
IN CALVES

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The goal in controlling bovine leukemia virus (BLV) infection is to create a population or subpopulation of non-infected cattle. Depending on the producer's market and management philosophy, the specific type of cattle in which infection is to be controlled may vary from only bulls or heifers destined for stud or international markets to the entire herd. Regardless of individual producer needs, it reasonable that efforts begin by controlling infection in calves.

In general, there are two main objectives to be accomplished in creating a population or subpopulation of BLV-free calves. One is to identify and remove sources of BLV transmission, and the other is to maintain BLV-free calves through test and segregation (1).

BLV Transmission

In considering the various means of BLV transmission, it is appropriate to begin with fetal transmission and progress through possible exposures calves may encounter as they age. In utero infection may take place in 5–10% of calves born to infected cows (2,3). The only factors suggested so far to be connected with an increased risk of in utero infection are presence of lymphosarcoma in the dam (4) and the fetal sex being male (2). Although information concerning these factors may not assist in controlling in utero transmission, knowledge of the sex of the calf and of lymphosarcoma in the dam may help guide post-partum segregation of calves. Herd rates of in utero infection can be reduced by controlling overall herd infection, thereby reducing the number of infected cows, and by using only BLV-negative embryo recipients.

A significant amount of transmission is believed to take place at the time of parturition (5), particularly when cows calve in a common maternity area. During parturition and immediately post-partum, considerable maternal and fetal blood may contaminate calves and/or cows. The behavior of cows to lick blood, membranes, and calves, whether their own or not, is likely to contribute to transmission during the highly susceptible (agammaglobulinemic) period of the calf. Post-partum transmission can be reduced by avoiding blood spillage and contamination during deliveries, by the early removal of the calf from the cow, and by fastidious sanitation to minimize exposure to blood and membranes of other cows or calves. Ideally, cows should calve in individual pens, thereby preventing exposure.
BOVINE LEUKEMIA VIRUS INFECTION

to other cows and calves. If individual pens cannot be provided, segregated calving areas, one for infected and one for noninfected cows, would help reduce the chance of transmission.

After calving, the next likely source of infection is the colostrum. Bloody colostrum or colostrum from mastitic quarters may contain sufficient infected lymphocytes to produce infection, in spite of the presence of anti-BLV antibodies (6). Transmission via colostrum can be controlled by feeding colostrum only from known BLV-free cows or by feeding pasteurized colostrum. A disadvantage in feeding colostrum only from negative cows is that some protection against infection may be lost (6,7).

Transmission via milk can be minimized by reducing or eliminating the feeding of mastitic milk from BLV-infected cows. This can be done by feeding low CMT milk from the tank, not from 'hospital' cows, by feeding pasteurized milk (8), or by feeding milk replacer (9).

Iatrogenic transmission can take place anytime after birth. Possible methods of iatrogenic transmission include ear tagging, tattooing, oral treatment via balling guns, use of nose tongues, deteating, and dehorning (10–12). Prevention of iatrogenic transmission should include disinfection of instruments and tools between animals and use of electric dehorners or dehorning paste. Iatrogenic transmission may result from calves licking blood on each other or on blood-contaminated structures, such as stanchions or fences. Efforts should be made to minimize bleeding and blood spillage, and to ensure that infected calves are not in physical contact with susceptible calves. Exposure to blood following a procedure, such as dehorning, may pose a greater risk of infection than the actual procedure (12).

Although arthropod vectors, such as mosquitoes and horse flies, have been shown to transmit BLV experimentally, data are not available to indicate that flies constitute an important natural means of transmission (13). Nevertheless, it would be prudent to practice appropriate fly control. Similarly, the lack of evidence for transmission following use of injection needles (12, 14) should not preclude use of individual sterile needles.

BLV Testing

Currently, agar gel immunodiffusion is used to test for antibodies to BLV. It is important that infection status of calves be monitored as often as possible to permit early identification and segregation of infected calves from susceptible calves. There are several approaches to consider in testing for BLV antibodies. Early identification of calves infected in utero requires testing of precolostral sera, which, under many management situations, may pose logistical problems. Another approach to identifying calves infected in utero or shortly after birth is to monitor antibody titers during the first 4–6 months (15,16). Calves with titers that do not decay can be considered to
be infected. Negative and suspect calves should be tested as often as possible, preferably every 2–3 months, depending on the incidence rate of infection. High rates, such as 1–2 new infections per month, may justify more aggressive testing.

Segregation

Control measures and testing can be facilitated by segregation of calves into one of three groups according to their infection status (17, 18). An infected group would consist of calves with precolostral antibodies, a non-decaying titer during the first six months of age, or a conversion from negative to positive status. A suspect group would consist of calves for which infection status is unknown, such as calves with BLV antibodies during the first six months, calves from cows with lymphosarcoma, or calves believed to have been exposed. A negative group would consist of calves that have repeatedly tested negative for BLV antibodies.

Segregation should be arranged to prohibit physical contact of calves within and among groups. Preferably, calves within a group should not be allowed contact with each other. The groups should be situated so that water and feed cannot be shared by calves in different groups. Routine feeding, treatment, and general working of calves should be done first among the negative calves, with sick calves handled last. Calves in the infected group should be fed and worked last. Noninfected and suspect calves should be fed colostrum and milk from negative cows, pasteurized colostrum and milk, or milk replacer. Infected calves can be fed mastitic colostrum and milk from infected cows.

Control of BLV infection in calves can be enhanced by control of infection in the entire herd. Programs aimed at producing BLV-free calves, therefore, should consider appropriate control of adult infection.

References

5. Van Der Maaten MJ, Miller JM, Schmerr MJF. Factors affecting the


REPORT OF THE COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUS

Chairman: Dr. B. I. Osburn, Davis, CA
Vice Chairman: Dr. L. D. Miller, Ames, IA

J.A. Acree, MD; G.A. Anderson, KS; T.L. Barber, CO; D.J. Carr, WI; T. Conger, AR; W.C. Davis, WA; E.J. Dubovi, NY; J.F. Evermann, WA; T.B. Falls, VA; R.W. Fulton, OK; C.A. Gipson, FL; C.M. Groocock, NY; R.D. Heilman, VA; R.B. Hillman, NY; T.J. Holt, NY; J.A. House, NY; T.H. Howard, WI; M.M. Jochim, CO; A.J. Luedke, CO; N.J. MacLachlan, CA; S. McConnell, TX; R.W. Mead, WA; J.O. Mechem, WY; H.E. Metcalf, MD; J.M. Miller, IA; D.R. Monke, OH; J. Nehay, CA; V.F. Nettles, GA; J.E. Pearson, IA; J.R. Pitcher, TX; R.D. Schultz, WI; J.L. Stott, CA; D.E. Suther, CA; P.R. Turner, TX; J. Van Der Maaten, IA; K. VanSteenbergh, MO; T.E. Walton, WY; S.i. Wechsler, WY; W.C. Wilson, WY; G.O. Winegar, MD.

The Bluetongue and Bovine Retrovirus Committee met on Tuesday, October 29, 1991 from 1:30 p.m. to 5:45 p.m. in the Town and Country Hotel in San Diego, California. There were 62 in attendance during the meeting.

BOVINE RETROVIRUS

The bovine retrovirus portion of the meeting was chaired by Dr. Lyle Miller, Iowa State University, Ames, Iowa. Information on carcass condemnations at federally inspected slaughter plants was presented by Dr. Janice Miller, USDA, ARS, NADC, Ames, Iowa. The number of condemnations in 1990 for lymphosarcoma among all cattle was 49 cases/100,000 cattle slaughtered and the rate for mature cows was 273 condemnations/100,000 cows slaughtered. These numbers far exceed the level of 30 cases/100,000 cattle in Denmark in the 1950's when it became the first country to embark on an eradication program. Speculation on the reasons for the increased condemnations included a higher infection rate for Bovine Leukemia Virus (BLV) as herds increase in size and young stock are commingled in larger groups. Also, there is preliminary evidence that dual infections with BLV and bovine lentivirus may enhance the oncogenicity of BLV.

Dr. Jeff Stott, School of Veterinary Medicine, University of California, Davis, CA, reported on the usefulness of the Polymerase Chain Reaction (PCR) and southern blotting technology to detect the BLV genome in peripheral blood lymphocyte cultures. The technique could be especially helpful in detection of viral infection in calves with colostral antibodies.

Dr. Mark Thurmond, School of Veterinary Medicine, University of California, Davis, CA reported on calf management procedures for dairymen to control BLV infection. His paper also was presented at the plenary session and will be printed in the proceedings.
Dr. Don Monke, Select Sires, Plain City, OH, discussed responses by Artificial Insemination (AI) centers to various international requirements for BLV control. Before the discovery of BLV, and the development of serologic tests to detect virus infection, repeated hematologic examinations were made to detect persistent lymphocytosis. Hematologic procedures rapidly became obsolete when the specificity and reliability of the agar gel immunodiffusion (AGID) test was known in the mid 1970's. A major problem with the serologic tests in AI centers is that the BLV status of young bulls that have colostral antibodies is uncertain until they are well over 1 year of age. In the process of "proving" sires, it is common for importing countries to bring in semen, artificially inseminate their cows and do the progeny testing in their country. Also, they often require a seronegative test of the donor bull at 2 years of age before any semen can be imported. Thus there can be nearly a 1 year delay in initiating the process of "proving" some sires. Other problems can occur when a seronegative test on the bull's dam is required and she is not readily available for testing or when questions arise in the case of a bull from embryo transfer, whether it is the genetic dam or the surrogate dam that must be tested. Often the surrogate dam has not been retained in the herd.

In some situations, all bulls in an AI center or within a subunit in the AI center must be seronegative before any semen can be exported. Many of the AI centers problems with BLV could be reduced or eliminated if a national BLV herd certification program was established and became successful. Programs in the State of New York and other countries provide examples that could be useful for a national program.

Mr. Richard Nelson, Holstein-Friesian Association, Brattleboro, VT, discussed the need for a single certifying body for voluntary programs to control or eradicate BLV infection in cattle herds. The Purebred Dairy Cattle Association (PDCA) has taken a position that all dairymen be urged to monitor their respective herds for BLV and identify seropositive animals. Those cattle will be managed to minimize the spread of infection to offspring and other cattle in the herd. The PDCA also urges breed associations to encourage the announcement of BLV serologic test results for each animal at the time of a public or private sale.

Requirements for international movement of genetics, whether it be germplasm, embryos or live animals, continue to become more restrictive for BLV. The need for programs to allow herd owners to establish and maintain BLV free herds will continue to increase.

Following additional discussion, action was taken to establish a committee to study various local and international BLV control programs and charge the committee to formulate a uniform and internationally acceptable program proposal for BLV presentation in U.S. cattle herds to USDA,APHIS for comment before the next annual meeting of the USAHA. The committee will include Lyle Miller, Ames, Iowa, Chairman; Ed Dubovi, Ithaca, NY; James Evermann, Pullman, WA; Don Monke, Plain City, OH; Richard Nelson,
BLUETONGUE AND BOVINE RETROVIRUS

Brattleboro, VT; Mark Thurmond, Davis, CA and others to be appointed later.

BLUETONGUE REPORT

Dr. Bennie Osburn (CA) chaired the Bluetongue session. Dr. J. E. Pearson, National Veterinary Services Laboratories gave an update on bluetongue and epizootic hemorrhagic diseases in the U.S.

Virus Isolation

In calendar year 1990, there were bluetongue (BT) isolates from 15 submissions to the National Veterinary services Laboratories (NVSL). In addition, isolates submitted to the NVSL were typed and BT was isolated and identified on four premises by the Arthropod–Borne Animal Disease Laboratory (Table 1). There was one epizootic hemorrhagic disease (EHD) isolate. As of September 15, 1991, there have only been two BT isolates, both from bighorn sheep (Table 2).

The Belize, Trinidad, and Dominican Republic isolates were from water buffalo that were being tested for entry into the United States. They were refused entry.
### TABLE 1. BLUETONGUE ISOLATES

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Type</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>*California</td>
<td>Bovine</td>
<td>BT13</td>
<td>?</td>
</tr>
<tr>
<td>*Alabama</td>
<td>Bovine</td>
<td>BT10 &amp; 17</td>
<td>?</td>
</tr>
<tr>
<td>*California (2 isolates)</td>
<td>Bovine</td>
<td>BT17</td>
<td>?</td>
</tr>
<tr>
<td>*California</td>
<td>Sheep</td>
<td>BT10 &amp; 17</td>
<td>?</td>
</tr>
<tr>
<td>*California</td>
<td>Deer</td>
<td>BT17</td>
<td>?</td>
</tr>
<tr>
<td>*California</td>
<td>Bovine</td>
<td>BT13</td>
<td>?</td>
</tr>
<tr>
<td>**Nevada</td>
<td>Sheep</td>
<td>BT13</td>
<td>July</td>
</tr>
<tr>
<td>**Utah</td>
<td>Sheep</td>
<td>BT11</td>
<td>July</td>
</tr>
<tr>
<td>**Nevada</td>
<td>Sheep</td>
<td>BT13</td>
<td>Sept</td>
</tr>
<tr>
<td>**Utah</td>
<td>Sheep</td>
<td>BT11</td>
<td>Sept</td>
</tr>
<tr>
<td>Trinidad</td>
<td>Water Buffalo</td>
<td>BT4,8,17</td>
<td>Sept</td>
</tr>
<tr>
<td>Belize</td>
<td>Water Buffalo</td>
<td>BT13,14</td>
<td>Sept</td>
</tr>
<tr>
<td>*California</td>
<td>Sheep</td>
<td>BT13</td>
<td>Sept</td>
</tr>
<tr>
<td>***Idaho</td>
<td>Sheep</td>
<td>BT17</td>
<td>Oct</td>
</tr>
<tr>
<td>Oregon (4 farms)</td>
<td>Sheep</td>
<td>BT17</td>
<td>Oct</td>
</tr>
<tr>
<td>***Idaho (3 farms)</td>
<td>Sheep</td>
<td>BT17</td>
<td>Oct</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>Zebu</td>
<td>BT11</td>
<td>Oct</td>
</tr>
<tr>
<td>*California (2 farms)</td>
<td>Sheep</td>
<td>BT17</td>
<td>?</td>
</tr>
<tr>
<td>*California</td>
<td>Sheep</td>
<td>BT10</td>
<td>?</td>
</tr>
<tr>
<td>*California (2 farms)</td>
<td>Bovine</td>
<td>BT17</td>
<td>Nov</td>
</tr>
<tr>
<td>*California</td>
<td>Bovine</td>
<td>BT10</td>
<td>Nov</td>
</tr>
</tbody>
</table>

*Isolates submitted from the University of California for typing

**Reported by Dr. Chris Chase, Arthropod-Borne Animal Disease Laboratory

***EHD – 1990 isolates submitted by Dr. James Everman, University of Washington for typing

### TABLE 2. BLUETONGUE VIRUS ISOLATIONS

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Type</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>Bighorn Sheep</td>
<td>BT10</td>
<td>April</td>
</tr>
<tr>
<td>New Mexico</td>
<td>Desert Bighorn Sheep</td>
<td>BT17</td>
<td>July</td>
</tr>
</tbody>
</table>
BLUETONGUE AND BOVINE RETROVIRUS

Bluetongue_Survey

Due to budget restrictions, only four states were surveyed in 1990–91. The 1991–92 survey was started October 14, and will include the 19 Northern states sampled previously, plus Alaska and Hawaii. The results of the 1990–91 survey were:

<table>
<thead>
<tr>
<th>State</th>
<th>Number of Samples</th>
<th>ID Pos (%)</th>
<th>Neutralization</th>
<th>BT</th>
<th>BT/EHD</th>
<th>EHD</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delaware/Maryland</td>
<td>702</td>
<td>13 (1.8)</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td>641</td>
<td>14 (2.2)</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>North Dakota</td>
<td>625</td>
<td>3 (.5)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Bluetongue Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA)

A meeting was held in Vienna, October 15, 1990, to discuss the sensitivity and specificity of the C-ELISA test. There were representatives from the United States, Canada, England, Australia, as well as from the Office of International Epizooties (OIE) and the International Atomic Energy Agency. The conclusion was that the sensitivity and specificity of the test was satisfactory. The following are the recommendations of the group:

1. OIE adopt the C-ELISA as the internationally recognized standard procedure to detect BT antibody.
2. The proposed protocol be accepted as a standard procedure for the reference test.
3. This test, using the reagents and procedure described, should be used as the bluetongue C-ELISA reference standard in national laboratories to standardize local tests.

Requirements for Export of Semen to European Committee (EC)

Semen from bulls negative for BT antibody using CELSIA test, and EHDV antibody using AGID test which are in a BT free area can be exported to EC. A vector free area is defined as those states with 2% or less AGID positive samples as determined by the annual BT survey. Other vector free areas may also be defined by USDA if agreed upon by EC.

Semen from serologically positive animals or semen collected in areas with vectors can be exported if virus isolation is negative in sheep and
REPORT OF THE COMMITTEE

embryonated chicken eggs using a very complex procedure. The bull must have a stable antibody titer during collection and for 21 days after the last collection using the neutralization and CELISA test.

In addition, member countries can refuse any importation of semen from serologically positive bulls.

After December 31, 1992, semen from bulls that are serologically positive will not be accepted unless the requirements are renegotiated.

Dr. Paul Gibbs, University of Florida, gave an overview of the epidemiology of bluetongue virus infections. His manuscript is included in the proceedings.

A review of OIE Workshop Proceedings of the Second International Symposium on Bluetongue African Horse Sickness and Related Orbiviruses was given by Dr. N.J. MacLachlan and Dr. B.I. Osburn. Dr. MacLachlan reported on the germplasm report which he co-chaired with Dr. Geoff Gard (Aust). He emphasized that many of the recommendations had not been accepted by all regulatory officials in a few European Countries. Recommendations of the Germplasm Committee and many international scientists were based upon uniform conclusions that persistent infection of ruminants is unimportant in nature. There is no evidence that bluetongue virus has been transmitted by seropositive bulls between regions of the United States or between countries that receive the germplasm.

Dr. Osburn summarized the Epidemiology Reports chaired by Drs. Tom Yuill, University of Wisconsin and Peter Kirkland, Australia. Distribution of orbivirus transmission can be determined by serological surveys of animals. Once an ecosystem which has evidence of bluetongue is identified by serology, the incidence of orbivirus infection of vertebrates overtime, the arthropod vectors transmitting infection and the serotypes being transmitted all need to be determined. Arthropod vector studies which determine the capacity and efficiency need to be determined.

The International Impact of bluetongue report was co-chaired by Drs. Kevin Doyle of Australia and Tom Howard of Wisconsin. Their report indicates that economic losses can be attributed to disease outbreaks, cancellation of events, germplasm and live animal sales, purchase of vaccines, death of wildlife, and trade associated losses such as quarantine costs, and fetal calf serum sales. The report also acknowledged ORISA's recommendation that bluetongue be dropped from the list of OIE Group A since the history of BT diseases is as a vectorborne infection rather than a contagious transmissible infection.

The Diagnostic Virology report co-chaired by Dr. Ahmad Afshar, of Agriculture Canada and Geoff Gard of Australia reviewed the recommended methods for isolating or identifying bluetongue virus and endorsed the OIE/International Atomic Energy Agency's proposal that the C-ELISA serve as the international standard for group serological testing.
BLUETONGUE AND BOVINE RETROVIRUS

A report on Vector Biology co-chaired by Drs. W. Tabachnick, ABADRL, Laramie, Wyoming; P. Mellors, England and H. Standfast, Australia was summarized. The report acknowledged the importance of Culicoides vectors as the means of spread and distribution of bluetongue virus infections. The need to define vector systematics, vector capacity and vector competence was emphasized.

Summary of the Molecular Virology report chaired by Drs. B. Eaton, Australia and H. Huismans, South Africa highlighted the methodology for identifying genetic and peptide sequences and discussed those sequences that were important for diagnostic purposes. They also indicated that these probes may be of assistance in identifying the origin of these viruses. The application of monoclonal antibodies and the development of innovative new vaccines was discussed.

Dr. Osburn then summarized the Regulatory Report which was chaired by G. Alexander, Australia and C. Portugal, Portugal. This report emphasized that the importing country must consider:

1.) Distribution and level and seasonality of incidence of bluetongue infection in exporting country
2.) The presence of vectors or potential vectors in its own country and seasonability of incidence; and
3.) Presence of susceptible hosts in its own country and their relations to vectors.

The report further emphasizes the following points based upon scientific data:

1.) The maximum viremic period in ruminants is less than 3 months.
2.) It is only when bulls are viremic that virus is in semen.
3.) There is no convincing evidence that natural bluetongue viruses ever cross the placenta to cause fetal infection and production of persistently infected, seronegative adult animals.
4.) There are highly accurate, sensitive and rapid tests available to detect bluetongue viruses/antigen in live animals, germplasm and products.
5.) The concept of regionalization is valid for insect borne virus infection.

The report further states that the risk in movement of live animals is that:

a. seronegative animals from seronegative populations are of no risk; however, these animals are susceptible to
bluetongue virus infections, and
b. movement of seronegative animals after negative virus isolation from blood is of no risk; however, these animals are susceptible to other serotype/strains of bluetongue viruses.

In regard to the movement of semen and embryos, this work group supported the germplasm report. The report further states that there is no evidence that fetal calf serum has ever been associated with an outbreak of bluetongue virus infection.

A resolution was then presented by Dr. Konrad Eugster, Texas. The resolution following discussion was amended and passed. The points emphasized in the resolution were to:

1.) encourage that bluetongue be removed from the Office International des Epizooties (OIE) Group A list of reportable diseases,
2.) that animal health trade requirements for ruminants and their products and germplasm be modified to acknowledge that bluetongue viruses in ruminants are less than 3 months in duration.
3.) that the demonstration of bluetongue virus in the blood rather than semen or other secretions/excretions should be considered as the definitive test in determining bluetongue virus status in ruminants and
4.) that research be initiated to determine the virulence of Central American and Caribbean bluetongue virus serotypes for North American domestic and wild ruminants and to determine vector competence by U.S. culicoide species.

The meeting was adjourned at 5:45 p.m.
Fiscal Year 1991 was the second year that the brucellosis program has operated under the Rapid Completion Plan. The advances that were made in virtually every measurable aspect of the program indicate that the procedures of the plan are sound and the goals realistic. The total number of reactors, the number of newly infected herds, and the number of the herds under quarantine were all less than the previous fiscal year. There were increases in the number of animals tested under the Market Cattle Identification program and in the total number tested for all purposes. One State advanced to Class A during the year and one State, plus Puerto Rico, attained Class Free status. A notable setback was the loss of Free status by one State that was unable, despite taking legal action, to depopulate a herd that had acquired brucellosis from imported animals.

On April 23, 1991, a long-needed amendment to the Code of Federal Regulations went into effect that increased the allowable rate of indemnity in herd depopulations. This amendment increased Federal indemnity for nonregistered exposed cattle and bison in depopulated herds from $50 up to $150 per head. This so-called incentive indemnity is a major provision of the Rapid Completion Plan and was considered essential for attaining the goal of eradication within a reasonable time period. The new rates resulted in an increased number of infected herds being depopulated during the last half of FY 1991. This increase was particularly evident in the Southeast where twice as many herds were depopulated after the increase than were depopulated during the first half of the year without the incentive. The new rates, plus increases in State indemnity in several States, provide a strong inducement for owners to select depopulation as the method for handling their infected herd problems.

At the end of August 1991, only 26 dairy herds were under quarantine for brucellosis in the United States with 16 of these equally divided between Texas and California. The task force in California's Chino Valley continues to make progress in reducing infection in that highly concentrated dairy area and only 6 herds still remain under quarantine. Much of this progress can be attributed to the credibility that has been achieved by the task force and to the cooperation of owners in adhering to testing schedules and in making herd management changes that reduce within and between herd exposure.

A decision by the U.S. Department of Interior (USDI) to prepare an Environmental Impact Statement (EIS) on the brucellosis problem in the greater Yellowstone Park area has renewed hopes for the eventual
resolution of this issue. The study is being carried out by representatives of the USDI, U.S. Department of Agriculture (USDA), Forest Service and the State of Montana with the USDA, Animal and Plant Health Inspection Service (APHIS), providing technical advice and suggesting options for eliminating the disease from the Park. Elk calves continue to be vaccinated with bio bullets containing strain 19 on six Wyoming feedgrounds by the Wyoming Game and Fish Department. This project is funded by APHIS as is research by Texas A&M on the efficacy of vaccinating bison for brucellosis. The latter project includes the development of a *Brucella neotome* vaccine which shows promise of providing immunity without producing titers that complicate test interpretation.

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**Brucellosis Eradication**

**State Classification of Cattle**

<table>
<thead>
<tr>
<th>Number*</th>
<th>Free</th>
<th>Class A</th>
<th>Class B</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>17</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**Herd Infection Rate**

- 0 < 0.025% < 1.5%

**Adjusted MCI Rate**

- < 0.05% < 0.1% < 0.3%

*Not Included:

- District of Columbia - Free
- Virgin Islands - Free
- Puerto Rico - Free
- Yellowstone National Park, WY - Not Classified

---

On September 30, 1991, 29 States, Puerto Rico, and the Virgin Islands held Class Free status; 17 States were Class A, and 4 States were Class B. In October 1990, Idaho advanced from Class A to Class Free, and Arkansas moved up to Class A. During the second half of the year, Oklahoma advanced from Class B to Class A; Puerto Rico recovered its Class Free status; and Indiana reverted to Class A status because of its inability to depopulate an infected herd which was found the previous year.
Twenty-three percent of all beef cows that have calved are now located in Class Free States, 54 percent in Class A States, and 23 percent in Class B States.

Of the nation's 10.2 million dairy cows, 60 percent are in Class Free States, 33 percent in Class A States, and only 7 percent in Class B States.
Only 26 dairy herds in 8 States were under quarantine for brucellosis as of September 30, 1991, with California and Texas each having 8 herds in this category.

Of the combined total of beef and dairy cattle, 32 percent are in Class Free States, 49 percent in Class A States, and 19 percent in Class B States.
Brucellosis Eradication

Number of Reactor Herds Found (According to State Classification)

Thousands

New State Classification (Effective May 1, 1982)
- Class Free
- Class B
- Class A
- Class C

<table>
<thead>
<tr>
<th>Year</th>
<th>Class Free</th>
<th>Class A</th>
<th>Class B</th>
<th>Class C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>20</td>
<td>19</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>1985</td>
<td>21</td>
<td>19</td>
<td>8</td>
<td>4</td>
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<tr>
<td>1986</td>
<td>24</td>
<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>
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<td>1989</td>
<td>27</td>
<td>17</td>
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<tr>
<td>1990</td>
<td>29</td>
<td>15</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>1991</td>
<td>29</td>
<td>17</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

States with dual status:
+ Wyoming - Class Free and Class A
+ Montana - Class Free and Class A
+ Texas and Florida - Class B and Class C
+ Texas and Florida - Class B and Class C
+ Arizona - Class Free and Class A
+ Texas and Florida - Class B and Class C
+ Arizona - Class Free and Class A
+ Florida - Class B and Class C
+ Florida - Class B and Class C

There were 1,256 reactor herds found in FY 1991. This was a decrease of 26 percent from the 1,701 reactor herds found in 1990. Of the total number of reactor herds in FY 1991, 801 were located in the four Class B States. The Class A States accounted for 455 reactor herds and 1 reactor herd was located in a Class Free State (Indiana).

Brucellosis Eradication

Percent of Total Reactor Herds Found

*Fiscal year 1991
Total herds: 1,256

45.62% States: 1 Herds: >500 Total reactor herds = 573

32.64% States: 5 Herds: 60 < 500 Total reactor herds = 410

Of these 1,256 herds, 97 percent were in 13 States and the remaining 3 percent were in 37 States. Texas, with 573 reactor herds, represented 46 percent of the total. The five States of Oklahoma, Florida, Mississippi, Louisiana, and Missouri had 115, 82, 76, 70, and 67 reactor herds respectively which combined, represented 33 percent of the national total.
Brucellosis Eradication

New Reactor Herds
October 1990 through August 1991

The reactor herd totals on figures 6 and 7 include herds initially found infected in FY 1991 plus infected herds carried over from the previous year in which reactors were disclosed during FY 1991. This traditional way of presenting reactor herd data is imprecise and has caused confusion by implying that all of the reactor herds were found during the fiscal year covered by the status report. When herds carried over from FY 1990 are subtracted, the actual number of newly infected herds in FY 1991 was 699 in 18 States.

Change in Number of New Reactor Herds
September 1990 through August 1991 (741) vs. September 1989 through August 1990 (793)

Ten of these States had reduced their number of newly infected herds from the previous year. The increase of 53 in Texas was due to area testing parts of 5 counties in 3 areas of the State where there was a higher prevalence of the disease.
Brucellosis Eradication

Milk Ring Test Results (BRT)

Fourteen affected dairy herds were located by Brucellosis Ring Test (BRT) surveillance. There were 2,113 suspicious BRT laboratory reports which resulted in 1,320 herds being blood tested for a herd test rate (HTR) of 63 percent. The HTR in FY 1990 and FY 1989 were 59 percent and 54 percent respectively.

Brucellosis Eradication

Market Cattle Testing Program

The 11.5 million MCI tests in FY 1991 was one-half million more than the number tested in FY 1990. Of these, 5.6 million samples (48.8 percent) were collected at slaughter plants, and 5.9 million (51.2 percent) were collected at stockyards.
The total number of cattle tested for brucellosis in FY 1991 was 15.1 million, an increase of 500,000 compared to FY 1990. There were 3.6 million animals sampled through herd tests on farms or ranches, and 11.5 million under the MCI program. Although the total number of tests increased by 3.4 percent, there was a decrease in the number of reactors of 17 percent from 24,000 in FY 1990 to 20,000 in 1991.

A total of 8.9 million calves were vaccinated for brucellosis in FY 1991, the same number that was vaccinated in 1990.
The swine brucellosis program continued to show progress in fiscal year 1991 with 3 States, Hawaii, Kentucky and New Mexico, achieving Validated Brucellosis Free area status during the year. At the end of the fiscal year, 38 States held validated-free status (Stage III), 7 States were in Stage II, and 5 States in Stage I.

Swine Brucellosis
Total Number of Swine Tested
October 1990 Through June 1991

Total: 1,303,028

- Slaughter: 379,011 (29.8%)
- Markets (First point): 827,632 (7.49%)
- On farm*: 63.51%

*Includes reactor MST tracebacks

A total of 1,303,028 swine were tested for brucellosis during the year with 63.51% (827,632) tested on farms, 29.8% (379,011) at slaughter, and 7.49% (96,385) at markets.
Swine Brucellosis

Reactors Disclosed
October 1990 through June 1991

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<tr>
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*Also includes reactors in herds as a result of MST traces

These tests disclosed 695 reactors for a reactor rate of 0.05%.

Swine Brucellosis

Newly Infected Herds
October 1990 through June 1991

All of the 68 newly infected herds in FY 91 were found in 9 States in the South Central and Southeastern areas of the country. Texas, with 39 herds, accounted for 57% of the total.
The source of infection was community spread in 27 cases (39.7%), purchased swine in 16 cases (23.5%), and from other sources in 8 cases (11.7%). One case was due to exposure to feral swine. These animals remain an important source of disease in areas where their populations are significant and where contact with domestic swine is possible. Sixteen herds (23.5%) acquired brucellosis from unknown sources.

There were 3,560 Validated Brucellosis-Free herds at the end of the fiscal year, 48 more than the 3,512 on the same date a year earlier.
Field Evaluation of "D–Tec Brucella A.", a Monoclonal Antibody-Based Competitive Enzyme–Linked Immunosorbent Assay (cELISA) For Serodiagnosis of Brucellosis in Cattle

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INTRODUCTION

Bovine brucellosis, a bacterial zoonotic disease, has been controlled in many parts of the U.S.A. and other countries; yet, a large number of samples are still being tested regularly to pursue and monitor the control program. Considerable numbers of samples with positive test results are reported from different geographic areas, many of which are subsequently proven to be false positive at considerable cost and effort. The incidence of false positives is much higher in the screening tests due to their low specificity. Several of the samples that are positive by the screening tests are found to be negative with other tests such as Rivanol, Complement Fixation (CF), and Particle Concentration Fluorescence Immunoassay (PCFIA) which have improved specificity (fewer false positives). However, many cattle positive by these tests have also been found to be not infected with Brucella abortus field strain. Persistent titers due to strain 19 vaccination or non-specific reactions due to unknown factors are presumed responsible for the false positive results.

The percentage of false positive results of any test increases considerably with the decrease of the prevalence of the disease. It is important that tests with higher specificity (fewer false positives) are used with the progress of the control program resulting in lower prevalence of the disease.

A monoclonal antibody–based competitive ELISA (cELISA) test for differentiating strain 19 vaccinated animals from B. abortus infected animals was developed at Texas A&M University by Dr. Garry Adams and coworkers and licensed for marketing by Coopers Animal Health. In a comparative study at Texas A&M University with samples from adult cattle experimentally vaccinated with strain 19 and/or challenged with the field strain (S2308), none of the samples from the vaccinated animals were positive by the cELISA test during the 3 to 18 week period studied following vaccination; whereas, 14% to 100% of those samples were positive (false positive) by Card, Rivanol, CF, and PCFIA tests. All tests including cELISA were equally effective in detecting infected animals when tested 11 weeks after experimental challenge as verified by cultures. Results of this study were presented to the Brucellosis Committee of the United States Animal Health
FIELD EVALUATION OF "D-TEC BRUCELLA A.

Association in its 93rd annual meeting in October 1989 where it was "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".

The cELISA test has been formulated into a commercial kit named "D-Tec Brucella A." by Synbiotics Corporation and Pitman-Moorel, Inc., successor of Coopers Animal Health. An evaluation of this test kit at Synbiotics and Texas A&M University with the experimental samples originally used at Texas A&M University gave results essentially identical to that found earlier by Dr. Adams and co-workers. These results indicate that the commercially formulated D-Tec Brucella A. kit is functionally equivalent to the original cELISA test.

D-Tec Brucella A. was evaluated to assess its usefulness in determining the brucellosis status of test eligible cattle in different phases of evaluation as recommended by the U.S.A.H.A. Results of these evaluations are presented here to obtain an appropriate recommendation for regulatory approval of the test kit as an official test for determining the brucellosis status of test eligible cattle.

MATERIALS AND METHODS

Test Kit: - D-Tec Brucella A. test kits Lot No. - RD-001 produced at Synbiotics Corporation in October 1990 with expiration date of March 21, 1992 were used for evaluation.

Description of the Test Kit: D-Tec Brucella A. is a monoclonal antibody-based cELISA test kit for determining the brucellosis status of test eligible cattle by testing serum or plasma. The assay may be run manually or adapted to most of the existing automatic or semi-automatic ELISA test systems and can be performed with minimal training. It contains reagents and controls sufficient for testing 220 samples in duplicate provided that 44 samples are assayed at a time. It is to be stored at 5-8°C temperature and has a shelf life of 18 months from the date of manufacturing.

PRINCIPLE OF THE TEST

D-Tec Brucella A. Diagnostic Test Kit is a competitive enzyme-linked Immunosorbent assay (cELISA) based on an unique monoclonal antibody which competes differentially with the antibodies produced in normal responses to strain 19 vaccination, B. abortus infection, or other non-specific factors for a specific antigenic determinant on the lipopolysaccharide (LPS) of B. abortus. Serum or plasma samples mixed with the biotinylated monoclonal antibody are incubated in ELISA plates coated with partially purified LPS of B. abortus. The amount of monoclonal antibody
immunologically bound to the LPS is measured by reacting avidin-peroxidase conjugate followed by substrate chromogen. The color produced is proportional to the amount of the monoclonal antibody bound to the polysaccharide and inversely proportional to the degree of competition between the monoclonal antibody and the sample antibody.

Antibodies due to strain 19 vaccination or other nonspecific factors compete poorly with the monoclonal antibody; whereas, antibodies elicited from B. abortus infection compete strongly with the monoclonal antibody. The samples from B. abortus infected animals when tested result in low binding of the monoclonal antibody to the LPS; and therefore, little color formation. The ratio between the absorbance (optical density) of the sample well to that of the negative control (S/N) expressed as Percent Inhibition (1−S/N) × 100 is used to determine the status of the sample.

Based on the results of the studies with field and experimental samples, it was decided to consider samples exhibiting inhibition of more than 80% as positive, those with inhibition of 54% or less as negative and those from 54% to 80% as suspect.

**STUDY METHODS**

For the Phase I (experimental) study, samples from cattle experimentally vaccinated with B. abortus strain 19 and challenged with field strain S2308 were tested with D-Tec Brucella A. The results were compared with the results of Card test, Rivanol test and CF test obtained at Texas A&M University Laboratory and the Texas Animal Health Commission Laboratory.

For the Phase II (double blind comparison) study, a panel of 203 sera in lyophilized form was obtained from U.S.D.A. of which only 65 bovine sera were found comparable. The rest were from other species, or had no results after lyophilization. The sera were reconstituted by adding distilled water and tested with D-Tec Brucella A and the results of the appropriate sera were compared with other tests and the known culture status of the animals.

The phase III (Field) study was conducted in two separate stages. In the initial stage or the "Pre-field trial" study, serum samples were obtained from the Federal/State brucellosis laboratories of 8 states (AR, CO, IN, LA, MS, NJ, ND, OK) representing at least three geographic regions and three brucellosis disease statuses. These laboratories were requested to provide all of the available positive and suspect samples along with some negative samples. The samples were tested blindly with "D-Tec Brucella A." at the Diagnostic Research Laboratory, Pitman-Moore, Inc, Terre Haute, In. in accordance with the procedure established for the test. Results of the D-Tec Brucella A. test were compared with the results of other brucellosis tests performed by the laboratories providing the samples.

The final stage of the phase III study or the official "Field Trial" was
FIELD EVALUATION OF "D-TEC BRUCELLA A."

done according to a protocol approved by the U.S.D.A. Brucellosis Laboratories of seven states (CA, CO, LA, MS, NJ, OK, and TX) participated in the study. Bovine serum samples received for brucellosis testing were tested with D-Tec Brucella A. test kit at the participating laboratory in addition to other brucellosis tests normally done in the respective laboratory. The Mississippi State laboratory was unable to perform the cELISA test due to failure of their ELISA reader, so their samples were tested by D-Tec Brucella A. method at the New Jersey State laboratory as provided in the approved protocol, and the results were reported back to the Mississippi Laboratory.

In all studies, the results of the D-Tec Brucella A. test were compared with the results of other brucellosis tests done at the respective state laboratories.

For comparative purposes, the results of the U.S.D.A. approved brucellosis tests were classified as positive (P), suspect (S) or negative (N) according to criteria stated in the Code of Federal Regulation CFR9, 1991. The D-Tec Brucella A. results were classified as positive when the competitive inhibition was above 80%, as suspect when greater than 54% but equal to or less than 80% and as negative when equal to or less than 54%.

Bacterial culture results for isolation of B. abortus were also taken into consideration for comparison. In order to determine the reproducibility of the D-Tec Brucella A. test kit at the participating laboratories, a set of 25 samples was sent to each laboratory.

Specificity of the D-Tec Brucella A. test was determined from the test results of samples from brucellosis–free states. Sensitivity was determined from the results of the serum samples from infected cattle verified by isolation of B. abortus field strain. For this study, a state was considered free or Class A only if it was classified as such for the past three years. All results classified as suspect were considered positive in determining specificity, sensitivity, and other calculations. Comparison between D-Tec Brucella A. test, CF test and PCFIA was done using Kappa values for agreement.(1,2)

RESULTS AND DISCUSSION

Phase I or Experimental Study: The bulk of the experimental studies were done with the original cELISA test at Texas A&M University, results of which were presented at the annual meeting of the Brucellosis Committee in 1989. At Synbiotics Corporation, D-Tec Brucella A. was used to test randomly selected samples from these experimental animals; the results are shown in Table I. In this study, several post–vaccination samples were found positive or suspect by D-Tec Brucella A. along with other tests, but in an earlier report all post–vaccination samples were noted negative by
the cELISA test, although a varying number of samples were positive by other tests. This difference is due to the fact that in the original study samples were classified as positive if Percent Inhibition was 85% or more with the remainder classified as negative; whereas, in the present study samples with inhibition of above 80% were classified as positive, those with 54% or less as negative and those in-between as suspect.

Phase II or Double Blind Study: Of the 203 check samples tested at PMI Research Laboratory, only 65 lyophilized bovine sera including 25 sera from the experimentally infected cattle had results of other tests.

All of the 25 experimentally infected animals were culture positive at the time of parturition occurring 37 to 62 days before taking the samples tested. Thirteen of these became culture negative at the time blood samples were taken, while 12 remained culture positive. All 13 of the culture negative experimental animals were serologically negative by D-Tec Brucella A., 7 by PCFIA, 11 by RIV, 8 by Card, and 6 of 12 by CF. Three of the 12 culture positive animals were serologically negative by Card, RIV, PCFIA, and D-Tec Brucella A., and two additional animals were negative by RIV and D-Tec Brucella A. and marginally suspect by PCFIA (S/N = 0.570 and 0.665). Two of those 5 animals were suspect by CF and the remaining three were anticomplementary. The serological status of those 25 experimentally infected animals at the time of parturition was not available. Experimental infection, conversion from culture positive at parturition to culture negative within 37 to 62 days afterwards in 13 of 25 animals and serologically negative results in at least 25% (3/12) of the culture positive animals by Card, RIV, PCFIA, and D-Tec Brucella A. would make these data unsuitable or questionable for comparative evaluation of different tests.

A comparison of the results of sera from the nonexperimental animals is presented in Table II. All culture positive animals were serologically positive by RIV, CF, PCFIA and D-Tec Brucella A. while 29% of the culture negative animals were serologically negative by Card, 58% by RIV, 33% by CF, 46% by PCFIA and 79% by D-Tec Brucella A.

Phase III or Field Study: The field study was done in two stages, the initial one designated as "Prefield Trial" and the other as "Field Trial". The results of these two studies are presented in a consolidated form in Tables designated by III for convenience. Particulars of samples tested are shown in Table III-1. Eleven thousand one hundred twenty (11,120) samples including 5,096 from calfhood vaccinates (C), 1,928 from adult vaccinates (AV) and 4,096 from non-vaccinates (NV) plus animals of unknown vaccination status from 10 states were included in the field study.

The samples of the prefield trial study were from the positive and suspect cattle available at the time of study. Some negative samples were
FIELD EVALUATION OF "D-TEC BRUCELLA A.

obtained from the same sources as the positive or suspect cattle sera with the majority of the negative samples from the brucellosis-free states (NJ, ND). These prefield test sera included all of the sera received by the cooperating laboratories of New Jersey and North Dakota.

In the official field trial study, the samples received by the participating laboratories during the study period were included in addition to the positive and suspect samples available in storage.

Test Results: Test results of non-vaccinates and calfhood vaccinates from the brucellosis-free states (ND, NJ) and Class A States (CA, CO) are presented in Tables III2, and III3, respectively. Specificity was estimated from the results of the free states (Table III2) assuming all samples were from non-infected animals.

The estimated reduction of false positive results of some other tests by D-Tec Brucella A. is shown in Table III-4. It was calculated from the test results of samples from the free and Class A states assuming that all positive results were false positive. All of those animals in the prefield trial were considered non-infected by the regulatory authorities based on other circumstances. Test results of non-vaccinates, calfhood vaccinates and adult vaccinates from Class B states are presented in consolidated form in Table III5-7. It should be noted that Texas used PCFIA for screening samples and only the positive and suspect samples were available for testing with D-Tec Brucella A. The Card test was used only as field test which accounts for the lower number of Card test results.

Culture Results: Test results of serum samples from the animals with culture for isolation of B. abortus during prefield trial and field trial are presented in consolidated form in Tables III-8 along with the applicable results of the experimental study presented in Table I. Results of post vaccinates in the experimental study were excluded since most of these were not test eligible because of too short of a period after vaccination. Sensitivity of different tests for strain 19 and field strain was determined from the results of animals with respective isolates. Relative specificity of different tests was calculated based on the results of the animals with no isolates. This is not the real measure of specificity of the test, but only a comparison among several tests. It should be noted that although a positive culture is proof of infection, a negative culture does not rule out infection. The non-isolate group contains both the non infected (true negative culture) and some infected animals (false negative culture), but the culture positive groups are infected animals (true positive).

Inter Test Comparison: Inter test comparison of D-Tec Brucella A. with CF, and PCFIA, with Kappa test for agreement are shown in Table III9. It should be noted that Kappa values for comparison between D-Tec
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Brucella A. and PCFIA were always less than 0.5 indicating poor agreement; whereas, those between D–Tec Brucella A. and CF varied from poor to moderate agreement, indicating closer agreement of D–Tec Brucella A. to CF than to PCFIA. The proportion of suspect results of PCFIA was considerably higher than other tests making the combined positive and suspect (practical positive) much higher than that of D–Tec Brucella A.

GENERAL DISCUSSION

The usefulness of a diagnostic test depends on the accuracy of test results which in turn depends on the "specificity" and "sensitivity" of the test. "Specificity" is the measure of its ability to detect correctly the negative cases and "Sensitivity" is the measure of its ability to correctly identify the positive cases. Another very important measure for determining the usefulness of a diagnostic test is the predictive value of its results. Predictive values of the positive and negative results indicate what percent of the observed positive and negative results are from the infected and non–infected animals, respectively.

Predictive value of positive results depends on the specificity of the test; whereas, the predictive value of the negative results depends on the sensitivity of the test. At a comparatively low prevalence, the predictive value of the positive result decreases greatly with the decrease of the prevalence of the disease, but that of a negative result is only slightly influenced by the corresponding decrease in prevalence.

Given a 95% specificity and 95% sensitivity of a test, the predictive value of positive results would be 16.1% at 1% prevalence of the disease which will decrease to 1.9% at 0.1% prevalence; whereas, the predictive value of negative result would be 99.89% and 99.99%, respectively. Theoretically, a test with 100% sensitivity and 100% specificity is the ideal test, but it is hardly attainable due to inherent overlapping of test attributes between the diseased and non–diseased animals. For practical purposes, highest possible sensitivity and specificity are sought for a good diagnostic test.

At present, there are several serological tests for determining brucellosis status of test eligible cattle. Some of these tests are used exclusively for screening and others are used primarily to aid definitive diagnosis but may also be used for screening if so desired. Many of the samples that are positive by the screening tests are found negative with Rivanol, CF, and PCFIA tests which have better specificity (fewer false positive). However, many animals positive by these tests have been found to be non–infected with B. abortus. Persistent titers due to strain 19 vaccination or non–specific reaction due to unknown factors are presumed responsible for the false positive results.

Although brucellosis has been eradicated from many states and
FIELD EVALUATION OF "D-TEC BRUCELLA A.

effectively controlled in others, large numbers of samples are still being tested regularly to monitor the disease control program. Considerable numbers of samples with positive test results are reported from different locations, many of which are found to be false positive at considerable cost and effort.

A test with a minimum possible false positive results (high specificity) and with little or no false negatives (maximum sensitivity) would be a great help in administering the brucellosis control program. As stated earlier, predictive value of positive results decreases greatly with the decrease of prevalence of the disease. In other words, the rate of false positives increases with the decrease of prevalence. This increase of false positives with decreased prevalence may be minimized by increasing the specificity of the test applied.

With the gradual decrease of brucellosis in the U.S.A., it is important that tests with higher specificity are used in order to minimize the number of false positives; and thereby, avoid the associated cost and wasted effort.

For the determination of real sensitivity and specificity of a diagnostic test, one must know the diseased or non-diseased status of each animal in the test population with certainty, which may be difficult to obtain. Therefore, only the relative sensitivity and specificity can be calculated based on the disease status of the test animals by a standard test or other suitable means. Since no test with sensitivity and specificity approaching 100% is available for brucellosis at present, the specificity of D-Tec Brucella A. test as well as some other tests was determined based on the test results of the samples from the states which are free of bovine brucellosis for the last 3 or more years; all the cattle from those states are considered non-infected. Sensitivity of D-Tec Brucella A. and other tests likewise was determined from the test results of the samples from animals with positive B. abortus culture, the best proof of infection.

Specificity: Specificity is the measure of correctly identifying the non-infected animals. Considering all animals from the free states to be non-infected, specificity of D-Tec Brucella A. was calculated to be at least 99.97% based on the results of 2,729 samples with only 1 suspect result, compared to a specificity of 98.9% for the Screening Test (Card test for NJ and Rapid Slide test for ND) with 30 positive results (Table III-2). Specificity of Rivanol was calculated to be 78.9% using test results from 71 samples, which is a biased underestimate. If all of the other non-tested samples are considered negative by Rivanol then the specificity for Rivanol would be 99.5%. Greater specificity means fewer false positive by the test and conversely a higher rate of false positive tests means lower test specificity.

Test results of non-vaccinates and calfhood vaccinates from Class A States where practically all animals are free of infection (Table III-3) indicate higher specificity of D-Tec Brucella A. compared to other tests assuming most or all of the positive results are false positive. Relative
specificity of different tests based on the negative culture (Table III8) also indicates much higher specificity for D–Tec Brucella A. in comparison to CF and PCFIA (50% vs 21% and 1.5% respectively).

Comparatively low relative specificity of all tests in this calculation is due to the fact that some of the culture negative animals were probably infected and had false culture negative results. Estimated reduction of possible false positive results of other tests by D–Tec Brucella A. (Table III4) is the reflection of its higher specificity or fewer false positive results. In this estimation all positive results of non–vaccinates and calfhood vaccinates from the free and Class A states were assumed to be false positive in absence of any proof of infection. If any of these animals were really infected and needed exclusion from the calculation, then the estimated reduction of false positive would be even higher. D–Tec Brucella A. was able to reduce the possible false positives of the screening test (Card test) by 95%, Rivanol test by 76%, CF by 78% and PCFIA by 77% (Table III–4).

Sensitivity: Sensitivity of a diagnostic test is the measure of its capacity to detect the infected animals correctly. Test results of animals with positive culture (field strain or strain 19 isolate) were used to determine sensitivity of D–Tec Brucella A. and other tests as isolation of causative agent is considered to be the proof of infection.

Sensitivity of D–Tec Brucella A. for B. abortus field strain, was estimated to be 98.8% based on the 84 field animals with positive culture (Table III8). If the results of 90 experimental animals with positive culture are included then its sensitivity becomes 99.4%, compared to 100% sensitivity for other tests. A single animal from Texas was field strain culture positive but tested negative by D–Tec Brucella A. and suspect by both C.F. and PCFIA. The single negative result of D–Tec Brucella A. compared to suspect by PCFIA and CF may be explained by the following facts rather than slightly lower sensitivity of D–Tec Brucella A.

1. The animal could be in a very early stage of incubating infection when viable organisms may be found but an adequate amount of specific competitive antibodies were not available to produce a positive result. The suspect results of CF and PCFIA were due to pre–existing non–specific reaction at the time of testing as have been found with many non–infected animals.

2. In the experimental study, it was found that a longer period (8 weeks vs 11 weeks) was needed after experimental challenge for all infected cattle to be positive by the monoclonal antibody–based cELISA compared to other tests which may be the cause of the negative result by D–Tec Brucella A. and suspect by C.F. and PCFIA.

Sensitivity of D–Tec Brucella A. for animals with B. abortus strain 19
FIELD EVALUATION OF "D-TEC BRUCELLA A.

isolates was calculated to be 88% compared to 99% for CF and 100% for PCFIA (Table III8). The monoclonal antibody based cELISA test was originally aimed at eliminating positive results due to residual titer of strain 19 vaccination. A comparatively lower sensitivity of D–Tec Brucella A. for persistence of strain 19 organism is not surprising.

Inter Test Comparison: The comparison between D–Tec Brucella A. and CF or PCFIA with Kappa values indicates that D–Tec Brucella A. is distinctly different from the PCFIA as both Kappa values were less than 0.5 (Table III9). Agreement between D–Tec Brucella A. and CF varied from poor to moderate.

Kappa test simply indicates that performance of D–Tec Brucella A. is different from PCFIA but cannot indicate which one is better. Approximately 60% (798/1319 Table III9) of the PCFIA positive animals were negative by D–Tec Brucella A., most of which are either false positive by PCFIA or false negative by D–Tec Brucella A. If the results of D–Tec Brucella A. and PCFIA of the animals with positive and negative cultures are examined (Table III8 it becomes clear that the difference between the two tests lies in the fact that PCFIA gives comparatively more false positives than D–Tec Brucella A. Since the sensitivity of D–Tec Brucella A. based on culture positive animals, was 99.4% for the field strain and 86% for strain 19, and the relative specificity based on culture negative animals was 50% for D–Tec Brucella A. and only 1.5% for PCFIA, the above mentioned 60% positive is mainly due to false positive results of PCFIA rather than false negative results of D–Tec Brucella A. Inter test comparison between D–Tec Brucella A. and CF similarly indicates that 49% (502/1020) of CF positive animals were negative by D–Tec Brucella A. and most of them if not all are due to false positive results of CF rather than false negative results of D–Tec Brucella A.

Lower sensitivity of D–Tec Brucella A. for strain 19 may account for some of these false positives but the bulk of them are due to the false positive reactions of PCFIA. Comparison between D–Tec Brucella A. and CF reveals a similar trend, meaning more positive (false positive) with CF than D–Tec Brucella A. but the difference is comparatively less. Approximately 79% of the culture negative animals were positive by CF compared to 98% by PCFIA and 50% by D–Tec Brucella A.; the difference being also reflected in the Kappa values.

Reproducibility: Results of the 25 samples sent to each participating laboratory (Table III10) indicates that all of the participating laboratories correctly identified the 8 positive samples and 14 negative samples but 3 samples were identified either negative or suspect by different laboratories each reporting one or two as suspect. Synbiotics Corporation calculated Inter–assay coefficient of the variance (C) to be 4% and Inter–
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assay C to be 6% when the test was developed as a commercial kit.

CONCLUSIONS

Based on the data presented, it is apparent that D-Tec Brucella A. would offer considerable advantage over the currently used tests in determining brucellosis status of test eligible cattle. Due to its high specificity (99.97%), D-Tec Brucella A. was able to reduce the false positive result of screening tests by 95%, and that of Rivanol, CF and PCFIA by more than 75%. The sensitivity of the test was calculated to be more than 99%. The high specificity of D-Tec Brucella A. was not achieved at the expense of its sensitivity.

The false positive results often encountered by other tests due to persistent strain 19 vaccination titers or other non-specific reaction were almost equally reduced by D-Tec Brucella A. Inter test comparisons strongly indicate superior performance of D-Tec Brucella A. over PCFIA and CF, the two most relied upon tests presently used by reducing false positives.

These data indicate that D-Tec Brucella A. qualifies as an official test for determining the brucellosis status of test eligible cattle.

ACKNOWLEDGEMENT

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REFERENCES

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Table II - USDA Double Blind Study Data

Comparative Results of the Lyophilized Check Sera From the Nonexperimental Cattle

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<th></th>
<th>Card</th>
<th>RIV</th>
<th>C.F.</th>
<th>PCFIA</th>
<th>D-Tec Br. A.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonexperimental Animals (40)</strong>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. Specificity</td>
<td>7/24* = 29%</td>
<td>14/24 = 58%</td>
<td>6/18 = 33%</td>
<td>11/24 = 46%</td>
<td>19/24 = 79%</td>
</tr>
<tr>
<td>Sensitivity (F.St.)</td>
<td>9/9** = 100%</td>
<td>9/9 = 100%</td>
<td>4/4 = 100%</td>
<td>9/9 = 100%</td>
<td>9/9 = 100%</td>
</tr>
<tr>
<td>Sensitivity (ST. 19)</td>
<td>6/7*** = 86%</td>
<td>7/7 = 100%</td>
<td>6/6 = 100%</td>
<td>7/7 = 100%</td>
<td>7/7 = 100%</td>
</tr>
</tbody>
</table>

**Comparison With D-Tec Br. A.**

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K-Values</td>
<td>0.33</td>
<td>0.75</td>
<td>0.43</td>
<td>0.59</td>
<td>---</td>
</tr>
<tr>
<td>Agreement</td>
<td>Poor</td>
<td>Excellent</td>
<td>Poor</td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

* = Number of animals with negative culture at the time of bleeding.
** = Number of animals with positive culture at the time of bleeding.
*** = Indicates number of animals involved.
### TABLE III - Consolidated Field Data

Particulars of Field Samples Tested

<table>
<thead>
<tr>
<th>Nature</th>
<th>Status</th>
<th>No. of States</th>
<th>NV</th>
<th>CV</th>
<th>AV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefield Trial</td>
<td>Free</td>
<td>2</td>
<td>1,814</td>
<td>120</td>
<td>0</td>
<td>1,934</td>
</tr>
<tr>
<td></td>
<td>Class A</td>
<td>2</td>
<td>101</td>
<td>84</td>
<td>0</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>Class B</td>
<td>4</td>
<td>121</td>
<td>88</td>
<td>9</td>
<td>218</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td></td>
<td><strong>8</strong></td>
<td><strong>2036</strong></td>
<td><strong>292</strong></td>
<td><strong>9</strong></td>
<td><strong>2,337</strong></td>
</tr>
<tr>
<td>Field Trial</td>
<td>Free</td>
<td>1</td>
<td>527</td>
<td>268</td>
<td>0</td>
<td>795</td>
</tr>
<tr>
<td></td>
<td>Class A</td>
<td>2</td>
<td>285</td>
<td>1,507</td>
<td>1,171</td>
<td>2,963</td>
</tr>
<tr>
<td></td>
<td>Class B</td>
<td>4</td>
<td>1,248</td>
<td>3,029</td>
<td>748</td>
<td>5,025</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td></td>
<td><strong>7</strong></td>
<td><strong>2,060</strong></td>
<td><strong>4,804</strong></td>
<td><strong>1,919</strong></td>
<td><strong>8,783</strong></td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td></td>
<td><strong>10</strong></td>
<td><strong>4,096</strong></td>
<td><strong>5,096</strong></td>
<td><strong>1,928</strong></td>
<td><strong>11,120</strong></td>
</tr>
</tbody>
</table>
TABLE III2 - Consolidated Field Data

Test Results of Field Samples from Brucellosis-Free States

<table>
<thead>
<tr>
<th>Nature</th>
<th>No. of States</th>
<th>No. of Samples</th>
<th>Screen Test</th>
<th>RIV</th>
<th>D-TEC BR. A.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>P - N = T</strong></td>
<td><strong>P - S - N = T</strong></td>
<td><strong>P - S - N = T</strong></td>
</tr>
<tr>
<td>Prefield</td>
<td>2</td>
<td>1934</td>
<td>13-1921=1934</td>
<td>8 - 4 - 1= 13</td>
<td>0 - 1 - 1933=1934</td>
</tr>
<tr>
<td>Trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field Trial</td>
<td>1</td>
<td>795</td>
<td>17- 778= 795</td>
<td>3 - 0 - 55= 58</td>
<td>0 - 0 - 795 = 795</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>2729</td>
<td>30-2699=2729</td>
<td>11- 4 - 56= 71</td>
<td>0 - 1 -2728 =2729</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2699/2729</td>
<td>98.9%</td>
<td>56/71 = 78.9%</td>
<td>2728/2729 = 99.97%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(*2729-15)/2729</td>
<td></td>
<td></td>
<td></td>
<td>(*)2729-15)/2729 = 99.5%</td>
</tr>
</tbody>
</table>

*Adjusted specificity considering all untested sample as negative.
TABLE III - Consolidated Field Samples

Test Results of Non Vaccinates and Calfhood Vaccinates from Class A States

<table>
<thead>
<tr>
<th>Nature Status</th>
<th>Vac.</th>
<th>Total No.</th>
<th>Test</th>
<th>RIV</th>
<th>CF</th>
<th>PCFIA/Cite</th>
<th>D-Tec Br. A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefield NV,CV</td>
<td>185</td>
<td>84 -101=185</td>
<td>26-0-58=84</td>
<td>5-0-0=5</td>
<td>4-1-1=6</td>
<td>3-0-182=185</td>
<td></td>
</tr>
<tr>
<td>Field Trial NV,CV</td>
<td>1792</td>
<td>438-1353=1791</td>
<td>50-70-316=436</td>
<td>60-158-243=461</td>
<td>8-2-138=148</td>
<td>19-6-1767=1792</td>
<td></td>
</tr>
</tbody>
</table>


% Negative 74% 72% 52% 90% 93%
Table III4 - Consolidated Field Data

Estimated Reduction of Possible False Positive of Other Tests by D-Tec Brucella A.

<table>
<thead>
<tr>
<th>Nature</th>
<th>No. of States</th>
<th>D-TEC BR.A. POS</th>
<th>Screen Test POS</th>
<th>RIV POS %Reduct.</th>
<th>CF POS %Reduct</th>
<th>PCFIA POS %Reduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefield Trial</td>
<td>3</td>
<td>4</td>
<td>97</td>
<td>95%</td>
<td>38</td>
<td>89%</td>
</tr>
<tr>
<td>Field Trial</td>
<td>2</td>
<td>25</td>
<td>455</td>
<td>95%</td>
<td>53(18)</td>
<td>66%</td>
</tr>
</tbody>
</table>

*Calculation (91-22)/91 = 76%

Number in ( ) indicates the number of positively D-Tec Br. A.
### TABLE III5-7 - Consolidated Field Samples

Test Results of NV and CV Samples from Class B States and AV Samples from Class A&B States

<table>
<thead>
<tr>
<th>Nature</th>
<th>Vac. Status</th>
<th>Screen</th>
<th>Total Test</th>
<th>CF</th>
<th>PCFIA</th>
<th>D-Tec Br. A.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vac. Samp.</td>
<td>P- N = T</td>
<td>RIV P- S- N = T</td>
<td>P- S- N = T</td>
<td>P- S- N = T</td>
</tr>
<tr>
<td>Prefield</td>
<td>NV</td>
<td>121</td>
<td>67-54=121</td>
<td>5-0-9 = 9</td>
<td>62-1-0 = 63</td>
<td>52-10-2 = 64</td>
</tr>
<tr>
<td>Field</td>
<td>NV</td>
<td>1248</td>
<td>301-520=821</td>
<td>30-0-273=303</td>
<td>211-98-347=656</td>
<td>140-261-352=753</td>
</tr>
<tr>
<td>Field</td>
<td>CV</td>
<td>88</td>
<td>75-13=88</td>
<td>11-0-15=26</td>
<td>37-16-16=69</td>
<td>35-41-2 = 78</td>
</tr>
<tr>
<td>Field</td>
<td>AV</td>
<td>9</td>
<td>9-0=9</td>
<td>1-0=1</td>
<td>8-1-0 = 9</td>
<td>8-1-0 = 9</td>
</tr>
</tbody>
</table>

| Prefield | NV | 121 | 67-54=121 | 5-0-9 = 9 | 62-1-0 = 63 | 52-10-2 = 64 | 47-8-66-121 |
| Field | NV | 1248 | 301-520=821 | 30-0-273=303 | 211-98-347=656 | 140-261-352=753 | 155-41-1052=1248 |
| Field | CV | 88 | 75-13=88 | 11-0-15=26 | 37-16-16=69 | 35-41-2 = 78 | 24-9-55 = 88 |
| Field | AV | 9 | 9-0=9 | 1-0=1 | 8-1-0 = 9 | 8-1-0 = 9 | 8-0=1 = 9 |

| Total | 3117 | 456-1678=2134 | 17-0-394=411 | 246-91-615=952 | 278-293-998=1569 | 178-56-2883=3117 |
| Total | 1248 | 301-520=821 | 30-0-273=303 | 211-98-347=656 | 140-261-352=753 | 155-41-1052=1248 |

<p>| Grand Total | 6414 | 1418-3192=4610 | 206-110-1637=1953 | 993-507-1800=3300 | 678-811-1501=2982 | 660-177-5538=6375 |</p>
<table>
<thead>
<tr>
<th>Nature</th>
<th>No. of States</th>
<th>Culture</th>
<th>Total # of Samples</th>
<th>C.F.</th>
<th>PCFIA</th>
<th>D-TEC BR.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Result</td>
<td></td>
<td>P-S-N=T</td>
<td>P-S-N=T</td>
<td>P-S-N=T</td>
</tr>
<tr>
<td>Prefield</td>
<td>3</td>
<td>NI</td>
<td>38</td>
<td>16-15 - 7 = 38</td>
<td>18-15 - 1 = 34</td>
<td>8- 2 -28 = 38</td>
</tr>
<tr>
<td>Field</td>
<td>4</td>
<td>NI</td>
<td>172</td>
<td>96-38 -37 =171</td>
<td>112-48 - 2 =162</td>
<td>57-37 -78 =172</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>NI</td>
<td>210</td>
<td>112-53 -44 =209</td>
<td>130-63 - 3 =196</td>
<td>65-39 -106=210</td>
</tr>
<tr>
<td>Field</td>
<td>4</td>
<td>ST.19</td>
<td>132</td>
<td>123-7 -1 =131</td>
<td>28- 2 - 0 = 30</td>
<td>99-15 -10 =132</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>ST.19</td>
<td>135</td>
<td>125-8 - 1 =134</td>
<td>30- 3 - 0 = 33</td>
<td>101-15 - 19=135</td>
</tr>
<tr>
<td>Sub-Total</td>
<td>5</td>
<td>F.ST.</td>
<td>84</td>
<td>81-3 - 0 = 84</td>
<td>67- 3 - 0 = 70</td>
<td>73-10 - 1 = 84</td>
</tr>
<tr>
<td>Experimental Study</td>
<td>N/A</td>
<td>F.ST.</td>
<td>90</td>
<td>85 - 5 - 0 = 90</td>
<td>90 - 0 - 0 = 90</td>
<td></td>
</tr>
<tr>
<td>R.Specificity</td>
<td>5</td>
<td>N1</td>
<td>210</td>
<td>44/209 = 21%</td>
<td>3/196 = 1.5%</td>
<td>106/210 = 50%</td>
</tr>
<tr>
<td>Sensitivity (ST19)</td>
<td>5</td>
<td>ST.19</td>
<td>135</td>
<td>133/134 = 99%</td>
<td>33/33 = 100%</td>
<td>116/135 = 86%</td>
</tr>
<tr>
<td>Sensitivity (F.ST)</td>
<td>5</td>
<td>F. ST</td>
<td>84</td>
<td>64/84 = 100%</td>
<td>70/70 = 100%</td>
<td>83/84 = 98.8%</td>
</tr>
</tbody>
</table>

*9% inhib.; suspect by C.F. and PCFIA (0.420) and positive by card test.
TABLE III9 - Consolidated Field Data

Comparison of D-Tec Brucella A. With C.F. Test and PCFIA Along With Kappa Values for Agreement

<table>
<thead>
<tr>
<th>Prefield Trial</th>
<th>Field Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D-TEC Br. A.</strong></td>
<td><strong>D-TEC Br. A.</strong></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>52</td>
</tr>
</tbody>
</table>

| **D-TEC Br. A.** | **D-TEC Br. A.** |
| +   | +   | PCFIA | K = 0.05 | 90 | 50 | +   | +   | PCFIA | K = 0.46 | 521 | 798 | 1319 | Poor Agreement |
| -   | -   | 2 | Poor Agreement | 2 | 2420 | 2422 |
| 90  | 52  | =142 | | 523 | 3218 | =3741 | |

K < 0.5 = poor agreement  
0.5 ≤ K < 0.7 = moderate agreement  
K ≥ 0.7 = good to excellent agreement
Table III10

D-Tec Brucella A. Results of the 25 Samples Tested at Different Laboratories for Reproducibility

<table>
<thead>
<tr>
<th>State</th>
<th>PMI</th>
<th>CA</th>
<th>CO</th>
<th>LA</th>
<th>NJ</th>
<th>OK</th>
</tr>
</thead>
<tbody>
<tr>
<td>#POS</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>#SUS</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>#NEG</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

(2) 2 samples which tested negative by PMI were not tested by Oklahoma
Chairman: Dr. 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; T.L. Beals; TX; P. Becton, FL; J.S. Cargile, TX; J.A. Cobb, GA; T. Conger, AR; T. M. Cook, DC, 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. Galagher, SD; P.C. Genho, FL; M.J. Gillisford, MD; F.D. Gregerson, CO; J.H. Hagler, TX; G.A. Hall, OK; R.L. Hartin, OK; E.R. Hinshaw, AZ; M. Huff, CO; F.S. Idtse, WI; J. Johnson, TX; A.W. Keating, IL; J.D. Kopec, MD; M. Lea, LA; C. Massengill, MO, W.G. Nelson, ID; R.E. Nelson, VT; D.L. Notter, KY; J.O. Pearce, Jr., FL; W. D. Prichard, OR; D. Raths, MT; V.B. Ricketts, CO; F.Y. Rogers, MS; P.K. Saini, MD; D. Satterwaite, NV; J.E. Slauder, MO; W.E. Stemler, IL; N. Stirling, SD; N.E. Swanson, WY; L.P. Thomas, WV; E.T. Thorne, WY; D.K. Thorpe, SD; K.J. Throson, ND; L.C. Vanderwagen, CA; R. Velure, ND; J.M. Williams, CO; L. Williams, NE; J.F. Wortman, NM; R.E. Yoxheimer, PA.

Chairman John B. Armstrong opened the meeting with the observation that there continues to be a decline in the number of known infected herds over the past year. He stated that the Brucellosis Committee has played a vital role in the progress that has been made with its dynamic leadership.

Thirty-six committee members attended the first session with excellent participation in the discussion of the various issues facing the program.

Dr. Granville Frye, of the Cattle Diseases Staff of Veterinary Services gave a status report of the brucellosis eradication program. As of September 30, 1991, there were 601 known infected herds in the United States. On April 21, 1991, federal indemnity for commercial beef herds had been increased from $50 to $150 per head, which has helped in the accelerated depopulation program.

Dr. E. Ray Hinshaw, the State Veterinarian of Arizona gave a review of the role of Brucella abortus St. 19 vaccination in the eradication program since the mid-1950s. He indicated that it is an integral part of the program today, with a diverse set of state and federal regulations regarding its use. But, perhaps, it is time to begin to phase out calfhood vaccination as the potential for exposure decreases. He recommended that a committee be appointed to evaluate calfhood vaccination and to develop a strategy for its future use in the program. (See Appendix A.)

Dr. John Keller, of Canada, gave a report on the use of calfhood vaccination in his country. In 1985, when Canada was declared free of brucellosis, calfhood vaccination lost its significance to the eradication program. In fact, it has a negative effect on the international movement of
REPORT OF THE COMMITTEE

stocker females out of Canada, as well as posing a public health risk of infection due to accidental injection of *B. abortus* St. 19 into the user (veterinarian). Of the last four field strain infected animals that have been disclosed in Canada, two of them were calfhood vaccinated animals.

Dr. Don Luschinger, of the Operational Support Staff of APHIS, discussed the effects of calfhood vaccination on the exportation of bovine females. Three countries (Taiwan, Poland, and Yugoslavia) will not accept calfhood vaccinated animals, while four (Columbia, Ecuador, Iran, and Tanzania) require their imported females to be calfhood vaccinated. Forty-two countries do not have any restrictions or requirements regarding vaccination. Stateside, 21 states require imported heifers to be calfhood vaccinated.

Dr. John Ragan, Tennessee State Veterinarian, gave a report on the resolution regarding calfhood vaccination which was passed by the LCI meeting in June. It consisted of four points:

1. States with a higher rate of infection, and/or higher exposure risk, should continue their present calfhood vaccination programs.
2. Plans should be developed to phase out vaccination entry requirements in Class A and Free States for cattle originating out of Class A or free areas.
3. The vaccination of native cattle should be the prerogative of each individual state.
4. After the nation is declared to be bovine brucellosis–free, the sale of *Brucella abortus* St. 19 vaccine should be abolished.

Dr. Garry Adams, of Texas A and M University, gave the results of the research on the competitive ELISA (cELISA) test which has been a cooperative effort between his group and Pittman–Moore. He concluded that the sensitivity of the cELISA was comparable with that of other presently used official tests; and, that the specificity was superior to that of the other serological assays. He urged the adoption of the cELISA as an official diagnostic test.

Dr. Lonnie King, Deputy Administrator of APHIS, gave a report on the federal brucellosis budget perspective. He said that the elements of the Rapid Completion Plan were implemented two years ago, even though it did not receive the desired level of funding. Those elements included enhanced first point of concentration testing, improved epidemiology, and adult vaccination or depopulation of affected herds. APHIS intends to design and distribute a logo to all animal health periodicals in an effort to maintain interest and support for the brucellosis eradication program.

Dr. King also gave an assessment on the activities of the PCFIA Advisory Committee which was appointed last Spring. The conclusions by that committee were not available yet, but he did indicate the aspects that
BRUCELLOSIS

they were given to consider on all advanced technologies:

1. Establish the criteria to be considered for the adoption of all new technologies.
2. Recommendations on how they should be funded.
3. Propose possible incentives to be utilized to encourage the development of new technologies.
4. Recommend the most appropriate technologies to be utilized for each problem.
5. Evaluate their use in terms of their potential expansion into other programs and problems.
6. Assess the appropriate role of the federal government in dealing with contractors offering the advance technologies.
7. Evaluate whether the use of PCFIA should be increased or decreased in the brucellosis eradication program.

Dr. Granville Frye presented the proposed changes in the U. M. and R. Standards regarding the management of heifers born into brucellosis affected herds. He suggested that exceptions granted heifers in affected dairy herds should be dropped. There was considerable discussion of the application of those proposed changes in the Chino Valley (California) infected dairy herds.

Dr. Don Pie&, of WSL and Iowa State University, gave a report on the Heat Inactivation Ring Test (HIRT) in reducing the sensitivity of the traditional BRT assay. Data presented indicated that HIRT was very effective in reducing the false positive BRT test results, thereby reducing the time and cost of testing negative dairy herds. He proposed that HIRT be adopted as an official supplemental test to the conventional BRT test.

Dr. Frye asked the group if infected bison herds should be considered in states that are applying for Class Free Status.

Dr. Terry Beals, Texas State Veterinarian, suggested that U. M. and R. Standards concerning the management of adjacent herds be more specific and explicit. The testing area around an affected herd should be expanded to at least one-half mile from the fence line perimeter of the affected pasture in Class B states, and to at least one mile in Class A or Free states. After the index herd is released from quarantine a second negative test should be required on those herds. In both instances, the designated epidemiologist should be granted some flexibility in the administration of those rules, depending on the particular circumstances of the particular affected herds.

Dr. Hailu Kinde, of the California Veterinary Diagnostic Laboratory, gave a report on the use of selective culture media to distinguish \textit{Brucella abortus} biovariety 1 from \textit{B. abortus} St. 19. His study showed that there was excellent correlation between growth on the differential medium (BAMCUP - tryptose agar, antibiotics, crystal violet, and penicillin G) and biotyping
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results. The BAMCUP inhibited the growth of *Brucella abortus* St. 19, biovar 4 and biovar 2, while allowing the growth of *B. abortus* biovar 1 (which is the only field strain known to be in California). (See Appendix B.)

Dr. Granville Frye introduced a proposed change in the National Reporting System which is used in the surveillance/classification system of states. He proposed that the adjusted MCI reactor rate be dropped in favor of the tabulation of the "Successful Closure of Cases": 95% in Class Free and A states, and 90% in Class B and C areas. In doing so, it would simplify the bookkeeping task by focusing on the successful tracing of an MCI reactor back to the herd of origin.

Tuesday's meeting of the Brucellosis Committee had 29 members in attendance.

Dr. Tom Thorne, of the Wyoming Game and Fish Department, gave a report on the brucellosis concerns in commercial herds of Cervidae (elk, red deer, deer) and camelids (llamas). He mentioned that there isn't a brucellosis problem in those species, but that the potential was present (since elk and red deer are susceptible to the disease). It was emphasized there is no consistency between the states on interstate test requirements or diagnostic technologies. He urged the committee to develop U. M. and R. Standards governing those species to promote consistency.

Dr. Brian Espe, A.V.I.C. of Oklahoma, recommended that a subcommittee be appointed to work on that project.

Dr. Garry Adams, Chairman of the Scientific Advisory Committee, presented his recommendations on the Heat Inactivated Ring Test (HIRT). They urged that HIRT be adopted as an official supplemental test procedure for bulk tank samples that show suspicious on the conventional BRT and that the interpretation of HIRT results be made in conjunction with other epidemiological factors. The Committee also recommended that additional HIRT data be collected for analysis, including sensitivity, specificity, and Kappa determinations, in non-vaccinated, calfhood and adult Strain 19 vaccinated herds of cattle.

That motion passed unanimously. Dr. Adams gave the views of the scientific advisory committee on the *Brucella abortus* St. 19 vaccination program. He pointed out that both Texas A and M and NADC are working the development of other vaccine types (that will not cross-react serologically to complicate diagnosis) that may well take the place of Strain 19 in the future.

He also gave a report on the Ad Hoc Tri-State Technical Committee that was assembled to study the Yellowstone Park bison brucellosis problem. In order to formulate a scientific approach to the problem the group proposed 35 ideas for research. In behalf of that ad hoc committee, Dr. Adams proposed that the Brucellosis Committee submit a resolution supporting the lethal collection of bison in the Yellowstone Park area for the purpose of establishing the infection rate, and to be able to correlate the serology with
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the infection rate. The resolution passed.

Dr. Jim Alexander, of the Scientific Advisory Committee, gave his recommendations concerning the proposed adoption of the cELISA as an official diagnostic test. After thoroughly evaluating the data presented by Dr. Adams the previous day, and the voluminous report given by Pittman-Moore, Inc., he recommended that it be adopted as a supplemental test rather than as an official diagnostic test, as was proposed in Dr. Adams' resolution. (See Appendix C.) Dr. Adams' resolution was defeated.

John Cargile made a motion to have it adopted as a supplemental test. That motion passed.

Dr. Paul Doby gave the report of the Swine Brucellosis Committee. He indicated that there were 68 newly infected herds (39 in Texas, 29 in Alabama, 6 in Florida, 4 in Georgia, 3 each in Arkansas and Louisiana, and 1 each in Hawaii, Oklahoma and New Jersey) disclosed in the past year. The feral swine subcommittee recommended that feral swine, moving interstate, be treated as infected (i.e., be consigned only to slaughter or a hunting preserve). (See Appendix D.)

Dr. Paul Becton gave the report of the special subcommittee (consisting of Dr. Becton, Dr. Ray Hinshaw, and Bill Gallagher) which was appointed to evaluate the calfhood vaccination program. He indicated that any changes in the program at this time would send the "wrong message" to the cattle industry. (See Appendix E.)

Dr. Granville Frye indicated that no changes would be made in the U. M. and R. Standards regarding the management of heifers in infected herds, since none had been proposed.

Dr. Frye's motion to treat cattle and bison equally in the U. M. and R. Standards received a considerable amount of attention. He stated that the present language in the U. M. and R. does not clearly reflect the intent of the brucellosis eradication program concerning bison.

Dr. D. K. Thorpe, State Veterinarian of South Dakota, proposed that his state's infected bison herd be treated the same as the bison in Yellowstone National Park (i.e., be disregarded in South Dakota's quest for Class Free status).

Dr. Frye replied that applying a different standard for a Free State would undercut the position of Veterinary Services concerning the infected bison in Yellowstone. He indicated that Yellowstone National Park is not a part of any state, whereas the infected bison herd in South Dakota was clearly part of the state.

Dr. Paul Becton asserted that the bison herd in South Dakota is not any different than a cattle herd of similar size on a similar amount of acreage.

Dr. Frye's motion was passed by the Committee.

The adjusted MCI reactor rate requires extensive bookkeeping involving negative results on MCI tests, and the important issues are the
traceback of the MCI reactors back to the herds of origin. Dr. Granville Frye proposed his motion to have the MCI rate replaced by a system that measures the successful closing of investigations of MCI reactors. The standard for successful closure rates in Free and A States would be a minimum of 95% and 90% for B and C States. His motion passed.

Dr. Terry Beals revised his motion for the testing management of adjacent herds around an infected herd. He replaced his 1/2 mile distance with 1 mile for states of all classifications. (See Appendix F, additions underlined.) The motion passed.

Dr. Beals' motion regarding the retest of adjacent herds after the index herd is released from quarantine was thoroughly discussed.

Dr. Claude Barton, the Southeastern Regional Brucellosis Epidemiologist, suggested that Beals' "second test" be replaced by "final test" in the final draft, because some adjacent herds will be tested multiple times while the index herd is still under quarantine. (See Appendix G.) The motion passed.

Dr. Terry H. Conger, the Brucellosis Epidemiologist in Arkansas, gave the report of the Brucellosis Subcommittee on Education. He indicated that the creeping complacency associated with the progress which has been made in the brucellosis eradication program must be counteracted by a continued effort to educate the cattle industry. The focus must be on promoting the calfhood vaccination of replacement heifers in areas where there is even a remote possibility of exposure to the disease. Dr. Conger proposed that the Committee submit a resolution that APHIS reestablish the funding for the production and distribution of educational material on brucellosis. Dr. Bob Hartin made the motion, which was seconded by John Cargile. (See Appendix H.) The resolution passed.

APPENDIX A

OFFICIAL CALFHOOD VACCINATION

History

A. Instigated in early 1950's as a brucellosis control program for dairies.
   1. Mandatory in infected herds.
   2. 60–70% immunity attained.
   3. Prevent abortion storm in herds should they become infected.
   4. Duration of immunity questionable but protect most through susceptible period of 1st and 2nd calving.
B. Dairy program later expanded to include beef herds following area testing of range cattle in 1957–58.
C. Interstate vaccination and testing requirements through UM&R.
   1. Originally addressed dairy cattle.
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2. Extended to beef cattle by some states.
3. USDA recognition of high risk states by classification (Free, A, B, C).
   a. Import requirements.
   b. Export requirements.
   c. Still separate dairy and beef.

Results of Calfhood Vaccination Program

A. Excellent program for reducing incidence of brucellosis on a national basis, especially in high risk areas.
B. Addition of various serological tests aided epidemiology to separate vaccination titers from field strain.
C. Refining the vaccine (low dose) reduced the incidence of post vaccination titers.
D. Involved the producer in the eradication program.
E. Disease control tool – not eradication measure.

Present Status of Calfhood Vaccination Program

A. Deeply integrated in brucellosis eradication program.
B. Diverse state requirements for mandatory vaccination.
C. Diverse Federal interstate requirements.
D. Diverse state import requirements regarding vaccination.
   1. Dairy only.
   2. Beef and dairy.
   3. Legible tattoos.
   4. Age of vaccination.
E. Vaccination does not eliminate the infected heifer only precludes the chances of early detection.
F. Vaccination titers constitute 80–90% of the time spent tracing animals reacting to the brucellosis test in Class A states and 100% in Free states.
G. Mandatory OCV has created problems for some states in their attempt to reach the minimum MCI requirement for Free status.

IV Economics of the Calfhood Vaccination Program

A. It costs approximately $5.00 per head to vaccinate a heifer for brucellosis.
B. Funding from various sources.
   1. Producers.
   2. State funded.
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3. Fee basis – State/Federal.

C. Current cost for calfhood vaccination.
   1. FY 1989–90 in excess of nine million heifers vaccinated at a cost of over $45 million dollars.
   2. Examples
      a. Minnesota (Class Free) 196,763 head = $983,815.00
      b. Nebraska (Class A) 359,000 head = $1,795,000.00
      c. Texas (Class B) 1,200,704 head = $6,003,520.00

D. Loss in value of animal with vaccination titer.

E. Some states require 'IF' branding and the OCV program also requires they be vaccinated.

V Current Purposes for vaccination

A. Control brucellosis.

B. To meet interstate import requirements of other states.

C. Used sales incentive in non–mandatory vaccination states that they may move anywhere in future.

VI Re–evaluation of OCV Program

A. Brucellosis cannot be eradicated through vaccination.

B. In all but Class B states, OCV has become a means to move heifers interstate because of import regulations.

C. The Canadian eradication protocol has proved successful.

VII Recommendations

A. Phase out the Official Calfhood Vaccination Program.

   1. Re–educate producers and officials who have supported OCV Program – tool only.
   2. Continue use in high risk areas or states only combined with adult vaccination.
   3. Eliminate mandatory vaccination programs in all Free and Class A states.
   4. Eliminate interstate requirements regarding vaccination and implement herd or state history evaluations, import tests and post–movement tests as needed.

B. Correlate with Rapid Completion Plan.

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1. Consider infected herd as an emergency disease situation in "Free" and "A" states.
   a. Strict quarantine enforcement.
   b. Limited-time herd plan or depopulation.

2. Adequate funding for depopulation incentive.

Summary:

The time has come to phase out the Official Calfhood Vaccination Program and concentrate on removal of sources of infection. of the $47 million dollars spent annually, seventy five percent is for the purpose of interstate movement only and the remaining twenty-five is for the control of brucellosis. The cattle industry, Federal and State governments deserve a better return on their expenditures than continued financial support of a program that has become scientifically a white elephant we continue to feed because we don't know what to do with it.

Bottom line - let's save the industry, State and Federal governments $50 million dollars a year and relegate brucellosis calfhood vaccination to a tool that the scientific and epidemiological communities can use where it is needed to control field strain brucellosis.

APPENDIX B

PRESumptive Differentiation of Brucella Abortus Field Strain from Strain 19 Using a Penicillin Supplemented Medium

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SUMMARY

Quarter milk samples (n=6433) from high titer cows (n=1613) based on rivanol and complement fixation tests were submitted for brucella culture. All animals were naturally infected with brucellosis and/or adult vaccinated with the reduced dose of strain 19 vaccine ranging 3 x 10⁸ to 5 x 10⁸ colony forming units (CFU) Each sample was plated on 3 different types of media: basal medium (tryptose agar) with antibiotics plus crystal violet (BAMCV); tryptose agar, antibiotics, crystal violet and penicillin G (BAMCVP); and tryptose agar plus antibiotics (BAM). All isolates from BAMCVP medium satisfied the preliminary identification criteria for field strain and later
confirmed as Brucella abortus biovar 1. All strain 19 isolates grew both on BAM and BAMCV but not on BAMCVP media.

Brucella abortus, strain 19, is differentiated from field strains by its ability to grow aerobically, failure to grow on medium containing 1:500,000 thionine blue, 1 mg of erythritol and inability to grow on 5 IU/ml of penicillin (1,2,3,4).

In this paper, we show that the addition of 5 IU/ml of penicillin to the BAMCV medium (1) ensured presumptive differentiation of B. abortus strain 19 from B. abortus biovar 1.

MATERIALS & METHODS

Quarter milk samples (n=6433) collected from 1613 cows were submitted for routine brucella culture to the San Bernardino branch of the California Veterinary Diagnostic Laboratory System between September 1989 and June 1991. These samples were from cows that were infection with B. abortus biovar 1 and/or adult vaccinated previously with the reduced dose of strain 19 vaccine 3x10^8 to 5x10^8 CFU. The samples were refrigerated overnight to obtain gravity cream. The selective media were prepared using standard techniques (1,5). A third medium containing antibiotics and crystal violet plus 5 IU/ml of penicillin G was included. With a sterile cotton swab, 0.1 – 0.2 ml of the gravity cream was spread over the entire surface of each of the three media. All plates were incubated under 10% CO₂ at 37 C. Plates were examined for growth at 4, 7 and 10 days. Three representative colonies were selected from each plate and presumptively characterized as B. abortus strain 19 or B. abortus biovar 1 based on CO₂ dependency, morphology, urease production, and catalase reaction. If all 3 colonies produced the same results, only one colony was selected for biotyping. Biotyping of Brucella sp. was performed as previously described (6).

RESULTS

Brucella sp. were cultured from 960 milk samples (15%). Five hundred sixty one isolates from 560 cows were biotyped. Four hundred one (71%) of the isolates were typed as B. abortus strain 19 and the remaining 160 (29%) as B. abortus biovar 1. All isolates from BAMCVP media were B. abortus biovar 1. All strain 19 isolates grew both on BAM or BAMCV medium (or both), but did not grow on BAMCVP medium. Mixed infection (B. abortus biovar 1 and B. abortus strain 19) was seen only once in this study.

DISCUSSION

This study showed excellent correlation between growth on the
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differential medium (BAMCVP) and biotyping results. There is no evidence that any biovar of B. abortus other than B. abortus biovar 1 exists in California. This biovar has always been resistant to penicillin. Canadian isolates of B. abortus biovar 4 and B. abortus biovar 2 (6) are susceptible to penicillin. In conclusion, a penicillin-supplemented medium is a valuable, simple and fast diagnostic tool for differentiating B. abortus strain 19 from field strains particularly in areas where biotyping capabilities are not available and the field strain is known to be penicillin resistant.

REFERENCES

5. Laboratory procedures for isolating, identifying and typing brucella. 1965, Diagnostic reagents manual 65F. Ames, Iowa, National Veterinary Services Laboratories, APHIS, USDA.

APPENDIX C
Brucellosis Scientific Advisory Committee
Comments on the Competitive ELISA ("D-Tec Brucella A.11"
October 29, 1991

Two members of the committee (James Alexander and Barry Stenshorn) considered the submission on this topic from Texas A&M University and Pittman-Moore Inc., with assistance of written comments from Dr. Bill Deyoe, who was unable to attend the meeting for health reasons.

Our evaluation was to some degree limited by the brief time available (the preliminary report was only forwarded to us on October 22nd; the complete submission received on October 27th was not available to Dr. Deyoe).

In addition to our deliberations on October 27th and 28th, we held an open discussion with the authors of the report in the presence of approximately 10 observers during the Scientific Advisory Committee's
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meeting in the Forum Room on the morning of October 28th. We also considered points brought out in the presentation by Dr. Adams to this committee yesterday, and matters raised in the ensuing discussion.

Criteria for Test Evaluation

The subcommittee noted the considerable effort made to satisfy the guidelines for the three phases of test assessment that were developed by this committee in 1987. In summary, these are:

Phase 1: - comparison with current tests on samples from 1000 cattle of known epidemiologic background

Phase 2: - double blind comparison on 500 randomly chosen serum samples provided by USDA from disease free and culture positive cattle

Phase 3: - over a one year period, test over 10,000 animals in labs under natural conditions. Samples should be from unexposed, vaccinated and exposed populations. At least 11 tissues were to be cultured from each of 300 animals.

Although the data submitted go a long way to satisfying these guidelines, it is noted that the criteria for Phases II and III were not completely met. USDA was able to provide only 65 samples for Phase II. In Phase III, which preceded Phase II, just over 200 cattle were cultured.

Observations

1. The subcommittee agrees that there is an important role for tests of increased specificity in the latter stages of brucellosis eradication.

2. There is good evidence from the studies in Free and Class A States that the CELISA has very high specificity (99.97%).

3. It is too early to draw definitive conclusions regarding the specificity of the CELISA relative to that of the PCFIA and CFT because:
   a. the PCFIA interpretations were done using criteria of POS <0.25 and SUSP <0.70, rather than the more recently adopted criteria (POS <0.30 and SUSP <0.60) which increased the PCFIA specificity,
   b. it is difficult if not impossible to reliably assess specificity by testing sera from culture negative cattle in high prevalence areas because of limits on the sensitivity of culture method, and
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c. Only a limited number of sera from free and A States were tested by both PCFIA CELISA.
The latter two points also apply to a comparison of the specificity of the CELISA with that of the CFT.

4. Although fewer than the recommended number of cultured animals have been tested to date, there are clear indications that the sensitivity of the CELISA is slightly less than that of the PCFIA or the CFT. The subcommittee agrees with the suggestion that this may be due, at least in part, to the relative insensitivity of the CELISA to low affinity antibody produced in the early days of response to infection or vaccination. However, it should also be mentioned that high specificity of the monoclonal antibody, presumably to the "A" epitope (we have not been presented with evidence on this point), might reduce the sensitivity of the test to antibody produced in response to biotypes of Brucella abortus that have predominantly "M" antigens (e.g., biotype 4 and the atypical biotype 2M).

Recommendations

1. The CELISA should be available for use as a supplemental test under the guidance of epidemiologists. It would appear likely to be most useful in the class A and Free States to reduce the costs of investigating false positive reactions, allowing saving and more efficient use of resources for trace-back investigations.

2. More information should be gathered regarding the sensitivity of this test through comparison with current methods using additional sera from culture positive cattle. This should include an evaluation of its sensitivity for detecting animals infected with biotypes carrying "M" surface antisens.

3. Until more is known about the sensitivity of this test, the subcommittee would caution against it being used for certifying animals for movement from Class B to Class A or from Class A to Free States.

4. In view of evidence that the test has high specificity and a slightly reduced sensitivity, animals in the current "suspect" range should be considered "reactors".

5. The manufacturer should add to the test a third control serum with activity in the current "suspect" range to supplement the negative and positive controls currently included in the test kit. It should also, as a matter of course, satisfy quality control criteria of USDA-APHIS with respect to the standardization and calibration of new lots of the antigen and of the conjugated

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monoclonal antibody (USDAAPHIS will need to develop appropriate standards to apply in this case).

Concluding Remarks

The committee views the CELISA as an important new tool for the use of epidemiologists.

The test operates on principles that make it quite distinct from current tests and therefore we would encourage continued careful study of its performance under actual field operating conditions. Consequently, our subcommittee would look forward to reviewing additional data on the performance of this test next year. In particular, more information is needed on its sensitivity and its specificity relative to the CFT and PCFIA.

While reiterating our view that this test offers an important new tool, we would point out that all technologies can be abused in one way or another. In this case, we would ask that the Brucellosis Committee take care to ensure that this test not be used in such a way as to detract from efforts to prevent the improper or excessive use of strain 19 vaccine. In particular, it should not be used to promote tolerance of S19 infected cattle. In the final analysis, S19 infection is a form of bovine brucellosis.

This concludes our comments on the CELISA.

James L. Alexander
Barry Stemshorn
October 29, 1991

APPENDIX D

Report of the advisory Committee on Swine Brucellosis Dr. Paul Doby, Chairman.

The committee met on October 28 with members and a number of guests present.

Dr. Ernest Zirkle of the New Jersey Department of Agriculture, reported on an outbreak of swine brucellosis in that state. The index herd had been depopulated earlier by shipping the breeding herd and maintaining feeder stock. Experience with this herd and with other contact herds reinforced the view that test and removal, without entire herd depopulation, is not a satisfactory method of eliminating the disease from a herd, Zirkle said.

Dr. Victor F. Nettles of the Southeastern Cooperative Wildlife Disease Study, reported on the formation of a Feral Swine Technical Group by APHIS. The report of that group will be published as an addendum to the report of the Wildlife Diseases Committee. He said swine brucellosis has been diagnosed serologically and/or B. suis cultured from feral swine in 10 states, and the overall prevalence of sero–reaction was 11%.
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Netties called attention to a number of recommendations of the group contained in the full text of the report.

Dr. W. F. Alexander of Oklahoma reported on importation of three feral pigs that tested negative in Florida but were positive when re-tested in Oklahoma, reinforcing the inadequacy of individual animal tests for swine brucellosis, especially used in feral swine.

Dr. Terry Beals, executive director of the Texas Animal Health Commission, reported on the program in that state, which is an effort to eradicate swine brucellosis and pseudorabies at the same time. In the year from July, 90 to June, 91, a total of 9,477 samples have been tested for swine brucellosis, with 137 positive. Traceback of the positives resulted in disclosure of 20 infected herds and 26 more infected herds were disclosed on testing of adjacent and contact herds. Of those 46 infected herds, one was tested and released from quarantine, 11 were sold to slaughter and 34 are under a testing program. He said most of those newly infected herds are garbage-fed and involve multiple owners in feeding complexes which are difficult and expensive to test, because owners cannot be found when herds are sampled. A change in regulations will require owners to provide facilities and labor for such testing or the owners will be charged with failure to comply with the regulations.

As of October, 1991, the state has 53 infected herds, of which 47 are garbage-fed. Only two positives have been found in feral swine in testing of large numbers of samples obtained from feral swine at slaughter.

Dr. W. C. Stewart of the APHIS staff presented the annual report on swine brucellosis. It showed a gain of three states – Hawaii, Kentucky and New Mexico – bringing the list of validated free states to 38, plus Puerto Rico and the U.S. Virgin Islands. Of the 12 remaining states, 7 are in Stage II and 5 are in Stage I. He said he expects three of those states – Missouri, Kansas and South Carolina – to attain validated free status soon, since none has had a case for several years.

A total of 68 newly infected herds have been disclosed during the current federal fiscal year: 39 in Texas, 10 in Alabama (most of them small garbage-fed herds around population centers), 6 in Florida, 4 in Georgia, 3 each in Arkansas and Louisiana, and one each in Hawaii, Oklahoma and New Jersey.

Sources of infection in newly infected herds during the current fiscal year were community spread 17, purchased swine 16, exposure to feral swine 1, other sources 8 and unknown 16. Stewart expressed concern about the high number in which the source of infection could not be identified.

Stewart pointed out that in the non-validated free states, more than 10% of the breeding swine have been tested during the current fiscal year, ranging from a low of 15% in Texas to a high of 78% in Oklahoma.
Goals for fiscal year 1992 cited by Stewart are: (1) validation of three more states; (2) production of a video on feral swine; (3) program reviews in eight states; (4) regional training courses in the southeastern region and in Texas. He said a proposal to increase indemnity rates for brucellosis-exposed swine is in process.

Stewart distributed copies of a new APHIS pamphlet on "Wild Pigs, Hidden Danger for Farmers and Hunters".

Dr. Janet Payeur, of the National Veterinary Service Laboratory, discussed results of her studies comparing brucellosis serology tests on experimentally infected swine. Her report is reproduced as an addendum to this committee report.

The chairman presented results of a survey of the states on whether or not they will accept swine from validated brucellosis free states without an individual animal test or individual herd validation status. Twenty-five states indicated they would accept pigs from Free states without further testing and nine said they anticipated changing regulations to permit such movements. The others indicated they would continue to require individual animal tests or herd validation status. The committee asked the chairman to send results of the survey out to each of the states and to write the 16 states that will not accept such movements from free states without testing and encourage them to change their regulations.

The following change in movement regulations was forwarded to the feral pig committee of USAHA and recommended to APHIS for implementation: for all movements, interstate or intrastate, all feral swine be treated as though they are from an infected herd, that they move under permit to slaughter at a USDA or state inspected plant or to a state approved hunting preserve.

The committee approved a motion urging the feral swine and wildlife committees develop guidelines for regulating hunting preserves that include feral swine to prevent spread of disease to domestic swine.

The committee asked APHIS, NPPC and the SE Cooperative Wildlife Disease Study to develop a model program to prevent spread of disease from feral to domestic swine in those states with native feral swine populations.

The committee commended APHIS and the SE Cooperative Wildlife Disease Study on the wild pig brochure and urged its wide distribution, especially to hunters.

The committee took note of the growing problem of finding slaughter plants to kill hogs from infected herds, but had no solution to suggest.

A resolution calling for increased indemnity and required depopulation in all infected herds was approved and forwarded to there solutions committee.
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APPENDIX E

The positive impact of official calfhood vaccination on the progress made in reducing the prevalence of Brucellosis throughout the country is well documented, especially in areas which had high risk of exposure.

Calfhood vaccinations as a criterion for interstate movement has been and continues to be primarily a requirement of the receiving state.

Federal interstate requirements are limited to female dairy animals moving into or out of class "B" or class "C" areas and to female beef animals moving into or out of class "C" areas.

At this time, there are no class "C" areas and only four class "B" areas, most of which will achieve class "A" within a few months.

It is our belief that any action by the committee to change the Uniform Methods and Rules as it related to calfhood vaccination for interstate movement could send the wrong signal to the cattle industry concerning the current risk of exposure to brucellosis in some areas of the country.

We recommend that no changes be made as these requirements will cease to exist when all states reach class "A" status.

APPENDIX F

BRUCELLOSIS ERADICATION
Uniform Methods and Rules

CHAPTER 1
GENERAL PROVISIONS – CATTLE, BISON, AND SWINE

Part 1
DEFINITIONS

Adjacent Herd

A herd of cattle or bison that occupies a premises that borders a "herd known to be affected" (this includes herds separated by roads or fordable streams) or a premises located within a specified area of a "herd known to be affected" or a premises that was previously occupied by a "herd known to be affected" within a stated period of time. The designated epidemiologist shall determine the period of time; the specified area will be one mile from the fence line perimeter of the "herd known to be affected". In all states—unless the designated epidemiologist approves a variance.
PART II
PROCEDURES
1 - 6 (No change)

7 Epidemiologic Procedures

A. (No Change).

(Page 64)
B. Adjacent Herds and Epidemiologically Traced Herd Testing

Adjacent herds, or herds sharing common pasture or having other contact with a herd affected with brucellosis, and herds containing previous purchases from or exchanges with an affected herd must have an approved action plan within 15 days in Class Free and Class A States and areas and 45 days in Class B and Class C States and areas from the time the affected herd is disclosed. (See Chapter I, Part III, C.)

The plan should be adapted to the circumstances and may require handling the herd as an affected herd. If a disagreement occurs on the method of handling the herd, the herd owner, the State animal health official, the designated epidemiologist and, if requested, the owner's veterinarian, will meet to resolve the differences.

After (when) a brucellosis affected herd is released from quarantine, the owners of the potentially exposed herds (located across the fence line, across the road, or across a fordable stream) must have a final negative test, unless a variance is granted by the designated epidemiologist.

APPENDIX H

Brucellosis Subcommittee on Education
Report

The brucellosis Subcommittee on Education convened on October 28, 1991. Thirteen individuals were in attendance. Discussions were held on some of the vital issues that must be dealt with if the task of eradicating the disease is to be completed in a timely and efficient manner. The element of creeping complacency must be squelched and reversed in order to prevent the loss of positive momentum in the eradication effort.

It was reaffirmed that the focus of the dissemination of information
and education should be on the promotion of calfhood vaccination of all replacement heifers in areas where there is even a remote possibility of exposure to the disease. The problem and importance of establishing and maintaining a continuing education program for both salaried regulatory personnel and veterinary practitioners was emphasized.

In acknowledging that the veterinary practitioner is the primary liaison between the regulatory sector and the private sector, the motion was made, and adopted, that the exchange of information on regulatory matters must occur on a regular basis between the Veterinary Medical Officers (VMOs) and the veterinary practitioners in their area, and that that requirement be incorporated into their performance standards.

The subcommittee also passed the following resolution:

Be it hereby resolved that the Brucellosis Committee encourage the continued production and distribution of educational material concerning brucellosis, such as that which has been produced and disseminated by the Kerr Center, and that funding for such endeavors be re-established by APHIS.
PRESUMPTIVE DIFFERENTIATION OF BRUCELLA ABORTUS FIELD STRAIN FROM STRAIN 19 USING A PENICILLIN SUPPLEMENTED MEDIUM

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3National Veterinary Laboratory Services, Ames, IA.

SUMMARY

Quarter milk samples (n=6543) from high titer cows (n=1613) based on rivanol and complement fixation tests were submitted for brucella culture. All animals were naturally infected with brucellosis and/or adult vaccinated with the reduced dose of strain 19 vaccine ranging 3 x 10⁶ to 5 x 10⁸ colony forming units (CFU). Each sample was plated on 3 different types of media: basal medium (tryptose agar) with antibiotics plus crystal violet (BAMCV); tryptose agar, antibiotics, crystal violet and penicillin G (BAMCVP); and tryptose agar plus antibiotics (BAM). All isolates from BAMCVP medium satisfied the preliminary identification criteria for field strain and later confirmed as Brucella abortus biovar 1. All strain 19 isolates grew both on BAM and BAMCV but not on BAMCVP media.

Brucella abortus, strain 19, is differentiated from field strains by its ability to grow aerobically, failure to grow on medium containing 1:500,000 thionine blue, 1 mg of erythritol and inability to grow on 5 IU/ml of penicillin.

In this paper, we show that the addition of 5 IU/ml of penicillin to the BAMCV medium (1) ensured presumptive differentiation of B. abortus strain 19 from B. abortus biovar 1.

MATERIALS & METHODS

Quarter milk samples (n=6543) collected from 1613 cows were submitted for routine brucella culture to the San Bernardino branch of the California Veterinary Diagnostic Laboratory System between September 1989 and June 1991. These samples were from cows that were infection with B. abortus biovar 1 and/or adult vaccinated previously with the reduced dose of strain 19 vaccine 3 x 10⁶ to 5 x 10⁸ CFU. The samples were refrigerated overnight to obtain gravity cream. The selective media were prepared using standard techniques (1,5). A third medium containing antibiotics and crystal violet plus 5 IU/ml of penicillin G was included. With a sterile cotton swab, 0.1 - 0.2 ml of the gravity cream was spread over the entire surface of each of the three media. All plates were incubated under 10% CO₂ at 37 C. Plates were examined for growth at 4, 7 and 10 days. Three representative
colonies were selected from each plate and presumptively characterized as B. abortus strain 19 or B. abortus biovar 1 based on CO₂ dependency, morphology, urease production, and catalase reaction. If all 3 colonies produced the same results, only one colony was selected for biotyping. Biotyping of Brucella sp. was performed as previously described (6).

RESULTS

Brucella sp. are cultured from 960 milk samples (15%). Five hundred sixty one isolates from 560 cows were biotyped. Four hundred one (71%) of the isolates were typed as B. abortus strain 19 and the remaining 160 (29%) as B. abortus biovar 1. All isolates from BAMCVP media were B. abortus biovar 1. All strain 19 isolates grew both on BAM or BAMCV medium (or both), but did not grow on BAMCVP medium. Mixed infection (B. abortus biovar 1 and B. abortus strain 19) was seen only once in this study.

DISCUSSION

This study showed excellent correlation between growth on the differential medium (BAMCVP) and biotyping results. There is no evidence that any biovar of B. abortus other than B. abortus biovar 1 exists in California. This biovar has always been resistant to penicillin. Canadian isolates of B. abortus biovar 4 and B. abortus biovar 2 (6) are susceptible to penicillin. In conclusion, a penicillin-supplemented medium is a valuable, simple, fast diagnostic tool for differentiating B. abortus strain 19 from field strains particularly in areas where biotyping capabilities are not available and the field strain is known to be penicillin resistant.

REFERENCES

5. Laboratory procedures for isolating, identifying and typing brucella. 1965, Diagnostic reagents manual 65F. Ames, Iowa, National Veterinary Services Laboratories, APHIS, USDA.
EXPERIMENTAL EPERYTHROZOOONOSIS IN LLAMAS

Dept. of Clinical Sciences Colorado State University, Fort Collins, CO 80523

Appearance of clinical cases of Eperythrozoonosis in North American llamas began in 1988. These cases were variably characterized by anemia, weight loss, and other clinical conditions including pneumonia, lameness and dermatitis. While some would appear to respond to tetracycline therapy, most would relapse and eventually succumb. As an attempt to understand this disease condition, experimental infection was proposed. Funding for this research was provided by the llama owner community.

Materials and Methods:

Initially, 6 llamas were utilized, all of which were tested thrice serologically and found to be negative to an indirect hemagglutination test using an Eperythrozoon suis antigen. Proposed infective material included heparinized frozen infected blood with high parasitemia. In addition, comparable blood was frozen in 10% Dimethylsulfoxide (DMSO) solution. Table 1 indicated the dosage, route of administration, subsequent observations and treatments.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age</th>
<th>1st Inj*</th>
<th>Observation</th>
<th>2nd Inj**</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adult</td>
<td>A–SQ</td>
<td>Transitory(+)</td>
<td>C–SQ</td>
<td>Transitory(+)</td>
</tr>
<tr>
<td>2</td>
<td>Adult</td>
<td>B–IV</td>
<td>–</td>
<td>C–SQ</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Juvenile</td>
<td>A–SQ</td>
<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
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<td>B–IV</td>
<td>–</td>
<td>C–SQ</td>
<td>–</td>
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<tr>
<td>5</td>
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<td>B–IV</td>
<td>–</td>
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<tr>
<td>6</td>
<td>Juvenile</td>
<td>B–SQ</td>
<td>–</td>
<td>C–SQ</td>
<td>–</td>
</tr>
</tbody>
</table>

A - 20cc heparinized blood; B - 20cc heparinized blood in 10% DMSO
C - 20cc fresh parasitized blood from animal 3–25 days after 1st injection

Blood smears were examined daily using Wright–Giemsa stain for presence of parasite, with summary results of infection patency appearing in Table 1. Complete hematological and standard biochemical parameters were also determined and will be reported in detail elsewhere. Serological samples from all six animals following the two infection attempts were evaluated by the E. suis IHA test and all found to be negative. A hemagglutination test using parasitized llama red cells did not give...
EXPERIMENTAL EPERYTHROZOONOSIS IN LLAMAS

couraging results. To assure production of highly parasitized llama red cells for ELISA antigen/conjugate production, the two adult animals (1 & 2) were splenectomized, with llama number 2 receiving 10cc of fresh heparinized blood SQ and IV at the time of surgery. Llamas number 1 & 2 were parasitemic by day 7 and 10 respectively. Post splenectomy, both llamas 1 & 2 were euthanized for antigen preparation and cryopreserved infective material.

To assess the effect of immunosuppression on animals that had not become parasitemic from 2 previous infection attempts, llamas 4, 5 & 6 were administered 1 mg/kg bw of dexamethasone sid for 6 days. 2 juveniles were observed parasitemic by 2 days after the commencement of immunosuppression. A third became parasitemic 5 days after the last injection. These three animals and llama number 3 were subjected to a course of tetracycline therapy which included 10 mg/kg oxytetracycline (OXY-100 R) IV and long acting tetracycline (LA-200R) at 20 mg/kg sq, the latter repeated every 3 days for a 5 day treatment. While the parasitemia would disappear temporarily, recrudescence was observed within 3–5 weeks. Animal number 3 died in spite of therapy, likely due to preexisting immunodeficiency. Animals 4, 5 & 6 were cleared of parasites using a prolonged course of arsanilic acid therapy.

An ELISA test was found to have good correlation with experimental infection, in that prior to the infection all were negative, i.e., <1:200 serum dilution. After infection, all animals became ELISA positive except llama number 3 which was immunocompromised from the outset, a condition now recognized with high incidence in the llama population.

As an attempt to understand the natural incidence in the llama population, ELISA tests have been performed on hundreds of samples from all over North America. Approximately 70% of all llamas tested to date are positive to the ELISA test. To further evaluate this significance, 3 healthy adult llamas that were ELISA+ were subjected to steroid immunosuppression (1 mg/kg bw 5 days SID). While evidence of steroid administration was dramatic (lymphopenia, eosinopenia, and 1 died of enterotoxemia), no parasitemia was observed.

Conclusions

- Eperythrozoon (EPE) is not readily established in healthy animals.
- Splenectomy or steroid immunosuppression facilitates infection.
- The *E. suis* hemagglutination test is of no value in llamas.
- The "*E. llamae*" ELISA test correlates well with experimental infection and recovery.

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Therapeutic regimen using oral/parenteral tetracyclines do not appear to be long term effective.

Many North American llamas are ELISA seropositive.

ELISA seropositive animals, when immunosuppressed, do not appear to develop EPE.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE, BISON AND LLAMA

Chairman: Dr. C. S. Card, Tucson, AZ
Vice Chairman: Dr. L. M. Siegfried, Ames, IA

W.F. Alexander, OK; A.A. Andersen, IA; F.R. Bauer, CA; R.E. Bohlender, NE; T.C. Bunting, IL; E.A. Carbrey, IA; H.M. Chaddock, MI; R.F. Conner, IN; R.A. Crandell, TX; G.L. Crenshaw, CA; D.P. Ferlicka, MT; A.M. Gallina, WA; J.E. Gillette, WA; L.R. Harrison, GA; L. Johnson, CO; A.J. Luedke, CO; C.S. McCain, OK; P.L. McDonough, NY; C.A. Mebus, NY; J.M. Miller, IA; P.A. O'Berry, IA; B.L. Osburn, CA; D.H. Schlafer, NY; J.A. Schmitz, NE; R.D. Schultz, WI; V.A. Seaton, IA; N. Stirling, SD; J.E. Strickland, GA; D.E. Suther, CA; N.R. Swanson, WY; R.M.S. Temple, OH; C.O. Theon, IA; R.D. Walker, KS; R.E. Werdin, MN; C.A. Whetstone, IA.

The Committee on Infectious Diseases of Cattle, Bison and Llama met from 1:30 - 5:30 p.m. on October 29, 1991, in the Senate Room of the Town and Country Hotel, San Diego, California. Chairman C. S. Card and Vice Chairman L. M. Siegfried conducted the meeting. J. E. Gillette conducted the session on llama diseases.

Committee members present were:

W. F. Alexander, OK; A. A. Anderson, IA; F. R. Bauer, CA; G. L. Crenshaw, CA; D. P. Ferlicka, MT; A. M. Gallina, WA; J. E. Gillette, WA; L. R. Harrison, GA; L. Johnson, CO; A. J. Luedke, CO; J. E. Strickland, GA; D. E. Suther, CA; N. R. Swanson, WY; R. M. S. Temple, OH; C. O. Theon, IA; R. E. Werdin, MN; C. S. Card, Chair; L. M. Siegfried, Vice Chair.

Approximately 70 committee members and guests attended the committee meeting.

Dr. Mark Anderson, CA, described the emerging abortion disease caused by a neospora-like protozoal agent. Abortion, mainly in dairy cattle generally occurs in mid-trimester of pregnancy (5 - 6 months). Fetal lesions, both gross and microscopic, were described. The disease may account for one-third of the diagnosed abortions in California; and while most cases occur in dry lot dairies, the condition has also been diagnosed in beef cattle. Presently, the disease is recognized by the Immunoperoxidase (IFAT) test, and characteristic microscopic lesions in skeletal and cardiac muscle and central nervous tissue. Aborting cows are not noted to be sick.

At this time, there is no method of preventing abortions, and the life cycle of the neospora-like protozoal agent is unknown. The IFAT is not a good test. The condition is recognized in CA, AZ, CO, TX, WY, SD, ID, Mexico, and Canada.
Dr. Will Hueston, who was assigned to the Weybridge Laboratory in England for six months last year, discussed the present status of Bovine Spongiform Encephalopathy. Presently, there are over 2,000 new suspect cases per month in England. The total confirmed cases is 30,654 (histologic confirmation) in 12,289 herds. Approximately 24.4% of all dairy herds and 2.7% of beef suckler herds are affected. The youngest case diagnosed was 22 months of age. Clinical signs can be grouped to include nervous apprehension, increased cutaneous sensation and locomotor problems.

Epidemiological studies are attempting to identify whether any of the following three hypothesis are causal:

1. Scrapie agent transferred to cattle – sheep/goat – tissues in feed stuffs
2. A genetic disease – Chernobyl – Freak of breeding
3. Chemically induced toxic disease.

The present study has identified feeds milled with meat and bonemeal (M & BM) in the diet of homegrown calves as a significant factor. Animal to animal transmission and maternal transmission are probably not significant factors. The disease is widespread geographically in England, mostly in dairy herds. Increased herd size is associated with increased incidence. There are no breed or sex differences. Continued and future research include investigations of rates of M&B BM in milled feeds, recycling of infected cattle materials into feeds, clinico–pathology studies, transmissibility to laboratory species and cattle and molecular biology studies.

Dr. Steve Bolin of Ames, Iowa, reported that antigenic variants of noncytopathic BVD virus have emerged in vaccinated cattle. These viruses are dissimilar from vaccine viruses in surface structures involved in viral neutralization by antibodies. Vaccinated cattle have insufficient concentrations of antibodies in serum to neutralize these viruses as early as four months after immunization with killed virus vaccines and 12 months after immunization with modified live virus vaccines. Diagnostic tests involving immunostaining of viral antigen in cell cultures may fail to detect these viruses under suboptimal conditions.

Virulent noncytopathic BVD viruses have been identified. These viruses induce severe clinical disease during primary, postnatal infection. Characteristic lesions are petechial and ecchymotic hemorrhages throughout the body. Infected cattle are often thrombocytopenic, have high fevers, and diarrhea. Death is a frequent outcome from infection with virulent noncytopathic BVD viruses. Microscopic examination of tissues often reveals lymphoid depletion in the spleen, lymph nodes and intestinal tract. Current vaccines appear protective for these viruses.

Dr. M. Saleh, Assistant Director of Egyptian Military Veterinary Services, Cairo, Egypt, reported on a Corynebacterium cutis lysate
(ultracorn) used as an immune stimulant in experimental calves. The material used with BCG reduced mortality and morbidity in calves compared to BCG injected and control calves. Increased weight gains were noted in ultracorn and BCG injected calves over a three-month period.

Dr. V. Schuller of the Federal Infectious Disease Institute in Vienna, Austria, commented that European countries are interested in the use of immunostimulants to enhance protection in food-producing animals.

Dr. Don Davis, Texas A and M, updated the committee on the investigations using Strain 19, and Brucella neotomae in American bison. Strain 19 in both adults and calves has been inadequate in protecting against a Brucella challenge. A live oral vaccine of B. neotomae is being tested in laboratory mice and young buffalo. The mouse model has been encouraging. Challenge studies in mice and buffalo will be completed during the coming year.

The use of B. neotomae in feed or by oral and subcutaneous injection provides a minimal serological response and has not been cultured from treated animals.

Dr. Bill Palin (IDEXX) discussed single well screening assays for both BLV and 1BRV. The tests are effective and will be commercially available in the near future.

Dr. C. O. Thoen, Ames, Iowa, discussed the progress of tuberculin testing investigations in llamas being conducted at Mexicali University, Mexico. Dr. Larue Johnson is an investigator and Dr. Thoen is a consultant for the research. The project will be continuing over the next year, and results will be presented formally by the Mexicali faculty members.

Dr. Don Mattison, Oregon State University, updated the committee on serological surveys of llamas. Over 270 samples from 20 herds had less than a 5% titers for common viruses of domestic livestock and horses. Dr. Mattison concludes that interspecies transfers are not common, and llama have several pathogens unique to the species. Health certificates are recommended and should include test results for immunoglobulin levels by radio immunodiffusion.

Dr. Larue Johnson, Ft. Collins, CO, gave an update of several diseases of camelids, including listeriosis, coccidiodymycosis, llama immunodeficiency syndrome (LIDS), toxoplasmosis, eperythrozoonosis, chlamydial abortion and meningeal worms.

The LIDS was described as a wide range of maladies including poor growth, dull hair coat, external parasitisms, hypoglobulinemia, anemia. Genetic predispositions and the possibility of a retrovirus were discussed. The condition is seen in animals from six months to two years of age. Clinical disease and death are a usual sequelae.

A resolution by Dr. Dan Suther, CA, was read and approved by the committee. The resolution pertains to the need for increased investigations into the emerging problem of protozoal abortions in cattle.
INTRODUCTION

Some 10,000 years ago, at the beginning of the agriculture age, mankind began to store surplus food, i.e. cereal grains, fruit, vegetables and later meat and fish. Storage of food was and continues to be a challenge. Microorganisms can spoil it and make it unsafe to eat. Insects and other pests compete for it. Some foods are seasonal and highly perishable; unless safe means of processing and storing them are available, periodic shortages can occur.

Over the centuries, great efforts have been devoted to finding ways to preserve food and eliminate pests. Drying was one of the first techniques developed. Fermenting, salting and smoking also have long histories. Later inventions, including freezing, refrigeration, canning, and the use of pesticides during production and storage, and preservatives during processing, have further increased the quality, quantity, and safety of our food supply by protecting it against contamination and spoilage.

Irradiation, a new technique, developed in the 20th century (since 1897), is an economical and hygienic means of food protection. It is already being used to a limited extent worldwide and has been approved for some uses in the United States. Irradiation or radiation has been studied by French, German, British, Russian, Japanese, Chinese and South African scientists for virtually this entire century, yet relatively few consumers have heard of it, even though many use irradiated products, i.e. medicines and medical implants. Objections have been raised by consumer advocates about the wholesomeness of foods treated by irradiation. The American Council on Science and Health (ACSH), the American Medical Association, Government and International Agencies: i.e. National Government Agencies, The World Health Organization (WHO), Food and Agriculture Organization (FAO), and the International Atomic Energy Agency (IAEA) have recommended its use and answered questions about the quality and safety of irradiated foods. It is generally agreed that irradiation of food is safe. The Codex Alimentarius as well as the World Association of Veterinary Food Hygienists and our World Veterinary Association have endorsed irradiation. The WHO, FAO and IAEA in Vienna act as the Secretariat for the International Consultative Group for Food Irradiation (ICGFI). Twenty six
FOOD IRRADIATION HYGIENE

countries participate in the ICGFI deliberations on implementing the radiation process worldwide so countries can have better food security. They make recommendations to the Codex Alimentarius for incorporation of the practices and standards into the Codex codes. To further understand the use of irradiation there follows some questions and answers about irradiation.

. WHAT IS FOOD IRRADIATION?

Food is irradiated by subjecting it to varying doses of ionizing radiation (also called ionizing energy), either from radioactive isotopes of cobalt or cesium, or from devices that produce controlled amounts of beta rays* or x-rays. This process does not make the food radioactive.

. WHAT CAN THIS PROCESS DO?

Irradiation has several useful effects in food processing. The most important is the reduction or elimination of living food borne bacteria and parasites that cause disease. Irradiation also maintains the freshness of food by inhibiting enzyme activity that breaks down cellular integrity. Different doses of radiation are used for different purposes, as shown in Table 1. The application of irradiation is similar to pasteurization in that it eliminates hazards in food or changes the physical properties of foods in desirable ways. The major applications of irradiation are summarized below.

STERILIZATION BY IRRADIATION

Ionizing radiation is currently used to sterilize more than 50 percent of the sterile disposable medical devices used in the United States. The same technique can also be applied to foods, and extensive research has been done on this application. A relatively high-dose radiation treatment can kill all the microorganisms that might grow in a food. The sterilized food can be stored in sealed containers for years at room temperature without being spoiled by microorganisms. This process is analogous to canning, in which heat treatment is used to achieve the same preservation status.

The United States Army sponsored extensive research on radiation sterilization, with the goal of producing better quality meals for troops in the field especially during the Vietnam era and they were also available during the conflict in the Gulf and Middle East. Human volunteers consumed these meals for periods longer than three years with no ill effects. Some of the Army's earliest experimental radiation-sterilized foods, produced in the 1950's and early 1960's, were less than successful in terms of flavor, aroma, and texture. However, improvements have been made in the irradiation procedure since then, and the radiation-sterilized meat and poultry products produced by current methods have been rated by experts as superior to their
canned counterparts in texture, appearance and in some cases, flavor, and vitamin retention.

Although radiation-sterilized foods are not available commercially in the United States, they have been enjoyed by American and Soviet astronauts and by some hospital patients, and those who are confined to special sterile environments.

Many foods, including meat, poultry, some types of fish (including oysters), and some vegetables, are suitable for radiation sterilization, but others are not. For instance, undesirable flavor changes occur in certain irradiated dairy products so these foods are unlikely candidates for commercial irradiation at the present time. The shelf stable fluid milk that is popular in Europe and now sold in the United States is not an irradiated product. This milk, which can be stored safely at room temperature for approximately 6 months, is preserved by an ultra-high temperature heat treatment.

"PASTEURIZATION" BY IRRADIATION/RADIATION

Heat processes that decrease the number of microorganisms in a food product without completely sterilizing it have many uses. Heat pasteurization of milk is one example. This process destroys any disease-producing microorganisms that might be present in the milk and delays growth of spoilage microorganisms, but it has little effect on the milk's taste and nutritive value, and does not sterilize it.

Irradiation at doses lower than those used in sterilization can be used to achieve similar kinds of "pasteurization". It can destroy food borne bacteria and parasites and delay the spoilage of highly perishable fresh fish and shellfish, and extend the shelf life of fruits and berries by delaying mold growth. Irradiation at pasteurization doses has little or no effect on flavor.

Many health authorities believe there is a need for a pasteurization-type treatment to ensure the safety of fresh meat and poultry. These foods are frequently contaminated with pathogenic microorganisms i.e. virulent coliforms, campylobacters and salmonellas, as well as Listeria and Yersinia which can cause illness and are killed by radiation pasteurization. It is common knowledge that microorganisms from meat and poultry cause many cases of food poisoning each year, usually in situations where raw meat or poultry is mishandled and there is cross contamination (e.g., handled on table tops or with utensils used in the preparation of other items). Radiation pasteurization ensures the wholesomeness of the finished product. Shellfish, including oysters, clams and mussels can all be successfully irradiated to eliminate disease causing bacteria including the vibrios which are of much concern currently to the industry and public health authorities. Vibrio vulnificus is frequently found in shellfish living in warm sea water and causes human illness of varying intensity. Better food handling practices would
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reduce the number of illnesses from consuming foods of animal origin.

DISINFESTATION BY IRRADIATION

Low-dose irradiation can kill or sterilize insects in grains and other stored foods.

Irradiation would facilitate the interstate and international shipment of fruits and vegetables. Many states and countries prohibit the importation of foods suspected of contamination with live insects, fearing the introduction of a pest problem along with the produce. During the 1980’s the Mediterranean fruit fly (Medfly) infested California and Florida. The reluctance of buyers in other states and countries to accept produce that might be contaminated with live Medflies led to substantial economic losses. Irradiation would be very useful in episodes of infestations for destroying the pests so the produce can be shipped to uninfested areas without the risk of spreading the insect pest.

One disadvantage of irradiation as an insect control process is that it does not leave any residual active agent to protect unpackaged food against reinfestation. There has been great enthusiasm about the possibility that killing insects with irradiation might increase the amount of food available in developing countries, where a substantial proportion of the food produced is destroyed by insects before it reaches human consumers. However, irradiation alone cannot accomplish this. It would have to be combined with effective methods of storage and/or packaging to prevent reinfestation after irradiation.

SPROUT INHIBITION BY IRRADIATION/RADIATION

A very low-dose irradiation treatment inhibits sprouting of vegetables such as potatoes, onions, and garlic, and can replace chemicals that are currently used for this purpose. Many nations, including the United States, have approved this treatment, and it is used commercially in Japan for white potatoes, and elsewhere.

DELAY OF RIPENING BY IRRADIATION/RADIATION

Low-dose irradiation delays the ripening and therefore extends the shelf-life of some fruits, including bananas, mangoes, papayas, guavas and avocados.

ELIMINATING THE TRICHINOSIS HAZARD IN PORK BY IRRADIATION/RADIATION

A low dose of radiation, similar to that used to inhibit vegetable
sprouting or delay fruit ripening, can eliminate the potential hazard of trichinosis/trichinellosis in fresh pork, without affecting the flavor or texture of the meat and without freezing or cooking it. Irradiated pork cannot cause trichinosis even if it is undercooked or eaten raw. The radiation treatment works by sexually sterilizing *Trichinella spiralis*, the parasite that causes trichinosis, so that it cannot complete its life cycle and cause the disease in humans or animals.

The United States is one of the few technologically developed countries that still has a problem with trichinosis transmitted by commercial pork. Although the disease is rare, (less than 1 per 100,000 pigs) it can be serious, and about 100 human cases occur each year. Most of these cases involve recent immigrants to the United States who are unaware of the need to cook American pork thoroughly, because they come from countries where trichinosis is not a problem. Southeast Asia has a problem because raw pork meat is very popular. Thailand is now irradiating pork for raw consumption. Many countries will not accept pork exported from the United States because it cannot be guaranteed free from the trichina parasite. Irradiation could play a major role in the U.S. pork industry's current effort to develop a certified trichina-safe pork supply, and it would make U.S. pork acceptable in international commerce.

**PHYSICAL IMPROVEMENTS**

Irradiation can produce desirable physical changes in some foods. For instance, bread made from irradiated wheat has a greater loaf volume when certain dough formulations are used. Irradiated dehydrated vegetables reconstitute more quickly.

**HOW CAN ONE TREATMENT DO SO MANY DIFFERENT THINGS?**

Irradiation causes a variety of changes in living cells. High doses kill the cells, thus killing microorganisms or insects. Lower doses alter biochemical reactions, such as those involved in fruit ripening, and interfere with cell division, which is necessary for the reproduction of parasites and the sprouting of vegetables.

**WHAT KIND OF RADIATION WOULD BE USED TO TREAT FOODS?**

The radiation used is called "ionizing radiation", because it produces electrically charged particles. Ionizing radiations, including x-rays, gamma rays, and beams of high velocity electrons (also called beta rays) produced by electron accelerators, have a higher energy than other non-ionizing
FOOD IRRADIATION HYGIENE

radiations such as light, microwaves, and radio waves.
Welded rods containing radionuclides that produce gamma rays (e.g. cobalt-60) and machines that produce x-rays and electrons at energy levels that do not induce radioactivity are the radiation sources that are used for food treatment.

. IS IRRADIATION THE SAME AS COOKING IN A MICROWAVE OVEN?

No. Irradiation involves the treatment of food with ionizing radiation. In a microwave oven, foods are exposed to microwaves, a type of non-ionizing radiation that generates heat in moist products.

. DOES IRRADIATION MAKE FOOD RADIOACTIVE?

No. The doses and energy levels of radiation approved for the treatment of foods are not high enough to induce radioactivity in the food.

. DOES IRRADIATION GENERATE RADIOACTIVE WASTES?

No. The process simply involves exposing food to a source of ionizing radiation. It does not create any new radioactive material. Of course, when radioactive isotopes of cobalt or cesium are used as the radiation source, they must be handled properly at the end of their useful life. But using these materials for food irradiation does not make their eventual disposal more difficult. The Atomic Energy Commission of Canada, now Nordion, is the principal source of cobalt 60 rods and the rods are usually returned to be recharged when spent. The Department of Energy has recalled cesium rods from all companies using them as a source of radiation for any purpose.

. ARE IRRADIATED FOODS SAFE TO EAT?

The safety of food irradiation has been systematically and comprehensively evaluated, more than any other technology known to man. Extensive studies of foods subjected to low-dose applications of irradiation, such as for sprout inhibition and insect disinfection, have shown that these foods are safe to eat. These irradiated foods have been proven safe to eat by long time (years) feeding trials to various animals and human beings. These studies are described in more detail below.

Most authorities deferred final conclusions on the safety of high-dose food irradiation treatments, such as used for sterilization, until the results of a large American study of radiation-sterilized chicken could be fully analyzed. The evaluation of the results of that study was recently
completed, and no evidence of a health hazard was found. This confirms earlier studies indicating that food exposed to high-dose irradiation does not cause cancer (1985).

How has the safety of irradiation foods been tested?

Assessing the safety of irradiated foods has involved investigations in a number of areas, including:

**General Toxicology**

Irradiation produces some substances in foods called radiolytic products. "Radiolytic" does not mean radioactive. It simply means that these substances are produced by ionizing radiation. Tests failed to show any of these substances, which are not unique to irradiated food, are harmful in the amounts consumed. In addition to testing for immediate toxic effects, the tests showed that these irradiated foods did not pose a cancer hazard, interfere with reproduction, cause birth defects, or pose other long-term hazards.

**Nutrition**

The nutritional value of foods should be protected as much as possible. Most processing methods have some effect on nutritional quality. Tests have been conducted to assess the nutritional quality of irradiated foods and to compare it to foods processed in other ways. Ideally, the nutritional quality of an irradiated food product should be at least as good as that of other foods that have undergone a similar degree of processing by other methods. Tests have shown that the nutritional quality is not significantly impaired in foods at doses approved by the FDA. The nutrient quality of the irradiated food is as good as foods processed by other methods used commercially today.

What kinds of tests were used to evaluate all these aspects of safety and what did the tests show?

As one might surmise, a very complex set of testing procedures was needed. The methods used included the following:

**Animal Testing**

The most common procedure for evaluating the safety of foods or
FOOD IRRADIATION HYGIENE

Food ingredients is to feed them to animals and observe the animals' growth, reproduction, and general health. Many such studies have been done on irradiated foods with a number of animal species. Some studies involved observations of several generations of animals.

Animal tests provide a good indication of the safety of a food or food substance. They are an effective way to detect general toxicological hazards, and properly designed tests may also detect cancer hazards and reproductive problems.

Worldwide, many animal feeding studies have been carried out with irradiated foods. These studies, all properly conducted, have uniformly shown no evidence of a health hazard.

One of the most notable of these was the six-year animal feeding study involving high-dose irradiated chicken that was conducted for the U.S. Army and U.S. Department of Agriculture (USDA). In this study, which has been described as the most extensive study of a processed food product in history, more than 600,000 pounds of chicken were fed to several generations of test mice, hamsters, rats, rabbits and dogs. The review of the massive amounts of data produced by this study was completed in 1985. It was concluded that there was no evidence that radiation-sterilized chicken meat would pose any toxicological hazard to humans. A similar study in the Netherlands showed no evidence of any toxicological hazard from eating irradiation sterilized ham.

HUMAN VOLUNTEER STUDIES

In a study using 9 human volunteers, irradiated food items provided 35–100% of the calorie intake. Seven such studies failed to show any significant changes in the volunteers consuming the irradiated foods. In China, eight experiments were carried out involving several hundred volunteers who ate irradiated foods for periods of 7 to 15 weeks. No differences were discernible between the test groups and the controls. An Indian study reporting polyploidy in malnourished children has been discredited but is still cited by opponents of irradiation processing.

Another area where irradiated food has benefitted humans is the use of radiation-sterilized foods for hospital patients receiving immunosuppressive medications, and for AIDS and other immuno-compromised patients. The U.S. and Russian astronauts use irradiated food during their space flights. These uses may not answer completely the question of safety of irradiated food in humans, but they do contribute to the overall evaluation that irradiated food is safe.

NUTRITIONAL STUDIES

Nutritional studies have shown that low-dose irradiation treatments
do not cause significant decreases in the nutritional quality of foods. In some cases, high dose treatment (sterilization) cause measurable losses of some vitamins. These losses are similar to those caused by other processing techniques that produce a similar degree of preservation, such as canning, and therefore are not considered to have any adverse health consequences.

MICROBIOLOGICAL STUDIES

Microbiological studies have shown that radiation sterilization can destroy microorganisms, including highly resistant bacteria, e.g. *Clostridium botulinum*, with the same or greater degree of certainty as the heat treatment used to destroy these organisms in commercially canned foods.

WOULD A FOOD IRRADIATION FACILITY ENDANGER THE COMMUNITY WHERE IT IS LOCATED? COULD THERE BE A "MELTDOWN"? WOULD PEOPLE IN THE AREA BE EXPOSED TO DANGEROUS RADIATION WHEN RADIOACTIVE MATERIALS ARE TRANSPORTED TO AND FROM THE FACILITY?

A food irradiation plant would not endanger a community, any more than do the 50 or more medical products irradiation plants and more than 1,000 hospital radiation therapy units now operating in the United States; nor would it pose any more hazard to a community than the hundreds of industrial x-ray units currently operating in many communities across the country. Certainly, a food irradiation facility must be constructed and operated properly to ensure that there are no radiation hazards. But this is not a new challenge; the necessary precautions are well understood because they have long been applied in other types of radiation facilities.

It is impossible for a "meltdown" to occur in a food irradiation plant. An irradiator is not a nuclear reactor. It is simply a processing plant containing a shielded area where foods are exposed to a source of ionizing radiation. The radiation sources used in food irradiation cannot explode.

Like all potentially hazardous substances, radioactive materials must be transported carefully, with appropriate safety precautions. In the United States, the Nuclear Regulatory Commission (NRC) has jurisdiction over the safe storage, use and disposal of radioactive materials. The Department of Transportation (DOT) has carrier requirements for the transport of hazardous materials, including radioactive cobalt and cesium. These substances have been transported to irradiation facilities and hospitals throughout the world for many decades without difficulty. In fact, the containers used for the transport of radioactive cobalt are so well shielded and damage resistant that the DOT permits them to be shipped by common carrier.
FOOD IRRADIATION HYGIENE

WOULD WORKERS IN A FOOD IRRADIATION PLANT BE EXPOSED TO HAZARDOUS RADIATION?

No. As a result of long experience in designing and operating medical equipment radiation facilities, the necessary precautions for worker safety in a food irradiation plant are well understood. These precautions are enforced by several Federal agencies in the United States. The Occupational Safety and Health Administration (OSHA) is responsible for regulating worker protection from all sources of ionizing radiation. Food irradiation plants that use cobalt or cesium as the radiation source must be licensed by the Nuclear Regulatory Commission (NRC) or an appropriate state agency.

HOW HAVE INTERNATIONAL HEALTH ORGANIZATIONS REACTED TO FOOD IRRADIATION?

Generally, they have been enthusiastic about its potential uses. After evaluation of the scientific data, they are also confident of its safety. A 1981 World Health Organization (WHO) document states:

"All the toxicological studies carried out on a large number of irradiated foods, from almost every type of food commodity, have produced no evidence of adverse effects as a result of irradiation.

"Radiation chemistry studies have now shown that the radiolytic products of major food components are identical....Most of these radiolytic products have also been identified in foods subjected to other, accepted types of food processing....The nature and concentration of these radiolytic products indicate that there is no evidence of toxicological hazard...

"The technological and economic feasibility of food irradiation on an industrial scale should be established. A wider variety of foods should also be studied with respect to their suitability for processing by irradiation."

The international organization concerned with food standards, the Codex Alimentarius Commission of the United Nations Food and Agriculture Organization (FAO) and the WHO, has endorsed the conclusions of an expert committee which reported in 1980 that all foods irradiated at substerilizing doses are safe to eat and require no further toxicological testing.

HOW HAVE U.S. HEALTH AND SCIENTIFIC ORGANIZATIONS REACTED TO FOOD IRRADIATION?

The Institute of Food Technologists (IFT), the principal scientific organization in the field of food science, has also evaluated food irradiation. IFT's Expert Panel on Food Safety and Nutrition concluded in 1983 that irradiation was safe and could provide consumers with more food of higher
quality. IFT’s findings are summarized in the publication "Radiation Preservation of Food," listed in the "Suggestions for Further Reading" at the end of this report.

The American Medical Association, in a 1984 letter to a Congressional committee that was considering legislation related to food irradiation, stated that food irradiation is safe, may be an important substitute for pesticides, and can control bacteria contamination in some foods.

The American Council on Science and Health, a consumer oriented study organization has endorsed the use of radiation pasteurization of food in different reports published since the uniform approval by government and international agencies.

CAST, the Council on Agriculture Science and Technology was an early advocate of radiation of food for human and animal use.

WHAT IS THE LEGAL STATUS OF FOOD IRRADIATION AROUND THE WORLD?

Over 35 countries have approved applications of irradiation; and irradiated foods are now marketed in about 20 countries. For instance, in Japan, each year at least 10,000 to 20,000 tons of potatoes are irradiated to prevent sprouting. A wide variety of irradiated foods has been approved in The Netherlands, and plants there are irradiating about 18,000 tons per day. About 8,000 to 10,000 tons of food per year are irradiated in Belgium. Two commercial irradiation plants in South Africa process mangoes, papayas, and vegetables. In 1983, as part of a special project, 50 metric tons of onions were irradiated and sold in supermarkets in Chile. Spices and onions are irradiated commercially in Hungary, and they have also been produced in small quantities in France, Israel, Czechoslovakia, and other countries. Appendix II gives a partial list of irradiated food products approved as of 1988 by various countries.

WHAT IS THE LEGAL STATUS OF FOOD IRRADIATION IN THE UNITED STATES?

In the United States, irradiation has been approved for five purposes: maturation delay of fresh foods; insect disinestation of any food; control of pathogens in poultry, and trichina in pork; control of microorganisms in dry enzymes; and disinestation of spices and other seasonings. The first two of these approvals (potato sprout inhibition and insect disinestation of wheat and wheat flour) came in the 1960s, but neither has been taken advantage of commercially. Irradiation of spices was approved in 1983 and was used commercially for a time until consumer opposition caused the company to stop.
FOOD IRRADIATION HYGIENE

Irradiation of pork requires the commercial plant to have a quality control program approved by the U.S. Dept. of Agriculture, and irradiation of poultry requires an amendment to USDA's regulations. Seafood is now being considered for irradiation at the request of the seafood marketing industry.

Food irradiation is regulated by the FDA under the terms of the 1958 Food Additive Amendment to the Food, Drug and Cosmetic Act. This law prohibits the use of a new food additive until its sponsor has established its safety and the FDA has issued a regulation specifying conditions of safe use. The law specifically includes "any source of radiation" in the definition of "food additive".

Congress apparently grouped irradiation with food additives in order to ensure that irradiation would have to meet the same high standards of safety that new food additives must meet.

In the late 1970's the FDA began to take steps to resolve the regulatory uncertainties that had long delayed approval of food irradiation in the United States. In 1979, it established a committee to review, evaluate and recommend criteria for safety evaluation of irradiated food. That committee reported its findings in June, 1980. It recommended a specific policy for determining whether an application of irradiation had been proven safe.

In March 1981, the FDA took the next step toward translating the committee recommendations into formal agency policy. It published an advance notice of its proposed procedures for the regulation of irradiated foods in the Federal Register and invited comments on its plan.

The next step in the regulatory process took place in February, 1984, when the FDA published in the Federal Register a proposed rule for low-dose food irradiation. The final step was the publication of the final rule in the April 18, 1986 Federal Register which does the following:

1. Permits the irradiation of fresh foods with doses up to one kiloGray (kGy) to "inhibit growth and maturation" (i.e., prevent sprouting, delay ripening).
2. Permits irradiation of any food at doses up to one kiloGray (kGy) for the purpose of insect control.
3. Requires irradiation to be declared on retail food labels.

The FDA rules provide total clearance only with respect to safety issues. Other requirements may be imposed by USDA in areas of its jurisdiction. For example, meat and poultry products would also require USDA approval as would any imported food irradiated to fulfill quarantine requirements.
NOW THAT IRRADIATION IS APPROVED, WILL IT BE USED?

Most experts predict that it will be used, but the extent will depend on many factors, including regulatory actions, consumer attitudes toward irradiation, the economics of irradiation and competing processes, and the nature of labeling statements.

Most irradiation applications are alternatives to other food processing methods (e.g., heating or treatment with a chemical). Irradiation will have to compete in the marketplace with these other techniques. If irradiated foods are more expensive than foods processed in other ways, without a quality or safety improvement to justify the extra cost, they are not likely to be successful.

There are a few instances where irradiation would not have competition. For instance, no other treatment currently exists which can guarantee that fresh raw pork is trichina-safe. (Trichinae can also be killed by heating or freezing, but these processes yield cooked pork and frozen pork, not fresh raw meat). In other cases, irradiation may be clearly superior to the available alternatives. For instance, now that Ethylene dibromide gas (EDB) has been replaced by less satisfactory fumigants and physical treatments, irradiation may be the best insect disinfestation method for some agricultural commodities such as spices and many tropical fruits.

WHAT ABOUT PRODUCT LABELING?

The current version of the FDA low-dose irradiation rule requires retail labeling consisting of an internationally agreed upon logo (symbol) and the phrases "treated with radiation" or "treated by irradiation."

Labeling required in the final regulation could hamper the commercialization of some applications of irradiation even if consumers are willing to buy foods labeled "irradiated". In many cases, irradiation would be competing with other techniques which need not be declared on the label (e.g. heat processing, freezing, use of fumigants). Thus, the food processor who chooses to irradiate faces record-keeping and labeling requirements that competitors who choose other treatments do not have. Also, some commodities suitable for irradiation (e.g., fresh meat, seafood, including shellfish, and produce) are often sold with no labeling or packaging at all.

There is no health reason why irradiated foods must be labeled. There is no known population subgroup that needs to avoid these foods, and people do not have to know that a food is irradiated in order to know how to handle it safely. On the other hand, comments submitted to the FDA suggest that many people want to know whether foods have been irradiated, and this desire is a strong argument in favor of labeling for informational (not warning) purposes.

FDA officials have stated that the purpose of any label adopted is to
FOOD IRRADIATION HYGIENE

be informative and not be a warning (as on cigarettes). They have pointed out that while a label declaring that a food is "irradiated" certainly tells the consumer something, it may not give him or her the correct message, since surveys show that the term "irradiated" is commonly misconstrued to mean that the produce is unsafe or unhealthful. Also, if irradiation is indicated on the label while other treatments are not, consumers might incorrectly assume that unlabeled foods are unprocessed. For instance, those individuals who might reject irradiated onions in favor of the unlabeled alternative might make a different decision if the alternative package were labeled "treated with (full chemical name) to prevent sprouting."

In some countries, including The Netherlands and South Africa, irradiated foods are identified by the special symbol on the label. In South Africa, the use of the symbol is required at the wholesale level but is voluntary on retail food packages. At first, few retailers wanted to use the symbol, but as the process has gained consumer acceptance, more retailers have used it, as a form of promotion.

CONCLUSION

Irradiation could have many benefits for consumers by increasing the variety of techniques that can be used to provide a safe, wholesome, convenient food supply. Extensive scientific testing has shown the proper use of food irradiation does not present a health hazard. All of the evidence indicates that consumers have nothing to fear from irradiated foods but instead can look forward to a greater variety of high quality food products and, in certain cases, safer products if this process comes into more widespread use in the world.

We as public health veterinarians, including food hygienists and nutritionists, have a responsibility to advise society that we endorse and support irradiation of foods just as we do irradiation of medicaments and equipment.

We as veterinary physicians, scientists, educators and hygienists have a responsibility to help the consumer understand irradiation/radiation and how it can improve our lives and protect our health. We have that obligation as responsible leaders in protecting the public health.

Appendix I. Some Terms Frequently Used in Discussions of Food Irradiation.*

Beta Rays (Electron Radiation): Streams or beams of electrons accelerated by a machine to energies of up to 10 MeV.
Gamma Rays: Electromagnetic radiation of very short wavelength, similar to "short" x-rays. Gamma rays are emitted by radioactive isotopes of cobalt and cesium as they decay spontaneously.
Gray (Gy): The unit (or level) of energy absorbed by a food from ionizing radiation. 1000 Gy = 1 Kilogray (kGy).

Rad: Like the Gray, a unit used to measure radiation dosage. The Gray, a newer unit, is superseding the rad. 1 Gy = 100 rad.

Radappertization (Radiation Sterilization): Sterilization of food by radiation processing. The resulting processed food can be stored at room temperature in the same way as thermally sterilized (canned) foods.

Radicidation (Radiation Pasteurization): Treatment of food with doses of radiation high enough to kill or render harmless all disease-causing organisms except viruses and spore-forming bacteria. Processed foods usually must be stored under refrigeration.

Radurization: Another type of radiation pasteurization, designed to kill or inactivate spoilage microorganisms, thus extending the shelf life of the refrigerated product.

X-rays: Ionizing electromagnetic radiation of a wide variety of short wavelengths. They are usually produced by a machine in which a beam of electrons bombards a metallic target in a high vacuum.

REFERENCES

FOOD IRRADIATION HYGIENE


Food and Drug Administration. Irradiation in the production, processing and handling of food; final rule. Federal Register 48(129). July 5, 1983. (This initial approval was limited to control of microorganisms in certain spices. Subsequent regulatory changes have expanded the approval to include additional spices and other seasonings and to allow irradiation for insect control.)

Food Additive Amendment of 1958 to the Federal Food, Drug and Cosmetic Act, Section 402.


CRA Info (the newsletter of the Council on Radiation Applications, Bethesda, MD), March 1985, p. 3.


SUGGESTIONS FOR FURTHER READING
STEELE


"Radiation Preservation of Foods," Institute of Food Technologists, Chicago, 1983. (Available for $1.00 from Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601. This article was also published in *Food Technology* 37(2):55, 1983.)


Coon, J.M., E.S. Josephson, and E. Wierbicki. "Food Irradiation," Council for Agricultural Science and Technology (CAST), Ames, Iowa, 1985. (Available for $1.50 from CAST, P.O. Box 1550, Iowa State University Station, Ames, IA 50010-1550. This article was also published in the April 1985 issue of the magazine *Science and Food and Agriculture*, published by CAST.)


## Appendix II: Some Foods Approved for Irradiation in 1988 or Earlier.*

<table>
<thead>
<tr>
<th>Country</th>
<th>Food Products</th>
<th>Country</th>
<th>Food Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Potatoes, strawberries, onions, garlic</td>
<td>Czechoslovakia</td>
<td>Potatoes, onions, mushrooms</td>
</tr>
<tr>
<td>Belgium</td>
<td>Potatoes, strawberries, onions, garlic, shallots, paprika, pepper, gum arabic,</td>
<td>The Netherlands</td>
<td>Asparagus, cocoa beans, strawberries, mushrooms, hospital meals, potatoes,</td>
</tr>
<tr>
<td></td>
<td>78 spices</td>
<td></td>
<td>shrimp, onions, poultry, soup greens, fish fillets, frozen frogs' legs, rice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and ground rice products, rye bread, spices, endive, powdered batter mix.</td>
</tr>
<tr>
<td>Canada</td>
<td>Potatoes, onions, wheat flour, poultry, cod and haddock fillets, spices and</td>
<td>Philippines</td>
<td>Potatoes, onions, garlic</td>
</tr>
<tr>
<td></td>
<td>certain dried vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>Potatoes, papaya, wheat, chicken, on chicken, onions, rice, fish products,</td>
<td>Spain</td>
<td>Potatoes, onions</td>
</tr>
<tr>
<td></td>
<td>spices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>Spices, herbs, hospital meals</td>
<td>Thailand</td>
<td>Potatoes, onions, garlic, dates, wheat, rice, fish, chicken</td>
</tr>
<tr>
<td>France</td>
<td>Potatoes, onions, garlic, shallots, spices, dried fruits and vegetables</td>
<td>U.S.S.R.</td>
<td>Potatoes, grain, fresh and dried fruits and vegetables, dry food concentrates,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>poultry, onions, prepared meat products</td>
</tr>
<tr>
<td>Germany (West)</td>
<td>Hospital meals</td>
<td>U.K.</td>
<td>Hospital meals</td>
</tr>
<tr>
<td>Hungary</td>
<td>Potatoes, onions, strawberries, spices, mushrooms, grapes, cherries, pears</td>
<td>U.S.</td>
<td>Wheat and wheat flour, potatoes, spices, pork, fresh fruits and vegetables</td>
</tr>
<tr>
<td>Israel</td>
<td>Potatoes, onions, poultry, 36 spices, fresh fruits and vegetables</td>
<td>Yugoslavia</td>
<td>Cereals, potatoes, onion, garlic, poultry, dried fruits and vegetables</td>
</tr>
</tbody>
</table>

Appendix III: Food Irradiation: Some Major Milestones

1895: von Roentgen discovers X-rays.
1896: Becquerel discovers radioactivity. Minsch publishes proposal to use ionizing radiation to preserve food by destroying spoilage microorganisms.
1904: Prescott publishes studies at MIT on the bactericidal effects of ionizing radiation.
1905: U.S. and British patents issued for use of ionizing radiation to kill bacteria in foods.
1905 to 1920: Much basic research conducted on the physical, chemical and biological effects of ionizing radiation.
1921: USDA researcher Schwartz publishes studies on the lethal effect of x-rays on Trichinella spiralis in raw pork.
1923: First published results of animal feeding studies to evaluate the wholesomeness of irradiated foods.
1930: French patent issued for the use of ionizing radiation to preserve foods.
1943: MIT group, under U.S. Army contract, demonstrates the feasibility of preserving ground beef by x-rays.

Late 1940s and early 1950s: Beginning of era of food irradiation development by U.S. Government (among Atomic Energy Commission) industry, universities, and private institutions, including long-term animal feeding studies by U.S. Army and Swift and Company.

1950: Beginning of food irradiation program by England and numerous other countries.
1958: The Food, Drug and Cosmetic Act is amended directing that food irradiation be evaluated as a food additive, not as a physical process. All new food additives, including irradiation, must be approved by FDA before they can be used. The U.S. Congress passes legislation which President Eisenhower signed in 1958. This legislation is still the law of the land.
Appendix IV: Regulatory Highlights - Food Irradiation

1959: Canada approves potato irradiation.
1960: U.S. FDA approves irradiated bacon, wheat and wheat flour and potatoes.
1964: U.S. FDA approved flexible packaging materials of food during irradiation processing.
1976: Joint FAO/IAEA/WHO Expert Committee on (safety/wholesomeness of) Food Irradiation (JECFI) approves several irradiated foods and recommends that food irradiation be classified as a physical process.
1980: Joint FAO/IAEA/WHO Expert Committee on (safety/wholesomeness of) Food Irradiation (JECFI) approves all irradiated foods treated with a maximum average dose of 10 kGy.
1985: U.S. FDA approves irradiation of pork to control trichinosis.
1986: U.S. FDA approves irradiation of fruits and vegetables and other foods up to doses of one kGy.
<table>
<thead>
<tr>
<th>Type of Food</th>
<th>Radiation Dose in kGy*</th>
<th>Effect of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat, poultry, fish, shellfish, some vegetables, baked goods, prepared foods</td>
<td>20-71</td>
<td>Sterilization. Treated product can be stored at room temperature without spoilage. Treated product is safe for hospital patients who require microbiologically sterile diets.</td>
</tr>
<tr>
<td>Spices and other seasonings</td>
<td>Up to maximum of 30</td>
<td>Reduces number of microorganisms and insects. Replaces chemicals used for this purpose.</td>
</tr>
<tr>
<td>Meat, poultry, fish</td>
<td>0.1-10</td>
<td>Delays spoilage by reducing the number of microorganisms in the fresh, refrigerated product. Kills some types of food poisoning bacteria and renders harmless disease-causing parasites (e.g., trichinae)</td>
</tr>
<tr>
<td>Strawberries and some other fruits</td>
<td>1-5</td>
<td>Extends shelf life by delaying mold growth.</td>
</tr>
<tr>
<td>Grain, fruit, vegetables, and other foods subject to insect infestation</td>
<td>0.1-2</td>
<td>Kills insects or prevents them from reproducing. Could partially replace post-harvest fumigants used for this purpose.</td>
</tr>
<tr>
<td>Bananas, avocados, mangos, papayas, guavas, and certain other non-citrus fruits</td>
<td>1.0 maximum</td>
<td>Delays ripening.</td>
</tr>
<tr>
<td>Potatoes, onions, garlic, ginger</td>
<td>0.05-0.15</td>
<td>Inhibits sprouting.</td>
</tr>
<tr>
<td>Grain, dehydrated vegetables, other foods</td>
<td>Various doses</td>
<td>Desirable physical changes (e.g., reduced rehydration times).</td>
</tr>
</tbody>
</table>

*kGy (kilogram). For more information on the units used to measure radiation, see Appendix I.
A REPORT ON THE APRIL 8–18, 1991 VISIT OF A UNITED STATES DEPARTMENT OF AGRICULTURE (USDA) TEAM TO FRANCE REGARDING THE OUTBREAK OF TRICHINOSIS IN HUMANS

Team Members:

Robert Fetzner, D.V.M.; Caldwell W. Nash; Charlotte I. Miller, Ph.D.

Report Editor:
Linda Russell, M.A.
DRAFT
June 20, 1991

United States Department of Agriculture
Food Safety and Inspection Service (FSIS)
International Programs
Washington, D.C.

Executive Summary

On March 19, FSIS was notified of a possible outbreak of human trichinosis in the Auvergne region of south central France, attributed to horsemeat of U.S. origin.

On April 8, USDA sent a team to France to meet with French Food Quality and Veterinary Service (FVS) officials and review the information collected on the origin of the outbreak. Team members were: Dr. Robert Fetzner, Director, Export Coordination, FSIS; Caldwell W. Nash, Compliance Specialist, Regulatory Programs, FSIS; and Dr. Charlotte I. Miller, Chief, Information Resources Management Branch, Animal and Plant Health Inspection Service (APHIS).

When the USDA team arrived in France, French investigators had confirmed 11 cases of human trichinosis, although up to 31 cases had been mentioned as possibly associated with the outbreak. The French investigators had linked the outbreak to a horsemeat deboning and cutting plant—Sovica-Lottin, in Angers, France. The meat had been bought at three branches of the Mammouth supermarkets. The deboning and cutting plant had received horsemeat from U.S., Canadian, and Argentine plants. The French investigators concluded the source as probably a U.S. firm, Central Nebraska Packing Company, North Platte, Nebraska (Establishment E-713).

The USDA team met with FVS and other French officials and visited the implicated cutting plant. After reviewing information provided, the team concluded that:
OUTBREAK OF TRICHINOSIS IN HUMANS

1. Some people were clinically diagnosed with trichinosis in France and those people had eaten horsemeat in the period just prior to their diagnosis. However, it was not demonstrated conclusively to the team that the horsemeat the people ate was the vehicle for the parasites that caused their infection.

2. The conclusion by French investigators that all the cases were linked by a common source is not confirmed, because the French investigators did not completely eliminate all other possible sources of infection. Only one sample of meat was tested for trichina. That sample was labeled as horsemeat, but not subjected to species verification testing before trichina testing. The label on the sample of suspect meat indicated that it was packed after the infection occurred. Also, the trichinoscopic method of detection used by Dr. Perre on that sample did not detect any trichinae, whereas the artificial digestion method conducted by Dr. Soule did detect a single larva. This fact leads the team to suggest that French testing via trichinoscopic methods might fail to detect trichina parasites in other meat samples.

Furthermore, there is a higher probability that some of the 1991 cases may be due to pork or wild boar meat, given the epidemiological history of trichinosis in France and the fact that individual cases of trichinosis are not required to be reported, as they are in the U.S.A. Dr. Ancelle's paper of January 1989 on an outbreak attributed to wild boar in 1985 also reports a dozen cases in 1988.

3. Even if the assumption of the French investigative team—that horsemeat is the source of the human infections—is accepted, there is inadequate data to conclude that a particular country, let alone a particular plant, can be implicated as the source of the parasite.

The United States instituted an intensive testing system for trichinosis after an outbreak of human trichinosis was reported in France in 1985. Every horse carcass slaughtered in the U.S. for export is tested for trichina. After 1.8 million negative tests, between 1985 and the present, the evidence is that the U.S. testing system and method are thorough and effective.

Conclusion:

Given these findings, the team cannot concur with the conclusions reached by the FVS that the trichinae outbreak can be conclusively traced to horsemeat or that the meat sample found to have a trichina larva was of U.S. origin.

However, USDA agrees with the FVS that the source of the trichinosis outbreak should be properly determined. Therefore, it is important that all possible sources of infection be studied to conclusively determine food vehicles for the parasites that caused infection, and that preventative
measures be undertaken to prevent other human trichinosis infections.

Background

On March 19, FSIS was notified of a possible outbreak of human trichinosis in the Auvergne region of south central France, attributed to U.S. origin horsemeat. Herbert Rudd, Agricultural Counselor, U.S. Embassy, Paris, France, telephoned Patricia Stolfa, Deputy Administrator, International Programs, FSIS, to discuss the situation. Mr. Rudd had been notified of the problem by Dr. Regis Leseur, Director of Food Safety, in the French Food Quality and Veterinary Service (FVS) of the Ministry of Agriculture and Forestry.

On April 8, USDA sent a team to France to meet with French Food Quality and Veterinary Service (FVS) officials and review the information collected on the origin of the outbreak. Team members were: Dr. Robert Fetzner, Director, Export Coordination, FSIS; Caldwell W. Nash, Compliance Specialist, Regulatory Programs, FSIS; and Dr. Charlotte I. Miller, Chief, Information Resources Management Branch, Animal and Plant Health Inspection Service (APHIS).

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Information Gathered in France

On April 10, the USDA met with FVS officials to discuss the team's agenda and review the French investigation so far. The team asked for the schedule to be expanded to include visits to the plant at Angers and the Mammouth supermarkets in Clermont-Ferrand and Montlucon. This was prompted by a message from Paul Peeters, owner of Equinox S.A., a Belgian firm which owns Central Nebraska Packing Company. Mr. Peeters said that the owner of the French cutting plant would state that the lack of inventory traceability within the plant made it impossible to determine the country of origin—Canada, Argentina, or the U.S.—of a meat sample discovered in the home freezer of one of the trichinosis patients.

FVS officials agreed to allow the team to visit Angers, but suggested there was not enough time to visit Montlucon. Nor did they concur that the
OUTBREAK OF TRICHINOSIS IN HUMANS

USDA team should visit the Mammoth supermarkets. The French officials expressed surprise and dismay that the team wanted to visit these locations and stated that the request was a symptom of distrust between the two inspection systems. However, the USDA team was merely concerned with evaluating available data to confirm the source of the problem; these restrictions hampered its ability to complete its mission.

During that meeting and subsequent ones, the FVS presented its conclusion that U.S. origin horsemeat could be clearly demonstrated as the cause of the outbreak of human trichinosis in the Auvergne region.

**Summary of Epidemiological Studies**

The outbreak appears to have begun in February 1991. The FVS and French health officials confirmed 11 cases of human trichinosis—two with positive biopsies and serological tests, and 9 with positive serological tests only.

The first case, identified as Mrs. C., was hospitalized on February 26. Later epidemiological studies uncovered earlier cases which had probably been misdiagnosed as influenza, since they occurred at the height of the influenza season.

The USDA team did not meet with patients directly; nor did the FVS provide the USDA team with the content of the food consumption survey instruments or responses. The epidemiologists who met with the team explained the basis of the analysis—the only common element among the persons who became ill was that they all consumed horsemeat, among possible meat sources of trichinosis infection, during the period February 6–20. However, some information that Dr. Rey provided indicates that one patient with a positive biopsy may have eaten the suspect meat as early as January 30.

The USDA team was concerned that French health officials had already published accounts of both the trichinosis outbreak and the investigation of the origin of the meat which was implicated in it. They considered this premature.

At the parasitology laboratory in Clermont-Ferrand, the team viewed two biopsy slides which were dated March 25 and 27. According to Dr. Rey, the positive biopsies from this trichinosis outbreak were April 2 for Mrs. C. and April 3 for a patient from Montlucon. Dr. Hubert promised to have the epidemiological studies completed and give the team a copy of the draft report before they departed. As of June 1, the USDA team had not received this draft.

The FVS explained that the first discovery of the trichinosis, and most serious case, had been traced by patient interview to meat eaten on either February 16 or 17 in the Clermont-Ferrand area. The only sample of
horsemeat analyzed in connection with the trichinosis cases came from this patient's freezer. According to Dr. Rey, all four members of the C. family had eaten from the same horsemeat roast. Mrs. C. had eaten two slices; Mr. C., four slices; the 7-year-old son, one slice; and the 4-year-old son, half a slice. The woman had become seriously ill, while the father and older son experienced only mild symptoms. The younger son was the least affected. The woman had eaten the first two, or the most thoroughly cooked, slices of the roast. The others had eaten the rarer center slices.

All members of the family exhibited eosinophilia. The woman's level was over 10,000 mm3 while the other three had levels over 1000 mm3. The normal level is between 200 mm3 and 400 mm3. Any level over 500 mm3 is the "earliest and most consistent laboratory sign" of trichinosis.

According to medical personnel, on hospitalization, both biopsy (March 2) and serological (March 1) tests were negative for the woman. However, on March 19, a serological test for trichinosis gave positive results and on April 2 a biopsy of her right triceps muscle tested positive for trichinosis, demonstrating an encysted larva. Serology tests on the man and older child showed positive. As of April 12, the younger son had not had a positive serological test.

The Trace-Back Process

The FVS reported that it conducted an investigation based on this family's diagnosis and determined a purchase date of February 16 from a Mammouth supermarket in Clermont-Ferrand. According to the FVS, supermarket personnel determined that the meat was from a shipment delivered February 13 from the cutting plant in Angers.

According to the FVS, the supermarket received horsemeat shipments every Wednesday and only from this supplier. Using this delivery date, the FVS reasoned that the meat must have come from the U.S. on a health certificate dated February 6 from Central Nebraska Packing.

The FVS said that, based on information from the epidemiological studies still underway, all persons suspected of being infected had eaten horsemeat either raw or rare and those who were asymptomatic from the same families had either eaten it well done or not at all. (This statement seems to contradict what Dr. Rey told the team about the C. family.) All persons suspected of being infected reported that they had bought horsemeat at one of three Mammouth supermarkets: the Aubiere, Domeraet, or Croix de Neyrat branches.

Dr. Tissot told the team that she would provide them with a copy of her findings describing the methods and reasoning used to trace the meat from the retail to the wholesale establishment and linking it with specific health certificates. However, the team has not yet received this report.
OUTBREAK OF TRICHINOSIS IN HUMANS

Analysis of a Frozen Meat Sample

A 594-gram sample of frozen meat, bearing the Mammouth Aubiere label of "Cheval: rosbif faux-filet" (boneless horse roast), was recovered by Dr. Rey from the C. family's freezer. The packing date on the label was March 2 and the consume-by date was March 5. Therefore, it was packed at the supermarket and purchased by the family after Mrs. C. was already tentatively diagnosed as having trichinosis.

Dr. Rey gave the entire sample to Dr. Perre of the Departmental Veterinary Service (DVS) of Puy-de-Dome for analysis. Dr. Perre divided it in half and conducted a standard trichinoscopic examination on one half at an industry facility in Clermont-Ferrand. He shipped the other half to the CNEVA laboratory in Maison Alfort to the pathologist, Dr. Soule. He performed an artificial digestion test for trichinae using the magnetic stirrer technique and a special trichinoscopic tray to display the products of digestion. As a result of this process, the remains of one trichinae larva was found. Dr. Soule removed it from the tray in order to photograph it. He provided the team with a copy of the resulting photo, showing no muscle tissues and no cyst membrane—possibly due to the digestive breakdown. According to Dr. Soule, the larva was too small to preserve or to subject to DNA testing.

Trichinella species and subspecies cannot be identified on photographic information alone. When asked, French experts stated that the photograph was consistent with the diagnosis of trichinella.

When asked by the team, CNEVA said that no species verification testing had been conducted on the meat sample. The FVS said that they determined the species based on veterinary expertise.

Origin of the horsemeat

On the team's visit to Angers, the origin of all horsemeat in the Sovica-Lottin cutting plant was reviewed with representatives of FVS, DVS of Maine-et-Loire, plant management and Mr. Peeters. At a meeting in DVS offices on April 16, the team asked specifically about the inventory of horsemeat in the plant just prior to the three deliveries to Mammouth supermarkets (February 6, 13, and 27) which FVS had implicated in the infection of Mrs. C., the frozen sample, and one other prior delivery.

The plant inventory for February included fresh, bone-in hindquarters from two U.S. and three Canadian plants, and boneless vacuum-packed cuts from the U.S., Canadian and three Argentine plants. Sovica-Lottin invoices shown to the team described the products in the delivery as "Arrieres P.A.D." meaning boned-out hindquarters ready to cut. Since the invoices specified "hindquarters", the FVS reasoned that the shipment must have come from either the United States or Canada.
However, the team asked the plant owner if vacuum-packed product could have been used to fill out the order if insufficient boned-out hindquarters were in the inventory. Before he could answer, Dr. Tissot interrupted him to ask him if he was ready to admit to falsification of invoices. [In the U.S. system, such a practice could also be considered "misbranding" since the invoice is considered a part of the product label.] Mr. Lottin then chose not to respond to the team's question.

Nevertheless, the FVS and DVS representatives agreed that the February 6 delivery could have come from either the U.S. or Canada. As for the delivery of February 13 implicated in Mrs. C.'s infection, they said that Canadian product was delivered to the cutting plant on February 12, the same day as the suspect shipment left the cutting plant, but that they did not believe there was time enough to process the Canadian product received at noon prior to the 3:30 p.m. shipment time. However, Mr. Lottin stated that it took only 15 minutes to debone a horse quarter, and that he could not assure the team that Canadian product was not included in the February 13 delivery. Inventory at the plant was also consistent with either Canada, Argentina or the U.S. as the origin of the February 27 delivery, implicated as the origin of the frozen sample.

Previously at a meeting in Paris on April 15, Mr. Peeters provided the team with copies of telefaxed statements dated March 29, 1991, from Mr. Lottin to his company describing the inventory of his plant during February and stating that he was unable to testify to the origin of the meat sold at the Mammouth stores in question. When questioned by the team as to why he made the statements, Mr. Lottin said that he did so in response to Mr. Peeters' questions about what he had told the FVS investigators. At the April 16 meeting, Mr. Lottin verified the authenticity of the documents.

The FVS and European Community (EC) veterinarians claimed these documents were obviously self-serving, since they were created after the embargo. However, the USDA team was concerned that the officials disregarded this information, which could be vital in tracing the origin of the outbreak.

After the meeting, the team visited the Sovica-Lottin plant. During the visit, the team observed the inventory of hindquarters and vacuum-packed cuts, noting that Canada and Argentina were the countries of origin.

The team also noted the presence of unlabeled thinly sliced fat identified by Mr. Lottin as pork fat from nearby pork plant. Mr. Lottin advised that the fat was used to wrap horse roasts and that this operation was conducted in the same environment as the deboning (not in a separate part of the plant).
OUTBREAK OF TRICHINOSIS IN HUMANS

Findings

The USDA team's findings are:

1. Some people were clinically diagnosed with trichinosis in France and those people had eaten horsemeat in the period just prior to their diagnosis. However, it was not demonstrated conclusively to the team that the horsemeat the people ate was the vehicle for the parasites that caused their infection. The long incubation period of trichinella in humans would permit any one of several eating events as the source of a particular infection.

2. The conclusion by French investigators that all the cases were linked by a common source is only a hypothesis, because the French investigators did not completely eliminate all other possible sources of infection. Only one sample of suspect horsemeat was tested. The trichinoscopic method of detection used by Dr. Perre on that sample did not detect any trichinella, whereas the artificial digestion method conducted by Dr. Soule did detect a single larva. This fact leads the team to suggest that French testing via trichinoscopic methods might miss sources of infection other than horsemeat. In addition, the body of scientific evidence indicates that the trichinoscopic method is much less effective than the artificial digestion method.

The team was concerned that no species test was ever performed on the only sample of suspect product that was tested to confirm that it was indeed horsemeat. In addition, that sample bore a packing date that was after the infection had begun.

Furthermore, there is a higher probability that some of these 1991 cases may be due pork or wild boar. For instance, Dr. Ancelle's paper of January 1989 on an outbreak attributed to wild boar in 1985 also reports a dozen cases in 1988. Since France does not consider trichinosis a reportable disease, there is an inadequate epidemiological history of the illness in that country.

The team noticed that pork fat was contained in the same area of the plant as the horsemeat, suggesting that pork muscle tissue might also be in that area and could be a potential source of the outbreak. However, no pork muscle was retrieved or tested for Trichinella spiralis.

3. Even if the assumption of the French investigative team—that horsemeat is the source of the human infections—is accepted, there is inadequate data to conclude that a particular country, let alone a particular plant, can be implicated as the source of the parasite.

The team found evidence that the deboning and cutting plant could not trace its inventory of horsemeat back to a specific country, and that its shipments could have been of meat from Canada or Argentina, as well as the United States.
The United States instituted an intensive testing system for trichinosis after an outbreak of human trichinosis was reported in France in 1985. Every horse carcass slaughtered for food in the U.S. is tested for trichina. After 1.8 million negative tests, between 1985 and the present, the evidence is that the U.S. testing system and method are thorough and effective.

Conclusions

Given these findings, the team cannot concur with the conclusions made by the FVS that the trichinae outbreak can be conclusively traced to horsemeat or that the meat sample found to have a trichinella larva was of U.S. origin.

However, USDA agrees with the FVS that the source of the trichinosis outbreak should be properly determined. Therefore, it is important that all possible sources of infection be explored and preventative measures undertaken to control all sources of trichinosis infections. FSIS is concerned about the French officials' apparent lack of confidence and has offered additional guarantees of testing quality and oversight, discussed with the FVS and EC representatives at the close-out meeting on April 17.
The Food Animal Hygiene Committee was called to order by Chairman, Dr. Alfred W. Bailey at 1:30 p.m., October 28, 1991. Thirty (30) persons including fourteen (14) Committee members were in attendance.

Dr. Sara Kahn, Veterinary counselor, Embassy of Australia, spoke to the Committee on "Australian Programs for Detection and Control of Residues in Food." Dr. Kahn emphasized the importance of meat exports to the economy of Australia. This issue results in a concerted effort to be sensitive to and comply with requirements of principle importing countries. She said that regulatory authorities throughout the world are re-evaluating their veterinary public health programs toward more scientifically based procedures and more efficient use of inspection staff resources. Greater emphasis is being placed on documentary evidence as to the health and residue status of animals prior to slaughter and on the development of a "quality assurance" approach to the control of residues.

Dr. Kahn discussed specific programs for detection of chemical residues in animal products and some of the results obtained over recent years. Dr. Kahn's paper is being submitted for publication in the USAHA proceedings.

Dr. Joseph C. Paige, Special Assistant, Division of Voluntary Compliance and Hearings Development, Center for Veterinary Medicine, FDA, Rockville, Maryland presented a paper to the Committee entitled "Residue Issues for the 1990's." Dr. Paige's paper will be presented for publication in the USAHA proceedings. Dr. Paige discussed the creation of U.S. Food Safety Policies and the agencies responsible for implementing these laws, particularly the Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS).

Dr. Paige informed the Committee that within the FDA, the Center for Veterinary Medicine (CVM) is responsible for regulating the manufacture and distribution of food additives and new animal drugs which will be administered to animals from which human foods are derived.
REPORT OF THE COMMITTEE

Dr. Paige's paper focused on the potential for drug residues which may result from the administration of food additives and new animal drugs to food producing animals.

Dr. Robert Fetzner, Director, Export Coordination Division International Programs, FSIS, USDA, gave a report on the visit of a USDA team to France in response to an outbreak of human trichinosis. FSIS was notified on March 19, 1991, that a possible outbreak of human trichinosis had occurred in the Auvergne region of south central France, attributed to consumption of horse meat of U.S. origin. USDA sent a team to France for an onsite review. The findings of this team made it impossible to concur with the conclusions of the French officials. It was not demonstrated conclusively to the team that the horse meat consumed was the vehicle transmitting the trichinae; and the French investigators did not completely eliminate all other possible sources of trichinae. However, Dr. Fetzner said that FSIS agrees with the FVS (French Food Quality and Veterinary Service) that the source of the trichinosis outbreak should be properly determined and preventive measures be taken to prevent other human trichinosis infections.

Dr. Fetzner's entire report will be published in the USAHA proceedings.
RESIDUE ISSUES FOR THE 1990'S

Presented by
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Center for Veterinary Medicine
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Food Animal Hygiene Committee
USAHA Meeting
October 30, 1991
San Diego, California

The creation of U.S. food safety policy dates back to the passage of the Federal Food, Drug and Cosmetic Act in 1938, the 1967 Wholesome Meat Act and the 1968 Wholesome Poultry Products Act, which had their basis in the Meat Inspection Act of 1906. Today the responsible agencies for implementation of these laws are the Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS) respectively. The Environmental Protection Agency (EPA), is responsible for safety and efficacy evaluations and for establishing tolerances for pesticides. Additional agencies responsible for food safety include the Agricultural Marketing Service (AMS), and the National Marine Fisheries Service (NMFS).

Within the FDA, the Center for Veterinary (CVM) is responsible for regulating the manufacture and distribution of food additives and new animal drugs which will be administered to animals from which human foods are derived. This paper will focus on the potential for drug residues, which may result from the administration of food additives and new animal drugs to food producing animals.

The results from a number of surveys show that there is an increasing consumer concern for food safety, with particular emphasis on the presence of illegal drug residues in our food supply. To the extent that regulatory agencies by nature of their mission must consider future program direction, contingency planning has become common place, thus the need for change is always readily apparent.

The illegal residue problem, is reason enough for FDA to forecast events that must take place in the future, if we are to continue to ensure a safe and wholesome food supply. If we are to consider changing policy and the use of policy tools to assess consumer initiated concerns regarding drug residues in the nineties, we must also face increasingly difficult decisions over resource constraints which limit our capacity to carry out the Agency's mission. To this extent, I have identified three major areas of assessment that I feel fall within the realm of issues to be addressed. These three areas are:
RESIDUE ISSUES FOR THE 1990'S

1. The use of information systems as tools for identifying policy issues.
2. The role of USDA/APHIS-VS in drug residue follow-up investigations (an Epidemiology/Prevention Role).

These projections and assessments are based on my experiences in the area of residue prevention and the liaison responsibilities that I have with the various regulatory agencies that have a role to play regarding residue reduction in food producing animals. I refer to food safety policy in the broadest sense which encompasses those governmental programs that most directly affect the consumption of food of animal origin.

Within CVM, that program is called the Illegal Residues in Meat and Poultry Program.

Policy making is not an isolated activity within the Center. The policy-making process for tissue residues involves the Division of Compliance, Office of Surveillance and Compliance, General Counsel, and the Director of CVM. Any decision that involves USDA/FSIS implementation is forwarded to the Administrator of FSIS.

Let me now review each of the three areas that I have mentioned above:

1) The use of information systems as tools for identifying policy issues.

The use of information management systems and subsequent data analysis is useful in the regulatory decision making process as it helps to identify unanticipated effects and trends, establish program priorities, and the order in which potential regulatory actions should be followed. The mission of the FDA's CVM is to ensure the safety of animal-derived human food products and the safety and effectiveness of animal drugs. The statutory responsibility of FDA/CVM comes under the jurisdiction of the Food, Drug, and Cosmetic Act. One of the main objectives of FDA's statutory responsibility concerning the presence of pesticides, drugs, and industrial chemicals in animal feed and/or animal tissues is:

(1) to gather information on levels and the incidence of drugs, pesticides and industrial chemical residues in the feed supply in order to identify emerging problems and address issues with appropriate control measures before they result in animal safety concerns;

(2) to evaluate present FDA regulations, and

(3) to disseminate to concerned groups, data about residues avoidance initiatives.

Currently, there is no single source within the various Federal agencies to which an individual can go for a description of the basic principles of toxicity or adverse health effects from various drug residues, production data, or geographical use patterns of specific animal drugs, or any information that would be useful in rendering a rational decision about projecting future safety concerns. Therefore, an information retrieval system based on the appropriate variables will allow us to develop priority setting capabilities.
A computerized hazardous residue information system can be developed to give regulators, practicing veterinarians, and livestock producers information concerning residue problems. An outcome might be that these individuals may be able to avoid hazardous residues in meat, milk, and eggs. Producers need assistance in clearly defining and understanding potential residue problems and acquiring knowledge regarding options and available procedures to assist in management decisions that will reduce residues in the food chain. At present, limited information is available to meet this goal. Searching the available literature is time consuming and the materials fast become outdated.

A computerized hazardous residue information system would make information retrieval easier, faster, and more comprehensive in meeting the increasing need for a comprehensive evaluation of the residue picture.

In recognizing this need, CVM developed a Tissue Residue Information Management System (TRIMS). This initial design and development of the system was to meet the following objectives:

1. To increase the knowledge base regarding illegal residue violations.
2. To determine the causes for tissue residue violations in food-producing animals.
3. To identify possible trends.
4. To determine the extent of follow-up investigations for residue violations with information focused on drug(s) use and animal slaughter classes.
5. To identify the risk factors associated with illegal residue occurrence and to design preventive measures that can be applied effectively and efficiently.
6. To assess the impact of preventive measures when they are implemented.
7. To provide data essential to planning, implementation, and evaluation of policies related to residue prevention and to establish priorities among these policies.
8. To enhance the implementation of the residue reduction program, follow-up investigations, and interagency interaction with USDA/FSIS, APHIS, PSA, and ES.
9. To disseminate the data and program analysis to FDA and state personnel, and other Federal agencies in the respective FDA Regions/Districts involved in tissue residue monitoring.

Efforts must be directed toward the development and management of information pertaining to the occurrence of illegal drugs and residues, scientific information on safety and quality, legislative authority and legal precedents. A major commitment of the Center is to develop data systems to facilitate compliance activities and use that information to suggest possible broad solutions to problems in the ensuing years.
RESIDUE ISSUES FOR THE 1990'S

2) The role of USDA/APHIS-VS in drug residue follow-up investigations.

From 1966 until 1972 the FDA and the U.S. Department of Agriculture (USDA) utilized a Memorandum of Understanding in a Cooperative Federal-State Salmonella program to control the Salmonella level in food. This same kind of agreement could be used to unite FDA and USDA agencies in a common cause for reduction of residue incidents via educational efforts. Two events led to setting the stage for this interagency cooperation. First, in Fiscal Year (FY) 1990, a major milestone was reached in the Brucellosis Program, when the number of herds under quarantine in the United States decreased to less than one thousand. The significance of this is that it made available a core of highly trained veterinarians with on-farm experience and interview skills that could function in other areas which focus on food safety. Second, the FDA, because of its congressionally mandated mission is charged with the investigation of illegal residues in meat, poultry, eggs, and milk even when the establishment of tolerance limits falls under the jurisdiction of other agencies (i.e. EPA). At this time, FDA and the states are able to reach only a portion of the violators through follow-up investigations.

In order to increase the number of follow-up investigations at the farm of origin, a cooperative agreement between APHIS and FDA would be most appropriate. The rationale for this agreement is set forth within the mission statements for both agencies. Food, including meat, eggs, milk and poultry products may become adulterated with residues of drugs, pesticides, or environmental contaminants as a result of drug or chemical misuse. The primary concern is to assure removal of adulterated food from consumer channels and to prevent further marketing of such adulterated food.

FDA's CVM approves drugs used for food producing animals, establishes tolerances for residues of animal drugs in edible tissues, and establishes tolerances (other than for pesticides) and levels of concern for unavoidable environmental contaminants that may adulterate food.

USDA/APHIS is charged with the protection of public health through ensuring the health and care of animals and plants by detecting, monitoring, managing, and excluding from entry into the U.S. exotic agricultural pests and diseases. APHIS provides scientific and technical services, facilitates agricultural exports, protects the welfare of animals, protects endangered species, and collects, analyzes, and disseminates information necessary for disease control programs.

The purpose of an MOU would be to set forth the working relationships between the Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA/APHIS) in order to promote more effective, efficient, follow-up investigations on first time tissue residue violators. The MOU would serve as the model that describes the process by which residue follow-up investigations will be handled when APHIS is involved. This role is to be primarily educational in nature and involves no regulatory activity on
the part of APHIS. FDA would continue to cover enforcement activities (repeat violators and situations which meet our criteria for setting priority for inspections) when necessary.

3) Regulatory Enforcement Strategy: A change in Paradigm

The FDA represents the nation's foremost agency for consumer protection; it is a scientifically based law enforcement agency. The enforcement function of FDA is two-fold: 1) to safeguard the public health, and 2) to ensure honesty and fair play between the regulated industry and the consumer.

Dr. David A. Kessler, the FDA Commissioner, has indicated that a major way to regain the credibility of the agency is to have a strong enforcement minded FDA. To us in CVM charged with the management of the Tissue Residue Reduction Program, stronger action is indicated for repeat violators.

The concerns expressed by consumers regarding residues and pesticides in our food supply will result in major changes in the way in which FDA prepares for a regulatory case. The mechanism by which violators are prosecuted is dependant upon the type of violation, jurisdiction (interstate), and documentation. A major drawback has been the time it takes to refer a case for criminal prosecution and/or injunction through our General Counsel to the Justice Department. Recently there has been a willingness on the part of the Justice Department and local U.S. Attorney's to take residue cases to court especially where a history of multiple violations can be clearly documented.

The current Tissue Residue Reduction Program has not been overly successful in applying legal sanctions against responsible violators. However, FDA's compliance program is being revised with the intent of taking a stronger regulatory posture against producers marketing animals containing drugs and/or chemical residues. Special emphasis is focused on individuals/firms responsible for offering animals for slaughter for food purposes (not for testing) which contain violative concentrations of drugs, pesticides or environmental contaminants of drugs, on more than one occasion, within 12 months after receiving USDA/FSIS violation notification letters.

It is evident that the Center is innovatively trying to take new approaches. Such innovative changes include recommendations to enjoin a person or firm that violates the FD&C Act after the individual or firm has been adequately warned about the violation and given ample time to make corrections. If the firm/individual has not taken steps to prevent the recurrence of illegal tissue residues, the District offices have been asked to recommend regulatory sanctions. In addition, the Office of General Counsel has agreed to suspend the need for another residue to add to the previously documented violative residues. This procedure is especially useful in cases where poor husbandry practices have been documented and the
firm/individual makes little or no attempt to correct violative conditions.

The basic principle of any change in enforcement strategy should focus the agency's limited resources on those drug residues having the highest potential for human health risk. This requires that enforcement actions be taken, dependent upon the degree of human health risk presented by the residue violation. Enforcement actions may also be taken against responsible individual(s) causing residue violations involving drugs presenting a lesser risk to human health.

Citing poor husbandry practices is a new approach to developing residues cases. Individuals will be cited for violations initially and given a warning letter. This constitutes a written communication from FDA notifying an individual or firm that the agency considers one or more products, practices, processes, or other acts, to be violative to the extent that failure of the responsible party to take appropriate and prompt action to correct the violation may be expected to result in enforcement action without further notice. It is hoped that this action will accomplish voluntary compliance and it establishes prior warning, an important ingredient for regulatory action. After the initial inspection, the individual(s) will be monitored to see if corrections are made regarding husbandry practices and if compliance is not achieved, the appropriate regulatory action will be initiated. It is hoped that this change in overall enforcement strategy will bring about a greater enforcement effort regarding residue violators and also reduce the time required for initiating residue cases.
VESICULAR STOMATITIS VIRUS (NEW JERSEY) INFECTION
AND REPLICATION IN BLACK FLIES (*SIMULIUM VITTATUM*)

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INTRODUCTION

The epizootiology of vesicular stomatitis (VS) in both its enzootic form in Central and South America and its occasional epizootic form in North America is poorly understood. While the disease is known to be contagious and to spread readily between susceptible animals in close contact, the mode of transmission of the virus over considerable distances, a characteristic of VS epizootics, is unknown (Mason, 1978). There is considerable field evidence suggesting that the vesicular stomatitis virus New Jersey serotype (VSV-NJ) may be transmitted by arthropods (Heiny, 1945, Hanson and Brandley, 1957, Mason, 1978, Francy et al., 1988). Recently an enzootic strain of VSV-NJ on Ossabaw Island has been shown to be biologically transmitted by a phlebotomine fly, *Lutzomyia shannoni* (Comer et al., 1990). Since these flies are not strong fliers, but remain within a limited area (Ward, 1990), it is likely that they play a role in maintenance of the virus in enzootic areas, but they are probably not the epizootic vectors of VS responsible for the rapid spread of the virus over long distances. Field evidence suggests that an arthropod vector capable of traversing substantial distances, especially along water courses, is important in the epizootiology of VS.

Candidate vectors for transmission of VSV during epizootics include members of the Simuliidae (black flies). This possibility seems reasonable since many simuliid species are pests of livestock, particularly cattle (Cupp, 1986). During the 1982 VS epizootic VSV-NJ was isolated from a pool of black flies, *Simulium vittatum/bivittatum* in Colorado (Francy et al., 1988). In 1990, based on their analysis of weather fronts and wind patterns, Sellers and Marouf postulated that VSV-infected black flies could be carried by winds from VS enzootic areas in Mexico into the USA. These observations, together with the fact that VS often occurs near the riverine habitats favored by black flies, were the stimulus for these experiments which we designed to determine whether black flies, and specifically *Simulium vittatum*, can take up VSV-NJ by feeding, support the replication of the virus, and secrete the virus through their saliva, three essential attributes if these flies are to serve as biological vectors of the virus.
VESICULAR STOMATITIS VIRUS IN BLACK FLIES

MATERIALS AND METHODS

The Virus

The vesicular stomatitis virus New Jersey (VSV–NJ) selected for use throughout these studies was the Camp Verde strain which was isolated from a bovine case of vesicular stomatitis (VS) during the 1982 VS outbreak in Arizona. This virus was the index strain for the North American epizootic of 1982–1986. The virus was propagated in VERO–Maru cells, and was used at the 4th to 6th cell culture passage levels. The virus was obtained from the National Veterinary Services Laboratories, Ames, Iowa.

Experimental Methods

The first experiments were designed to determine whether black flies could be infected with VSV–NJ by feeding, whether the virus was maintained or replicated in the flies, and whether the virus reached the salivary glands and was present in the saliva. In these per os experiments flies were infected and then evaluated for the presence of virus at various times post–feeding, both whole flies and saliva being tested for the presence of virus (see below).

The last two experiments were designed to confirm that virus replication was in fact occurring by demonstrating the presence of an "eclipse phase" in the virus replication process. In these experiments flies were fed VSV–NJ, collected at day 0, and then daily for 5 days, frozen, and then thawed and evaluated for the presence of virus (see below).

Infection of Flies

One–to two–day–old Simulium vittatum adults from a continuous colony (F₇₅ to F₈₂) were used in these studies (Bernardo et al., 1986). Female flies were fed cell–culture fluids containing from 1.2 to 3.3 million plaque–forming units/ml (pfu/ml) of VSV–NJ using a membrane feeding system (Bernardo and Cupp, 1986).

Cages containing approximately 50 flies each were placed against a parafilm membrane which served as the interface between the flies and a heated (37 C) food source. The system was set up in an incubator set at 17 C. When the infected cell culture fluids were warmed to 37 C, a temperature differential was created which induced probing of the membrane and subsequent feeding by the flies. Engorgement rates were approximately 50%.
**Virus Reisolation and Titration**

A baseline sample of nine or ten engorged females were collected immediately after each feeding and the level of virus ingested was determined by titration of the flies on VERO-Maru cells using a previously described plaquing technique (Maré and Graham, 1973).

Whole flies were ground in sterile glass grinders in 0.5ml of cell culture medium containing a standard antibiotic solution of penicillin, streptomycin and amphotericin B, and the mixture was clarified by light centrifugation. Ten-fold serial dilutions of the supernatant fluid was then used as the cell culture inoculum which was adsorbed onto confluent cell culture monolayers and incubated at 37°C/1 hr. After removal of the inocula, the cells were overlaid with Iscoves medium containing 2% fetal calf serum and 0.8% gum tragacanth. Six days later the cells were fixed by adding 20% formalin to the overlay medium, and then stained with 5% crystal violet. Plaques were counted and the virus titers expressed as plaque-forming units/ml (pfu/ml), this reflecting the amount of virus present/ml in the original fly suspension.

Subsequent collections of flies were treated in the same way, except in the case of the "eclipse phase" experiments where all flies were frozen overnight before being tested for the presence of virus.

The presence of virus in saliva was determined by allowing tethered VSV-infected flies to feed from capillary tubes containing cell culture medium RPMI-1640 containing 20% fetal calf serum and penicillin/streptomycin (Muller, 1987). Flies were allowed to feed until replete and then removed from their tethers and placed in antibiotic-containing cell culture fluid and titrated for virus as described above. The contents of the capillary tubes were dissolved in 0.5ml of cell culture fluid and titrated for virus. Since the flies frequently swallowed their saliva during the act of feeding, this portion of the experiment was designed to demonstrate secretion of virus only, and was not considered to be quantitative.

**RESULTS**

**Infection of Black Flies by Feeding**

The flies fed avidly on the virus-containing cell culture fluids as long as the serum content was maintained at a high level (20% was used after flies refused medium containing 2%). Flies fed on fluids containing 1.2 – 3.3 x 10^6 pfu/ml of VSV–NJ consistently contained virus immediately after feeding (95% infection rate, 36/38 flies +ve) with the titers ranging from 1.7 x 10^2 pfu/fly to 2.8 x 10^4 pfu/fly. The median titer of the 36 flies testing positive immediately after feeding was 7.0 x 10^3 pfu/fly.
VESICULAR STOMATITIS VIRUS IN BLACK FLIES

Maintenance of Virus in Infected Flies

In an experiment designed to determine whether VSV taken up by black flies by feeding would be maintained, or possibly increase in titer, the 11 flies tested for virus on day "0" all tested positive, with titers ranging from \(4 \times 10^3\) to \(1.4 \times 10^4\) pfu/fly (Fig 1). The average titer was \(8.7 \times 10^3\) pfu/fly. Ten days after feeding 27 flies were collected; 10 were immediately titrated for virus, and 17 were frozen to be tested later. One of the 10 titrations was lost due to bacterial contamination, but 6/9 of the remaining flies tested positive for VSV with titers ranging from \(2 \times 10^2\) to \(1.1 \times 10^5\) pfu/fly. Ten of the 17 frozen flies later tested positive for virus (but were not titrated). Thus 16/26 (62%) of the flies were positive at day "10" (Fig 1).

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<tr>
<th>Day 0 fly titers</th>
<th>Day 10 fly titers</th>
<th>Day 10 saliva tit.</th>
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<tr>
<td>14,000</td>
<td>110,000</td>
<td>120</td>
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<tr>
<td>13,000</td>
<td>70,000</td>
<td>30</td>
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<tr>
<td>11,000</td>
<td>40,000</td>
<td>220</td>
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<tr>
<td>10,000</td>
<td>2,800</td>
<td>0</td>
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<td>8,000</td>
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<td>5,000</td>
<td>6/9 flies +ve</td>
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<td>4,000</td>
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<td>Frozen flies 10/17</td>
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<td>8,700 pfu/fly ave</td>
<td>16/26 (62%) +ve</td>
<td>3/9 salivas +ve</td>
</tr>
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</table>

Fed VSV at 2 million pfu/ml

Figure 1: VSV–NJ reisolation from black flies 10 days postfeeding
Excretion of VSV in Black Fly Saliva

Three of the nine saliva samples taken from the flies on the 10th day postfeeding were positive for VSV. Titers ranged from $3 \times 10^1$ to $2.2 \times 10^2$ pfu/fly (Fig 1). In an earlier experiment in which very few flies fed due to the low serum content (2%) of the feeding medium, three flies were sampled for virus in their saliva on the 9th day postfeeding, two were positive with titers of $4 \times 10^1$ and $1 \times 10^4$ pfu/fly respectively. The negative saliva came from a fly which also tested negative.

Demonstration of VSV Replication in Black Flies

Even though three of the flies in the previous experiment had titers far higher than the highest titer immediately postfeeding, it was deemed necessary to demonstrate a viral "eclipse phase" in addition to virus increase over input virus as proof of viral replication. In the first experiment designed to do this a large batch of flies were fed VSV with a titer of $3.3 \times 10^6$ pfu/ml. Eight of nine flies titered for virus immediately postfeeding were positive for VSV with titers of $4 \times 10^3$ – $1.8 \times 10^4$ pfu/fly, the mean titer of the positive flies being $1.3 \times 10^4$ pfu/fly. Batches of nine flies were collected at 18, 42, 66, and 90 hours postfeeding, and 10 flies were collected on day "10". Three flies were collected on day "11" and one on day "12".

The percentage of positive flies decreased from 89% (8/9) at zero hours to 22% (2/9) at 18 and 42 hours, and then increased to 71% (5/7) at 66 hours, and 100% (7/7) at 90 hours. On the 10th day, 80% (8/10) of the flies were positive. At both 66 and 90 hours two fly tests were lost due to heavy bacterial contamination. The percentage viral retrieval is depicted in Figure 2.

A second way of assessing viral replication is to compare the highest titer of virus in flies at various times postfeeding. Immediately after feeding the fly with most virus had a titer of $1.8 \times 10^4$ pfu, at 18 hours, $1 \times 10^3$ pfu, at 42 hours, $1.8 \times 10^3$ pfu, at 66 hours, $1.2 \times 10^3$ pfu, and by 90 hours the titer had risen to $7.9 \times 10^3$ pfu. The highest titer on day 10 was $1.1 \times 10^5$ pfu, on day 11, $8.6 \times 10^4$ pfu, and on day 12, $3 \times 10^4$ pfu. These findings are graphically summarized in Figure 3.

Comparison of the mean fly titers at various times postfeeding is another way of demonstrating viral replication. The mean titer of the nine fed flies immediately after feeding was $1.2 \times 10^4$ pfu/fly. By 18 hours the mean titer had dropped to $1.2 \times 10^2$ pfu/fly, at 48 hours it was $2.1 \times 10^2$ pfu/fly, at 66 hours, $3.5 \times 10^2$ pfu/fly, and by 90 hours it was up to $1.4 \times 10^4$ pfu/fly. On the 10th day the mean titer was even higher at $3 \times 10^4$ pfu/ml. The mean fly titers after feeding are summarized in Figure 4.
VESICULAR STOMATITIS VIRUS IN BLACK FLIES

**Figure 2**: Percentage of VSV-Positive Black Flies after Feeding

**Figure 3**: Highest Fly Titers Postfeeding
Figure 4: Mean Postfeeding VSV Titers in Black Flies

In a second experiment designed to demonstrate viral replication with an "eclipse phase" flies were fed medium containing VSV at a titer of $1.2 \times 10^6$ pfu/ml, and collected for virus titration at 0, 24, 48, 72, 96, and 120 hours postfeeding. The highest titered flies (expressed as pfu/fly) were: 0 hrs = $8.5 \times 10^3$, 24 hrs = $1.1 \times 10^3$, 48 hrs = $7 \times 10^3$, 72 hrs = $1.2 \times 10^4$, 96 hrs = $3.9 \times 10^4$, and 120 hrs = $2.3 \times 10^4$ (Figure 5).

Figure 5: Highest Fly Titers Postfeeding (Second Experiment)
VESICULAR STOMATITIS VIRUS IN BLACK FLIES

DISCUSSION

Uptake of VSV by black flies (Simulium vittatum) could be consistently demonstrated (95% success rate), and this established an excellent baseline against which to measure subsequent vector/virus interactions.

These experiments clearly demonstrate that replication of VSV-NJ occurs in Simulium vittatum, and that high titers of virus can persist for up to 12 days after feeding. Figure 6 is a summary of several experiments demonstrating the difference between the amount of virus taken up by black flies and the titer of virus present 9–12 days later, an increase of up to 5x the amount of virus ingested. In one experiment (VSV-4) summarized in Figure 6 flies were inoculated intrathoracically with VSV-NJ.

Figure 6: Comparison of Highest Fly Titers at Day 0 and Days 9–12 in Four Different Studies
Further proof of viral replication was obtained by demonstrating the existence of an "eclipse phase" occurring soon after virus uptake. Three different parameters were used to assess the presence of this phenomenon, namely the percentage of flies infected at various times postfeeding (Figure 2), the highest fly titers at those same times (Figures 3 & 5), and the mean virus titer of all flies collected at those times (Figure 4). Using each parameter a major reduction in virus activity could be demonstrated between about 18 and 48 hours postinfection. This represents the time during which ingested virus is disassembled and new viral RNA and proteins are formed, and precedes the assembly of new infectious virions.

Secretion of virus in saliva was also clearly demonstrated, the highest titer obtained being $1 \times 10^4 \text{ pfu}$, a substantial amount of virus. Since the method for collecting saliva results in dilution and probably considerable swallowing of virus, the results obtained here probably represent a very low estimate of the amount of virus actually present in the saliva. In one feeding experiment 50% (3/6) of flies which harbored virus in their bodies had virus in their saliva, and in the other trial in which poor feeding occurred due to low serum in the feeding medium, 100% (2/2) of the flies with virus in their bodies had positive saliva. In a preliminary experiment in which flies were inoculated intrathoracically with VSV, 80% (8/10) of the salivas collected on day 10 were positive for virus.

We believe that we have convincingly demonstrated that female black flies (Simulium vittatum) readily ingest VSV-NJ, that the virus replicates in a high percentage of flies (62–100%), that the virus persists for at least 12 days, and that more than 50% of infected flies may secrete virus in their saliva.

This evidence is strongly supportive of our hypothesis that black flies play an important role in the epizootic transmission of vesicular stomatitis.

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VESICULAR STOMATITIS VIRUS IN BLACK FLIES

ADVANCES IN VACCINATION FOR FOOT AND MOUTH DISEASE

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In world terms, foot-and-mouth disease (FMD) is the most economically important viral disease of farm animals, not only because of the loss of productivity which follows infection, but also because of the trade embargoes imposed on a country in which the disease is present. The disease is now largely confined to less developed countries, but it would be unwise to consider that the industrialized countries are safe. Rapid and large-scale international movement of people and foodstuffs means that there is an ever-present threat to countries which are currently free from the disease.

The United States has not had an FMD outbreak since 1929, but until vaccines became available which could be applied on a large scale, Western Europe had tens of thousands of outbreaks each year. It was the successful application of the Frenkel tongue epithelium vaccine (Frenkel, 1947), followed by products made in tissue culture cells, which led to the control of the disease on that continent. In fact, there have been no reported FMD outbreaks in Western Europe since 1989.

Foot-and-mouth disease vaccines prepared by imine inactivation of virus harvests from either tongue epithelial fragments or baby hamster kidney tissue culture cells are powerful immunogens. Properly prepared and mixed with an aluminium hydroxide-saponin or oil adjuvant, one parental vaccine inoculation is sufficient to elicit levels of neutralizing antibody which will protect cattle and swine against a challenge dose of 10,000 ID₅₀ FMD virus injected directly into the tongue (for cattle) or foot (for swine), or against natural infection from animals already clinically affected by the disease. These vaccines, which are stored as concentrates in liquid nitrogen, have a shelf-life of at least 18 months and form the reserve bank to be used in an emergency in several countries which do not normally vaccinate against the disease.

If FMD vaccines are so effective, it is fair to ask why the disease has not been eradicated worldwide in the past 40 years. One of the reasons may be the existence of wild-life reservoirs in some countries. More important, however, is the fact that FMD virus occurs as seven distinct serotypes which are constantly varying antigenically, posing problems regarding the selection of the most suitable vaccine to be used in any particular outbreak. There are additional problems in controlling FMD in less developed countries, not least of which are the greater logistical problems associated with more difficult terrain and geographical conditions in general, making it difficult to maintain the cold-chain so that the potency of the vaccine is retained until it is inoculated. Moreover, there is still the suspicion among many farmers that the vaccines are not safe, with the consequence
that they are often purchased but not used. In view of the unequivocal scientific evidence which has emerged from Western Europe during the last decade that most of the outbreaks there have been caused by improperly inactivated vaccines (King et al., 1981; Beck and Strohmaier, 1987), it seems that these suspicions may not be unfounded.

An important question, therefore, is whether we can make absolutely safe and effective FMD vaccines. The answer to the first part of the question is yes. It is the second part of the question which poses the problem - a problem which I believe can be solved through research of the kind we are now engaged in at Plum Island. To describe this approach, it is first necessary to summarize the properties of the virus and the structural features which elicit the protective immune response.

THE VIRUS AND VIRUS-RELATED PARTICLES

Foot-and-mouth disease virus consists of a particle, 300 Å in diameter, made up of a molecule of single-stranded RNA (molecular weight $2.6 \times 10^6$) and 60 copies of each of four proteins (molecular weights about $24 \times 10^3$ for VP1, VP2 and VP3, and about $10 \times 10^3$ for VP4). In addition to infectious particles, in virus-infected cells there are empty particles of a similar size but devoid of RNA. These empty particles contain the same proteins except that VP4 and VP2 are covalently linked. Also present is a particle (the so-called 12S pentameric sub-unit) comprising five copies of each of VP1, VP2 and VP3 and many copies of the viral RNA polymerase (also known as the virus infection associated [VIA] antigen).

Infectious virus particles and empty particles are immunogenic, whereas the 12S particle is weakly immunogenic and the VIA antigen is devoid of activity. Structural relationships between these particles have allowed us to build a model which explains many of the immunogenic properties of the virus.

APPROACHES TO NEW VACCINES

(a) VP1 - The first approach to a new-style vaccine stemmed from two observations. Firstly, it was shown that immunogenicity of the infectious virus particle of FMD virus serotype 0 was considerably reduced by treatment with trypsin (Wild and Brown, 1967). Trypsinisation resulted in cleavage of VP1 but the particle was otherwise unaltered. The second observation was that only VP1 of the four capsid proteins elicited any neutralizing antibody (Laporte et al., 1973). These observations led to experiments at Plum Island to express VP1 in Escherichia coli cells (Kleid et al., 1981). Although the level of expression was excellent, the genetically engineered product had low immunogenicity, probably because the three-dimensional folding of the
protein molecule differed from that which it assumes when it forms part of the virus particle.

(b) Fragments of VP1 – Comparison of the amino acid sequences of several serotypes of FMD virus showed that certain regions of VP1 varied considerably. Reasoning that antigenic variability of the virus would be reflected in amino acid sequence variability in the VP1 protein it was found that peptides corresponding to the highly variable 138–160 amino acid region of the VP1 molecule were immunogenic. One inoculation of 50 ug of such a peptide protected guinea pigs against challenge infection. By presenting the peptide on hepatitis B core particles or as a tetramer or octamer, protection can be obtained with much smaller amounts of peptide (Clarke et al., 1987; Francis et al., 1991) Unfortunately, antibody responses in cattle and swine to the peptide alone are considerably lower than those in guinea pigs. The problem seems to be the lack of a T cell epitope appropriate for cattle and swine on the peptide. However, preliminary work has shown that protection of swine can be achieved by presenting the peptide on the hepatitis B core particle, the latter presumably providing the necessary T cell help.

(c) Empty Particles – A third approach is being made which involves the biosynthesis of the empty particles referred to earlier. Empty particles which have been produced in E. coli cells and in the baculovirus Autographa californica, are immunogenic in both guinea pigs and swine (M.J. Grubman, personal communication).

(d) As Part of a Live Vector – By inserting the gene coding for a sequence of amino acids, including the 138–160 VP1 region referred to above, into the genome of bovine rhinotracheitis virus, a hybrid virus has been obtained which elicits protective levels of neutralizing antibodies in calves (Kit et al., 1991) The success of this approach is clearly of importance but its application in the field will depend on the absence of immunity to the vector.

All of these approaches would provide a vaccine which could not cause FMD, thus allaying suspicion regarding the safety of the product.

THE CHEMICAL BASIS OF ANTIGENIC VARIABILITY

It has been known for many years that the specificity of the immune response of the host to an antigen depends on the shape of the B cell epitope. We showed several years ago that the amino acids at positions 148 and 153 of the 141–160 immunogenic peptide are of particular importance in determining the specificity of the response (Rowlands et al., 1983). With four viruses of serotype A which differed only at those positions in the entire capsid protein region, clear differences could be found in their cross-reactivity in the neutralization test. This observation has been extended by analysis of three more viruses from the same serotype.
ADVANCES IN VACCINATION FOR FOOT AND MOUTH DISEASE

Moreover, analysis of the 141-160 peptides corresponding to these viruses by circular dichroism and nuclear magnetic resonance has provided a structural basis for the serologic observations (Siligardi et al., in press). This approach should provide us with the information to design synthetic vaccines of defined specificity and with the opportunity to synthesize a vaccine which will provide protection against all the serotypes of the virus.

CONCLUSION

Knowledge of the structure of the virus at the molecular level has provided us with the opportunity to identify those regions of the particle which elicit a protective immune response. These regions can be synthesised chemically or biochemically, thus avoiding the use of the infectious virus particle and consequently any chance of causing infection. Moreover, an understanding of the structural basis for antigenic variation is being reached which should enable us to design more efficient vaccines.

Fred Brown joined USDA-ARS at Plum Island in 1990 as a consultant to the Structural Biology Group. He graduated in chemistry at the University of Manchester in 1944 with a 1st Class Honor Degree. Following graduate studies on starch and other complex polysaccharides (PhD 1948), he participated in studies on vitamin E and thyroglobulin metabolism before joining the Animal Virus Research Institute, Pirbright in 1955 to study exotic virus diseases. His major interest has been the study of structure-function relationships with several viruses, including those causing FMD, vesicular stomatitis, vesicular exanthema and rabies. Of particular importance have been the identification of an immunogenic peptide on FMD virus and the solving of the virus' 3-dimensional structure. He was elected a Fellow of the Royal Society in 1981.

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REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

Chairman: Dr. C.J. Maré, Tucson, AZ
Vice Chairman: Dr. W.W. Buisch, Scotia, NY

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Dr. John Maré, Chairman, opened the meeting, welcoming all of those in attendance. He thanked Dr. John L. Hyde for the leadership he provided to the Committee over the past few years, and introduced Committee Vice-Chairman Dr. Bill Buisch. It was noted that the Committee needed to develop a "Statement of Purpose" and that in the future resolutions needed to have substantial impact. otherwise, we as a committee should consider and submit them as recommendations.


Dr. Peter J. Fernandez, USDA/APHIS/IS, discussed the world status of animal diseases. A summary of his remarks is given at the end of this report.

Status of Screwworm Eradication in Central America and North Africa

Dr. John Wyss, USDA/APHIS/IS, reported that Mexico was officially declared free of screwworms in 1991 and that the program is now in Guatemala and El Salvador with plans to enter Honduras in November, Nicaragua in 1992 and hopefully arrive in Panama in 1994. In addition, he noted that the United States–Mexican Commission for the Eradication of Screwworm is now producing up to 48 million sterile flies per week for the Screwworm Eradication Program in Libya.
REPORT OF THE COMMITTEE

Managing Foreign Animal Disease Risk in a Global Trade Environment

Dr. Alex B. Thiermann, USDA/APHIS/IS, noted the importance of the Uruguay Rounds at GATT to assure more open trade while addressing the needs for rules and regulations to conduct trade safety. He stressed the need for standards harmonization based on science, and indicated that the affected industries support them in this endeavour. However, they do not wish to see any increased level of risk to our country due to trade. Animal Health issues cannot be traded but rather need to be resolved.

The Pathogenesis of African Horsesickness

Dr. W. W. Laegreid, USDA, ARS, noted that historically very little is known about the pathogenesis of African Horsesickness (AHS). Their laboratory focused on the peracute pulmonary form of the disease. Basically they addressed the question of whether the different forms of AHS are due to different serotypes. They used a virulent isolate from the current outbreak in Spain and a partially attenuated isolate from Plum Island which had been passaged in mice and cell culture and originated in the Middle East in the 1960's. They concluded that AHS virus infects endothelial cells and that the different clinical signs which are observed with different types of AHS viruses are a function of their ability to infect these cells.

Update on African Horsesickness Vaccines

Dr. James A. House, USDA/APHIS/S&T, noted that the 1987 outbreak of AHS in Spain encouraged Plum Island to evaluate possible vaccines. Currently there are three types of vaccines: Adult Mouse Brain, Cell Culture Modified Live Virus, and Inactivated. He discussed viremia studies using the Tissue Culture Modified Live Virus and noted that with three ponies vaccinated and challenged, none died and all had a slight fever. In another study using Inactivated Vaccine nine of nine vaccinates survived challenge and zero of three controls survived challenge. He also reported that in a two dose trial using inactivated vaccine, there were no significant levels of viremia fifteen days post vaccination with the second dose.

Then Dr. Campos-Lopez of Mexico asked whether the United States would allow horses at the Barcelona Games to return. Dr. Whiting, USDA/APHIS noted that such horses could be quarantined in the United States for sixty days or could spend sixty days in other European countries and then return to the United States under the requirement for horses from the last European country they are in. Regionalization was then discussed and Dr. Whiting noted that the United States cannot legally accept regionalization with two diseases, Foot-and-Mouth Disease and Rinderpest, but that with other diseases there is no such constraint.
FOREIGN ANIMAL DISEASES

Non–immunological Diagnosis of Hemoparasitic Infections

Dr. Gale Wagner, Texas A&M University, noted that Giemsa staining of the parasite was currently necessary for the diagnosis of most Hemoparasitic Infections. In his opinion, there was a need for a better diagnostic tool for the clinical disease (not carrier animal). In serologic tests, antibody activity is not indicative of infection. DNA probes are very good and can detect 1000 Babesia bovis parasites in 20 ul of blood. This, however, is an elaborate test and takes several days to run. The QBC (Fluorescent Probe) is also very good and can detect 10,000 B. bovis parasites in 20 ul of blood. It is a single test which can be used in the field and only takes hours to run.

Update on Bluetongue Virus Epidemiology Research in Central America and the Caribbean

Dr. Johnny Gonzalez, OIRSA, reported that Bluetongue serotypes 1, 3, 4, 6, 8, 12, 14 and 17 have been found in Central America and the Caribbean. They have also found that no clinical bluetongue problems occur in resident animals. In addition, Culicoides insignis is a vector for bluetongue (and possibly other diseases) and there is apparently a stable distribution of bluetongue dependent on the vector.

Vesicular Stomatitis (New Jersey) Infection and Replication in Black Flies (Simulium vitalum)

Dr. John Maré, University of Arizona, gave a brief discussion of the paper he was to present in the general session on Vesicular Stomatitis Infection and Replication in Black Flies. A copy of his paper is in the proceedings. In the discussion which followed his presentation concern was expressed about the lack of funding for preemptive ecological studies of this type, and about our continuing tendency to ignore VS until an epizootic occurs.

Overview of Animal Health Activities in Israel

Dr. Aaron Shimshony, Director of Veterinary Services, Israel, discussed two outbreaks of Foot-and-Mouth Disease, type O, in Israel during March of 1991. Israel vaccinates all cattle, sheep and goats annually. In case of an outbreak, the spread of the disease is prevented by strict quarantine measures.

Bluetongue in exotic breeds of sheep is controlled by annual vaccination with a quadrivalent live–attenuated vaccine including serotypes
REPORT OF THE COMMITTEE

2, 4, 6 and 10 produced by the Onderstepoort Veterinary Institute, South Africa. Only one clinical case was noted in 1990 and three were noted in 1991.

There were two outbreaks of Sheep Pox, an increase in the number of rabies cases in 1989/90 and a decrease in 1991. However, more dogs than foxes were found positive even though vaccination of dogs is compulsory. Although Bovine Tuberculosis was eradicated in 1973, it has been confirmed this year on 5 farms with all animals slaughtered or condemned and destroyed.

Ephemeral Fever was reported in dairy and beef farms in October-November 1990. This disease has not been reported in Israel since 1951.

The beginning of 1991 was characterized by the Gulf War. Measures were taken to protect pets and food animals against chemical and biological warfare.

Classical Swine Fever in Mexico

Dr. Hector Campos, Director General of Animal Health, Mexico, presented a paper on the current situation of the Classical Swine Fever Program in Mexico. The states of Sonora, Baja California, and South Baja California, were declared free of the disease. All of the northern states of Mexico will be in eradication phase by the end of year 1991, as well as the states of southeast Mexico. The structure, strategy and operation of the program was presented as was the epidemiological situation of the disease during the last ten years. The goal of the program is to eradicate Classical Swine Fever from Mexico in a period of four years.

Viral Hemorrhagic Disease Campaign in Mexico

Dr. Armando Mateos, Exotic Animal Diseases Program, Mexico, presented a paper prepared by Dr. Angel Flores, also of Mexico, in which they described the campaign against Viral Hemorrhagic Disease of Rabbits. It is their prediction that this disease, which has had major impact in Mexico will become increasingly important world-wide in the near future.

Australia's Animal Health Status

Dr. Sarah Kahn, Veterinary Counsellor at the Australian Embassy, discussed the current status of animal health in Australia. Australia is free of all OIE Schedule A diseases and most of the Schedule B diseases. By the end of 1991 they expect to declare that they are free of Tuberculosis which was eradicated at an estimated cost of over $700 million. Eight serotypes of bluetongue virus have been isolated from insects and animals in northern Australia, but no clinical disease has been observed. Additional
bluetongue research is being conducted on the ecology of bluetongue in Australia, and on the development of new diagnostic tests, vector competence studies, and the development of vaccines.

A national Animal Health Information system is being developed. This system is to use currently available data and will provide information for use in risk assessment, reporting internationally, exports, and for monitoring the effectiveness of Australia's animal health programs.

CANADA'S 1991 FOREIGN ANIMAL DISEASE REPORT

Dr. W. S. Bulmer, Director of the Animal Health Division, Agriculture Canada, reported on foreign animal disease issues of the fiscal year ending March 31, 1991.

The major disease occurrence of interest was an outbreak of Velogenic Newcastle Disease (NDV) in wild waterfowl in Canada's prairie provinces during the late summer of 1990. Double-breasted cormorants, pelicans and gulls were the main species involved. Monoclonal antibody analysis of isolates from the outbreak, classed the recovered viral agents as NDV Group A viruses.

Nesting sites of affected species, where outbreaks had occurred in 1990, were monitored in 1991. Initial findings indicate that NDV antibody was present in approximately sixty percent (60%) of eggs but was not detectable in the serum of two-month-old juvenile birds. Based on monoclonal antibody binding and pathogenicity index testing NDV isolates from Canadian pigeons were found to be typical pigeon PMV-1 viruses. On the basis of monoclonal antibody binding profiles, an avirulent NDV isolate from a Saskatchewan turkey flock was unrelated to the isolate which caused disease in pigeons on the same premises.

During the 1990–92 fiscal year, fifty (50) samples were forwarded to federal laboratories as exotic animal disease submissions. A large proportion, thirty-six (36) submissions, were of avian origin, being consigned with a possible diagnosis of Newcastle disease or avian influenza. Only two samples were submitted from animals with vesicular lesions.

Serological monitoring of sentinel cattle in the Okanagan Valley of British Columbia for antibody to bluetongue virus (BTV) indicates that the Valley has remained free of the virus for another year, the most recent incursion of BTV into Canada occurring in 1988.

For the first time since the 1988 destruction by fire of Agriculture Canada's Grosse Ile laboratory, the departmental Exotic Animal Disease Course was conducted with a laboratory component. Following a one-week lecture segment in Ottawa, the course participants spent October 22 to 26, 1990, at USDA's Plum Island facility.

Progress in the development of Agriculture Canada's planned virus disease laboratory in Winnipeg, Manitoba, continues on schedule. Currently,
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the project is in the design development stage and the scheduled completion date for occupation of the facility is June 1997.

Emergency Programs Report

Dr. M. Andy Mixson, USDA/APHIS/VS, gave the Emergency Programs Report. A copy of his remarks is provided at the end of this report.

Grey Book Update

Dr. John L. Hyde brought the Committee up to date on the status of the Grey Book Revision. A subcommittee reviewed the current edition and made suggestions. All authors and reviewers are currently being notified with the suggestion that all assigned chapters be returned in final form within four months. Ms. Ella Blanton, Executive Director of the USAHA, discussed the options being considered at this time in discussions with the printer. It is hoped that the new version will be available in the summer of 1992.

Business Meeting

The first item of business was development of the "Statement of Purpose" for the committee as requested by President Pat Smith. A copy of the final draft is submitted with this report. This was followed by discussion of the agencies to which recommendations and resolutions emanating from the FAD Committee are usually submitted. A general statement addressing this issue was prepared and is submitted with this report.

The final item of business was discussion about membership of the Committee. Among the 60 current members there are an unknown number who seldom if ever attend the annual meeting. After discussion on how to prune the committee down to contain only "active" members, it was agreed that in future the attendance log would be evaluated each year, and that those members not heard from in 3 years would be removed from the Committee on the assumption that they are no longer interested in being active members of the Committee.

EMERGENCY PROGRAMS REPORT
FY 1991
DR. M. A. MIXSON

FOREIGN ANIMAL DISEASE (FAD) INVESTIGATIONS

During Fiscal Year (FY) 1991, (October 1, 1990, to September 30, 1991),
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258 investigations of suspect foreign animal diseases (FAD) were completed. These included 102 (39.5 percent) for vesicular conditions, 22 (8.5 percent) for swine septicemic conditions, 7 (3 percent) for mucosal conditions, 54 (21 percent) for exotic Newcastle disease in pet birds and poultry, 4 (1.5 percent) for avian influenza, 34 (13 percent) for encephalitic conditions, and 35 (13.5 percent) for undesignated conditions. The only FAD found was exotic Newcastle disease in pet birds which was quickly eliminated.

There have been no cases of exotic Newcastle disease in commercial poultry in the United States since 1974.

Foreign Animal Diseases (FAD) Training

One Foreign Animal Diseases Diagnostician training course was held during April 29 – May 10, 1991. Twenty-five Federal and State veterinary medical officers attended the course. Two FAD Seminars were conducted in FY 1991, one in Salt Lake City, Utah, during January 15–17, 1991, and another in Tulsa, Oklahoma, during February 5–7, 1991. Two seminars are conducted each year and are presented biannually in each VS Region.

Wildlife Seminar for FADD

The Wildlife Seminar for FADD was held August 27–29, 1991, at the University of Georgia in Athens, Georgia. The annual course is sponsored by the Southeastern Cooperative Wildlife Disease Study in collaboration with Emergency Programs.

The annual Professor’s FAD Seminar was held at FADDL, Plum Island, New York, November 5–9, 1991. This program is presented by Emergency Programs and FADDL for teachers of infectious diseases at colleges of veterinary medicine and diagnosticians from animal and poultry diagnostic laboratories.

Military Support to Emergency Animal Disease Programs Course was held in Hyattsville, Maryland, April 1–4, 1991. Eighteen Army veterinarians completed the course.

Two courses in Foreign Animal Disease awareness were held for Federal and State Animal Health Technicians (AHT’s). One course was held in McAllen, Texas, September 10–12, 1991, and the other in Oklahoma City, Oklahoma, September 24–26, 1991.

A new course, Threats and Implications of Foreign Animal Diseases, was held at the School of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, July 8–12, 1991. Approximately 40 Federal and State veterinarians and managers attended the course.
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Emergency Preparedness

The Regional Emergency Animal Disease Eradication Organization (READEO) in all four Regions were fully staffed and maintained. READEO workshops were held in Columbus, Ohio, May 28–31, 1991, (Northern Region); in Tampa, Florida, April 15–19, 1991 (Southeast Region); and in Arlington, Texas, August 12–16, 1991, (Central Region).

All of the emergency preparedness activities that are required of APHIS under provisions of Departmental Regulation 1800–1 were coordinated. Emergency Programs’ Staff completely renovated and updated the files at three APHIS executive emergency relocation sites which were visited to verify the inventory of emergency administrative resources.

VS and the State of Florida conducted an emergency preparedness exercise in the Southeast Region, from July 22–26, 1991. The exercise involved Emergency Programs Staff and the Southeastern READEO, other units of APHIS, the Florida Department of Agriculture and Consumer Services, the University of Florida, the U.S. Army, the Southeastern Cooperative Wildlife Disease Study, and the Florida and national equine industries. The disease outbreak, NADA, (Spanish for "nothing") was designed to simulate African horse sickness. The equine industry was actively involved in the planning and execution of the exercise. Evaluation of the exercise took place in Hyattsville the week of August 5, 1991.

Exotic Newcastle Disease

On March 29, 1991, a veterinary practitioner in Las Vegas, Nevada, reported a suspected case of exotic Newcastle disease in a young parrot to VS. A FADD investigated the report, confirmed the clinical diagnosis, and sent specimens to the National Veterinary Services Laboratories (NVSL), Ames, Iowa, where the Velogenic Viscerotropic Newcastle Disease (VVND) virus was isolated and characterized. Based on investigative information, the bird was purchased earlier in March from a vendor near the grounds of a swap meet at Spring Valley, California. Based on investigations and surveillance there was no spread to other birds. These isolates were not pathogenic to laboratory chickens.

On April 19, 1991, the California Veterinary Diagnostic Laboratory at San Bernardino, California, isolated a Newcastle disease virus from young yellow–nape Amazon parrots.

Subsequently, NVSL at Ames, Iowa, confirmed the isolate as a VVND virus. The parrot owners, a couple at Tarzans, California, reported the young birds were purchased as a group of 70 from a person in Venice Beach, California. Epidemiological investigations were conducted; however, the vendor could not be located and the exact source of the yellow–nape parrots was never found.
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In May 1991, another outbreak of VWND occurred in caged pet birds located in Indiana, Illinois, Michigan, and Texas. The point source for these outbreaks was determined to be an aviary in Houston, Texas. The birds in Indiana were distributed to an aviary and private residence in Illinois. Ten of the birds became ill shortly after purchase and died and/or were euthanized for diagnostic purposes. Seven birds were submitted to NVSL where a VWND virus was isolated from five of them. On June 12, 1991, the last case of VWND was confirmed positive. A total of four premises were infected and 29 additional premises were exposed. Birds on the four positive premises which were isolated from the infected birds were swabbed three times. All birds on these 29 premises were swabbed twice and remained negative for Newcastle disease. The point source aviary and one infected aviary in Indiana were depopulated at a cost of approximately $60,000. The total cost of eradicating the disease was $160,000.

All outbreaks of VWND in caged pet birds were quickly eliminated and no domestic poultry was affected.

Avian Influenza (AI)

The AI surveillance program in the live-bird markets in the Northeast and Florida was continued in FY 91. Al $H_2N_2$ and $H_8N_2$ viruses were isolated from separate environmental samples collected at a market in Jamaica, New York. Neither isolate was pathogenic to chickens.

In June 1991, New Jersey veterinarians sampled 25 live-bird markets, 2 dealers' premises, and 2 auction markets by collecting environmental and cloacal swabs for culture. An $H_6N_3$ virus was isolated from a cloacal sample from a chicken and from an environmental sample, and $H_2N_2$ viruses were isolated from swabs from 2 guinea fowl and 4 environmental samples.

These isolates were not pathogenic to laboratory chickens.

A sentinel chicken placed in a live-bird market in Miami, Florida, was positive for AI on serology. All avian species on the premises were depopulated and the premises cleaned and disinfected. Sentinel chickens are still being placed in livebird markets in Southern Florida.

On June 10, 1991, the Virginia Veterinary Diagnostic Laboratory, Harrisonburg, Virginia, identified turkey serum samples as positive for AI. Specimens were forwarded to NVSL, Ames, Iowa, where $H_{10}N_2$ antibodies were identified, and an $H_{10}N_7$ virus was isolated on June 17, 1991. The virus isolate was not pathogenic for chickens. Area testing in Rockingham County revealed that no other poultry flocks were infected.

NVSL, Ames, Iowa, reported that serums collected from a flock of turkeys at Worthington, Minnesota, as a part of their surveillance program, were positive for $H_7N_3$ antibodies. After the bird recovered, they were sent to slaughter.
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NVSL reported that H\textsubscript{3}N\textsubscript{3} Al virus was isolated from specimens collected on January 11, 1991, from turkeys at Longmont, Colorado. The isolate was inoculated into susceptible chickens and found to be non-pathogenic. This and other flocks owned by the same company remained asymptomatic and were sent to slaughter.

Tick-Infested Hedgehogs From Africa

On April 20, 1991, a shipment of 25 tick-infested desert hedgehogs from Togo, Africa, was refused entry into the United States at John F. Kennedy Airport, Jamaica, New York. On April 21, 1991, another 100 hedgehogs had entered the United States, but were not detected. They were traced to Miami, Florida. Ticks collected from the hedgehogs in Florida and New York were identified as Amblyomma nuttalli, species, Haemaphysalis multisomi, and Rhipecephalus sanguineous. Fleas were found on the hedgehogs and were identified as Ctenocephalides crataepus, Echidnophaga gallinacea, and Synosternus pallidus. All animals in contact with the hedgehogs, along with the associated premises, were treated with an acaricide. A permit is now required to import hedgehogs from FMD-free countries.

SECRETARY'S ADVISORY COMMITTEE ON FOREIGN ANIMAL AND POULTRY DISEASES

The Secretary of Agriculture's Advisory Committee on Foreign Animal and Poultry Diseases met in Ames, Iowa, August 27-29, 1991. Eighteen resolutions, comments, or recommendations were made by the Committee. The recommendations covered international trade issues, research and diagnostic laboratory facilities, emergency disease preparedness, communications between APHIS and native American tribal officials, frequency of meetings, animal disease threats, risk assessments, and environmental concerns.

WORLD STATUS OF ANIMAL, DISEASE 1990/1991

Foot-and-Mouth Disease (FMD) – Americas:

As in past years, no cases of FMD have been reported from North America, Central America, the Caribbean, or the Guyanas. Chile last reported FMD in August 1987. On October 29, 1990 Chile was declared free of FMD. In South America, during 1990, the Pan American Foot-and-Mouth Disease Center (PANAFTOSA) identified 3 outbreaks of FMD type A and 12 outbreaks of FMD type O from Bolivia and 1 type C and 24 type O outbreaks.
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from Ecuador. Ecuador also reported 3 type A FMD outbreaks and 3 type O FMD outbreaks for the end of 1990. In 1991, the International Office of Epizootics (OIE) received reports of 5 type 0 Ecuadoran outbreaks from PANAFITOSA, for January and February. Brazil reported the presence of FMD types 0, A, and C for the last two months of 1990. From March 1990 to March 1991, Brazil has also informed the OIE of 851 untyped FMD outbreaks. All the Brazilian cases reported, affected only bovids. Compared to the two previous years the reported outbreaks represent a 26.8% and a 29.8% reduction, respectively. During the last three quarters of 1990 the OIE reported that Colombia experienced 118 FMD outbreaks due to type A virus and 23 outbreaks due to type 0 virus. For January through May 1991, Colombia has reported 58 type A outbreaks and 47 type 0 outbreaks. In 1990, Argentina reported a total of 774 untyped outbreaks, 156 type 0 outbreaks, 88 type A outbreaks and 4 type C outbreaks. Untyped outbreaks for the first quarter of 1991 were 58. One type 0 and three type A FMD outbreaks were reported by Argentina for January and February of 1991. Three FMD type 0 outbreak reports were made by Venezuela in 1990. The ten Venezuelan type A outbreaks reported for 1990 affected a total 9103 cattle. As of mid-1991, the OIE has received no reports of FMD from Venezuela. Uruguay reported 10 type 0 outbreaks, 11 type A outbreaks and 11 outbreaks untyped from April through June of 1990. Both Paraguay and Uruguay reported one outbreak of untyped FMD for September. Paraguay later reported one untyped FMD bovine outbreak occurring in November with Uruguay reporting one type C outbreak for February 1991. During January and February 1991, Paraguay identified 2 outbreaks of type O FMD.

Foot-and-Mouth Disease – Europe:

No foot-and-mouth disease outbreaks were reported from Europe in 1990. On August 1, 1991, Bulgaria reported an outbreak of FMD the source of which is still unknown. Preliminary investigations revealed that the strain isolated was 0/3/BUL/1/91. All European Economic Community countries have banned imports of animals, meat, meat products and feedstuffs from Bulgaria.

Foot-and-Mouth disease – Eurasia:

Turkey continued to report type O and type A FMD throughout 1990, and from January to April 1991, over half-million animals have been affected. In the USSR, type A FMD was identified in 1990 outbreaks in the region of Tyumen and in the Uzbek S.S.R. The USSR submitted data for a July 1990 FMD type 0 outbreak involving 270 swine; all affected pigs were destroyed or slaughtered. The USSR also reported a bovine FMD type 0 outbreak which resulted in the destruction of all 161 dairy cows involved. All
susceptible animals at risk in the control zone were vaccinated and a ban was placed on movement of live animals and animal products.

Foot-and-Mouth disease – Asia:

In the Middle East, Israel's Ministry of Agriculture reported two outbreaks of FMD for April 1990. The first outbreak occurred in the Golan district and the other in the Kineret district. Both outbreaks were FMD type 0–1. Control measures implemented included: quarantine, restriction of animal movement, and vaccination. Jordan submitted reports indicating untyped FMD activity for the first half of 1990. Bahrain reported one untyped FMD occurrence for May, 1990, and type 0 during March 1991. During January and February of 1991, Syria reported five untyped outbreaks of FMD. Other Middle East countries reporting FMD were Iran, Oman, Yemen, and Saudi Arabia. Bhutan and Nepal outbreaks were identified as Type 0 FMD and Asia 1 was also isolated from an outbreak in Nepal during June 1990. FMD in a quarantine station in Malaysia proved to be virus type Asia 1. Type O FMD was reported from Sri Lanka, Myanmar reported outbreaks of types O, A, and Asia 1 and Pakistan reported FMD types O, A, C, and Asia 1. Outbreaks in Thailand were caused by types O, A, and Asia 1. Hong Kong's FMD outbreaks in 1990 were attributed to virus type O as was an outbreak in The People's Republic of China. Kampuchea reported one Asia 1 FMD outbreak, and type C FMD was identified as the causal agent of an outbreak in the Philippines. According to the OIE, FMD virus types 0, A, and Asia 1 are enzootic in India, Myanmar, Nepal, Pakistan and Thailand and probably in Bangladesh and Bhutan.

Foot-and-Mouth disease – Africa:

At the end of 1989, the type O FMD epizootic in Libya and Tunisia spread to Tunisia, Algeria and Morocco. FMD type O virus was last isolated in Morocco in 1965, but ten outbreaks of FMD type O occurred in 1991. Type O FMD was also the cause of numerous outbreaks of FMD in Egypt. FMD remains enzootic in much of Africa, countries reporting FMD being Ethiopia, Sudan, Chad, Burkina Faso, Senegal, Ghana, Togo, Ivory Coast, Nigeria, Niger, Mali, Benin, Djibouti, Kenya, Uganda, Burundi, and Zambia.

Vesicular Stomatitis (VS):

Mexico reported 13 outbreaks of Vesicular Stomatitis New Jersey (VS–NJ) in 1990 and 1991 all from the states of Chiapas and Veracruz. VS–NJ outbreaks were also reported from Costa Rica, El Salvador, Belize, Guatemala, Honduras, Nicaragua, and Panama. El Salvador also reported one Indiana serotype (VS–IN) isolation, and the Indiana serotype of VS

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predominated in Panama. Colombia reported bovine cases of VS-IND and VS-NJ, while Venezuela reported only VS-NJ outbreaks.

Swine vesicular Disease (SVD):

No SVD was reported to the OIE in 1990. Swine vesicular disease was diagnosed in Italy during January, February and April 1991.

Rinderpest (RP):

Rinderpest (RP) remains a major problem on the Indian Subcontinent. In 1990–1991 Sri Lanka reported 55 outbreaks of RP, and India 125. The OIE received reports of 125 outbreaks of RP from India. Kenya has reported RP outbreaks in 1990 and 1991, mainly in the Rift Valley, Ethiopia has suffered at least five major outbreaks, and RP was reported from Uganda in 1990. As in 1989, no RP outbreaks were reported from west Africa. The USSR reported seven cases of RP in the republic of Georgia in January of 1990. The outbreak was quickly controlled as a result of stamping-out methods and vaccination of susceptible animals. An outbreak of RP occurred in Oman in January, 1991.

Peste des Petit Ruminants (PPR):

Peste des Petit Ruminants (PPR) remains a serious problem in sheep and goats especially in West Africa. Countries reporting the disease include Central African Republic, Ivory Coast, Mali, Senegal, Nigeria, Togo, Ghana, Mauritania, Guinea, Ethiopia, and Oman. The presence of PPR in Ethiopia and Guinea represents the first reported occurrence of the disease in those countries.

Contagious Bovine Pleuropneumonia (CBPP):

In the enzootic areas of West, Central and East Africa CBPP remains a serious problem. outbreaks continued to occur in Nigeria, Benin, Burkino Faso, Ivory Coast, Ethiopia, Kenya, Uganda, Tanzania, Kuwait, and Myanmar. The reappearance of CBPP in Europe first in Portugal, and later Spain and Italy is of concern, especially to members of The European Economic Community. The 1990 outbreak of CBPP in Italy was the first in more than 90 years.

Lumpy Skin Disease (LSD):

Lumpy Skin Disease (LSD) remains widespread especially in East, West, and Southern Africa. The contiguous nations of South Africa,
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Botswana, Zimbabwe, Mozambique, Swaziland, and Zambia all reported LSD activity. The disease was also present in Madagascar, Kenya, Uganda, Ethiopia, Mauritania, Ivory Coast, Nigeria, Chad, and Egypt.

Rift Valley Fever (RVF):

Rift Valley Fever outbreaks were limited to the Southern African nations of Zimbabwe, Malawi, Mozambique, Zambia, and South Africa.

Bluetongue (BT):

The presence of BT continued to be documented from South Africa and the United States throughout 1990 and the first half of 1991. The disease was also reported from Zimbabwe, The Sudan, and Israel. Serious underreporting of BT is suspected.

Sheep and Goat Pox (SGP):

Turkey continued to report the most cases of SGP to the OIE. The disease was also causing concern in the North African countries of Algeria, Tunisia, and Morocco. In the Near and Middle East it occurred in Israel, Jordan, Kuwait, and Oman, in West Africa in Senegal and the Ivory Coast. SGP was also reported in The Sudan, in Pakistan, Myanmar, and Sri Lanka.

African Horse Sickness (AHS):

The four contiguous Southern African nations of Botswana, South Africa, Namibia, and Zimbabwe reported outbreaks of AHS. Spain and Morocco reported AHS outbreaks in September of 1990. The AHS outbreaks identified in Spain were restricted to the province of Malaga. The virus serotype implicated in these outbreaks was the same as that isolated in previous years; serotype 4. A mid November report from Morocco cited 51 outbreaks all above the 34th parallel. Control measures for both countries included vaccination and restriction of animal movements. Other countries reporting AHS activity were Senegal, Nigeria, Sudan, and Ethiopia.

African Swine Fever (ASF):

Only five African countries reported ASF activity in 1990–1991, namely Malawi, Uganda, Senegal, Zaire, and Mozambique. In both these years the three European countries with ASF were Portugal, Spain, and Italy. The disease remained active into 1991, with Italian reports of ASF continuing from Sardinia through the first trimester of 1991. All 177 Portuguese cases
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either died or were destroyed. In 1991 Spain reported a total of 51 outbreaks of ASF from its southern regions with 8,846 pigs affected. Of the animals involved, 151 died due to the disease and the remaining 8,695 were destroyed.

Hog Cholera (HC):

According to reports to the OIE Europe continued to log cases of HC through 1990 and into 1991. The disease was reported from The Netherlands, Belgium, Austria, France, Germany, Italy, Hungary, Czechoslovakia, and Yugoslavia. The USSR reported 2 outbreaks from Lithuania and Moldavia involving a total of 6,880 swine. In Africa, Madagascar reported an undetermined number of cases and HC was reported from Guinea-Bissau. In the Americas HC was reported from Mexico, Guatemala, Honduras, El Salvador, Nicaragua, Colombia, Venezuela, Ecuador, Peru, Bolivia, Paraguay, Uruguay, and Brazil. The disease was also widespread in Asia and occurred in India, Sri Lanka, Nepal, Bhutan, China, Hong Kong, Taiwan, South Korea, Vietnam, Laos, Kampuchea, Thailand, Malaysia, and the Philippines. Oceania remains free of HC.

Teschen Disease (TD):

Madagascar and the USSR were the only countries to report TD in 1990/1991. At the 59th General Session of the OIE, it was agree that TD would be removed from the List A disease category as of January 1992.

Fowl Plague (FP):

Myanmar reported outbreaks of FP in 1990, and the disease was reported in 1991 by Senegal, Nigeria and Pakistan.

Newcastle Disease (ND):

African nations reporting ND included: Botswana, Cote-D'Ivoire, Egypt, Kenya, Madagascar, Namibia, Algeria, Tunisia, Sudan, Ethiopia, Kenya, Uganda, Malawi, Mozambique, Mali, Senegal, Nigeria, Ghana, Gabon, Burkina Faso, Zambia, and South Africa. In Europe, Albania, Austria, Yugoslavia, Portugal, and Italy all reported ND. Haitian outbreaks of ND in February and March affected 89,000 birds with 67,000 deaths. ND outbreaks were also reported from Brazil, Mexico, and Colombia. Asian outbreaks of ND were reported from Iran, Kuwait, Myanmar, Hong Kong, Turkey, Pakistan, Bahrain, and the Philippines. Japan reported 7 outbreaks and the disease was also reported from Hong Kong, Indonesia, Myanmar, Sri Lanka, Pakistan, and South Korea.
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Velogenic Viscerotropic Newcastle Disease (VVND):

In the second quarter of 1990, Canada reported an outbreak of velogenic viscerotropic Newcastle disease (VVND) from northern Saskatchewan Province producing a "die-off in wild cormorants, some white Pelicans and gulls". The last recorded outbreak of Newcastle disease from Canada was in 1973. Ten outbreaks of VWND in South Korea affected a total of 74,880 birds and caused 58,930 deaths. Outbreaks of VWND were also reported from Myanmar, Taiwan, Indonesia, Sierra Leone, Botswana, and Mauritius.

Viral Hemorrhagic Disease (VHD):

Sweden recorded its first outbreak of VHD (also known as Necrotic Hepatitis of Rabbits) in June 1990. Malta reported its first 11 outbreaks in March. During 1990, Belgium, Denmark and Luxembourg also reported VHD for first time. In Mexico, a total of 8 active outbreaks had been recorded until late 1990. The outbreaks occurred in the states of Hidalgo and Mexico and the Federal district. The last cases recorded in Mexico were on October 10, 1990. An outbreak of VHD of rabbits was reported to the OIE in mid-April 1991 by Israeli veterinary officials. The outbreak occurred in the Nes Ziyyona Biological Institute in an imported consignment of laboratory rabbits and spread to in-contact animals. All 28 animals in the cage were destroyed.

Contagious Equine Metritis (CEM):

The United Kingdom's (UK) Ministry of Agriculture, Fisheries and Food (MAFF) reported in October 1990 the isolation of a streptomycin-sensitive strain of Taylorella equigenitalis, the etiologic agent of CEM, from a non-thoroughbred horse in Dumfries and Galloway Region of Scotland. The last isolation of CEM in the UK was in January of 1986.

Bovine Spongiform Encephalopathy (BSE):

Enzootically affected areas in the UK, Great Britain (12,828 cases), Northern Ireland (100 cases) and sporadically in Ireland (14 cases) continued to report disease in 1990. The OIE also received a report of a case that occurred in the Falkland Islands in 1989. The Federal Veterinary Office in Berne, Switzerland notified the OIE of an histologically confirmed case of BSE. On February 28, 1991, BSE was confirmed in a cow that had died January 16, 1991, at Plouha, France, after showing signs of the disease. The herd of 68 dairy cows from which the affected cow came was purchased by the French National Center for Veterinary Pathology Studies for research
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to be carried out at Lyon. Since this outbreak additional cases of BSE have been isolated in France and Switzerland. The UK, Ireland, Oman, Switzerland, and France are the only countries known to have had cases of BSE.

Screwworm (SW)

The last reported case of SW in Mexico occurred on July 10, 1990. On February 25, 1991, the US – Mexico Joint Commission for the Eradication of Screwworms, formally declared the successful eradication of SW from Mexico. It is expected that Guatemala will soon begin dispersal of sterile flies over the last quarter of its land area. The present Screwworm program continues to operate in Belize and Guatemala and negotiations for new cooperative agreements to begin sterile fly dispersal in El Salvador and Honduras are underway. The Food and Agriculture organization reported that in 1990 a total of 12,068 cases of new world SW were identified in Libya. Six cases of SW have been reported from N. Africa in 1991, and no new cases have been reported since April. If no more positive larval cases appear, the eradication phase will terminate in October 1991. A total of 880 million sterile insects have been released thus far in North Africa. Animal inspections in neighboring Egypt, Tunisia and Chad have not produced any cases.

Porcine Reproductive and Respiratory Syndrome (PRRS):

In February 1991 the European Economic Community (EEC) placed a two-month quarantine on farms affected by PRRS (mystery swine disease, mystery pig disease, mystery reproductive disease). The disease was first described in Germany in December 1990 and involved some 1,000 farms by the end of February 1991. Holland also reported PRRS 1991. A prospective causative agent of the disease has been isolated by Dutch scientists. The clinical signs are similar to the MSD described in the US in 1987. The name "abortus blauw" used in the Netherlands is due to abortion of fetuses and cyanotic coloring of ears which characterize the cases observed. PRRS was reported from the United Kingdom (UK) in June, and a recent USSR outbreak involved 3300 swine.
Anaplasmosis is an acute or chronic, infectious, non-contagious rickettsial disease of ruminants, particularly of economic significance in cattle. Calves less than a year of age are somewhat resistant, and in enzootic areas, often develop immunity after initial exposure. Older, susceptible cattle imported into enzootic areas, will usually develop the acute disease upon exposure. Clinical disease follows an incubation period of 20–40 days, and the clinical signs are associated with the progressive anemia which may be fatal. Packed cell volumes may drop as low as 14% before cattle are noticed as being sick. The acute disease usually lasts from 4 days to 2 weeks, resulting in either death or recovery. With recovery, the parasitemia rapidly decreases and the PCV slowly returns to normal. Acute infections can be controlled in individual animals with the use of tetracycline. However, while reducing the impact on mortality, tetracycline does not eliminate all infections. Therefore, whether acute infections are ameliorated naturally or by drug therapy, a key feature of this disease is the lack of sterile immunity resulting from infection. While cattle surviving an initial infection are, for the most part, immune to subsequent homologous exposure, they remain persistently infected with low level parasitemias.

A definitive diagnosis can be made by visual detection of the parasites within blood. However, parasitemias are too low in subclinical or chronic infections for diagnosis by this method. For this reason, several serologic assays have been developed including agglutination, complement-fixation (CF), and indirect immunofluorescence (IF).

The use of serology to detect cattle which have been infected and chemotherapy to remove infection have provided some control of the disease. For instance, the CF-test has helped reduce the spread into non-enzootic areas of the U.S., despite extensive movement of breeding stock to different geographic locations, and tetracycline has significantly decreased the economic severity of the disease in terms of mortality.

In the U.S., anaplasmosis is considered a regional problem. While the extent of anaplasmosis in the U.S. is not fully defined, there are two large enzootic regions. In the southeast, the primary vectors are biting flies; mechanical vectors, while in the intermountain west and west coast, ticks are the primary vectors and the association with the parasite is biological.
indicates, they are very efficient vectors, primarily through intrastadial or interhost transfer of male ticks which acquire Anaplasma from one host, become dislodged, and acquire another host\textsuperscript{4}. Due to this enzootic distribution, anaplasmosis control has been shaped by regional considerations, rather than a uniform state-to-state approach. For the most part, the northern tier and northeast states are considered non-enzootic and thus are most concerned about importing carrier cattle. In contrast, states in enzootic regions are most concerned about importing susceptible cattle.

Over the past several years, a degree of complacency has replaced the previous sense of urgency about the spread of anaplasmosis, as has been evidenced in the last three national anaplasmosis conferences, covering the last 15 years. For example, there has been little or no discussions about how the prevalence may have changed. In turn, there have been no evaluations during this time of the successes or failures of those regulations that do exist. This situation is the result of three primary factors: 1) a belief that the CF-test was capable of detecting most all carriers; 2) reports of successful elimination of carrier infections with the appropriate use of tetracycline; and 3) a realization that the incidence of new cases within the enzootic regions had decreased. It is now known that the CF-test is not reliable in detecting carrier cattle\textsuperscript{7,14}. One reason for the lack of sensitivity is due to the fact that bovine Ig\textsubscript{G} does not fix guinea pig complement\textsuperscript{15}. Thus, when the specific Ig\textsubscript{G}, titer decreases below a certain point, the test fails to detect what may still be a carrier animal.

Since the first report of the clearance of carriers with a long–acting oxytetracycline formulation\textsuperscript{21}, it has been shown that if cattle are re-exposed during drug administration, then the treatment is ineffective\textsuperscript{12}. It has also been shown that even without re-exposure, not all animals can be cleared\textsuperscript{7}. Finally, incidence rates alone in the absence of reliable prevalence data do not provide adequate information on the epidemiology of the disease, and should not be considered sufficient evidence to conclude that the disease is under control. There is a serious lack of reliable prevalence and incidence data for the U.S., and what little prevalence data exists has been obtained with the CF-test. Using a test with low sensitivity usually results in an underestimate of the true prevalence.

USDA–ARS scientists have been and are continuing to collaborate with Washington State University investigators in an active anaplasmosis research program. The major objectives of this research are to develop an improved vaccine based on a recombinant or synthetic subunit and to develop improved diagnostic capabilities. New diagnostic procedures will provide the epidemiologic information to define better control strategies and to assess vaccine effectiveness in reducing first incidence, and perhaps ultimately prevalence of infection. One aim is to develop diagnostic tests that can discriminate between active carrier infections and vaccinates. Two approaches were chosen: 1) a method that can detect the actual presence
of the organism in carrier cattle, and 2) a method that can detect serum antibodies with both improved specificity and sensitivity from carrier cattle. The two methods chosen were nucleic acid hybridization and an ELISA or similar test for antibody using a defined antigen. Nucleic acid probes have been developed and there is considerable progress toward a new serologic test with a defined antigen.

NUCLEIC ACID PROBES—The ability to identify, remove and relocate (recombinant DNA) genes and the controlled expression of their products has led to many medical and agricultural applications. Nucleotide sequences within the genomic DNA can be selected based on criteria required by the research problem. Whole gene sequences, partial gene sequences, or sequences without readily apparent functions, but with other useful attributes such as highly repeated sequences can be used for studying the basic biology of the organism and for several more utilitarian applications. One practical use is the development of nucleic acid probes and their application in diagnosing infectious diseases. This technique is becoming an attractive alternative or supplement to conventional serologic assays, especially in cases where sensitivity and/or specificity is a problem. Thus the ability to detect the actual presence of the parasite becomes an attractive adjunct to the use of even a sensitive serologic assay. Nucleic acid (DNA or RNA) probes are rapidly being developed to identify many of the most important vector-borne parasites of domestic animals, both in the vertebrate and invertebrate host.

The development and use of nucleic acid probes are based on the premise that each species possesses unique DNA sequences that differentiate it from even closely related organisms. It is this uniqueness that gives probes a very high level of specificity. Probe sensitivity, on the other hand, usually depends on the number of copies of the probe's sequence that occur in the genome of the target organism. Generally, the higher the copy number of the sequence, the more sensitive the probe.

We recently evaluated a DNA probe to detect Anaplasma marginale in bovine blood and infected ticks. The 32P-labeled probe, consisting of a 2 kilobase DNA fragment within the gene coding for an A. marginale isolate—common, major surface peptide (MSP-1), is species specific and can detect parasitemia levels as low as 0.000025% (about 4,000-fold more sensitive than stained blood smears) in carrier cattle. It also can detect the parasite (as few as 1000 organisms) in as little as 0.4% of a whole midgut from an infected tick. Based on the same gene fragment, an RNA transcript was also used as an RNA probe, and retained the same specificity and sensitivity. The advantage of using RNA as a probe is that RNA/DNA hybrids tend to be more stable. This probe was used to detect and quantitate the low, microscopically undetectable levels of parasitemia in chronically infected carrier cattle. Results of these studies showed that parasitemia
levels varied greatly among carriers and for individual animals tested on different dates, indicating that individuals within a group of cattle may differ significantly in their ability to transmit the parasite by way of arthropod vectors. We have compared the DNA probe with the CF test and an IIF assay, for diagnosing anaplasmosis in suspected carrier cattle from an enzootic area. The probe and IIF were in 90% agreement and identified 98% and 92% respectively, of the herd as infected. By contrast, the CF test identified only 9% of the positive animals. The DNA probe and IIF test thus appear to be much more sensitive than the CF test in detecting carrier infections. This DNA probe has also been used in several studies involving the nature of the developmental cycle of the parasite within the tick vector, and factors affecting tick vector competency.

A cloned DNA probe for *A. ovis*, which consists of a 9.6 kilobase fragment of *A. ovis* genomic DNA, has also been constructed. This probe detected *A. ovis* parasitemia levels of 0.0035%, while showing a limited degree of hybridization with partially homologous sequences present in *A. marginale*. The probe was also used to conduct an *A. ovis* prevalence study in goats from Kenya, and revealed a prevalence ranging from 22% to 87% depending on the location. The probe was also used to demonstrate the presence of *A. ovis* in the midgut and salivary glands of tick vectors. Finally, both the *A. marginale* and *A. ovis* probes were very recently employed in studies concerning the role that wild ruminants play in the epidemiology of anaplasmosis in the U.S.

**DEFINED ANTIGEN SEROLOGY**—The enzyme-linked immunosorbent assay (ELISA) has found wide application in infectious disease diagnosis. This antibody detection method is superior in sensitivity compared to most other serologic assays. Other advantages are that ELISAs are quantitative, non-subjective, automated, and have the potential for increased specificity depending on the efforts made in antigen preparation and characterization. Recently, ELISAs have been reported for the diagnosis of bovine anaplasmosis. While these reports suggest an improvement in sensitivity, problems remain with specificity due to the fact that antigen preparations used were either crude or at best, only partially purified. The attributes of MoAbs provides the means to identify highly immunogenic, species-specific, and either strain-common or strain-specific antigens. The MoAbs can then be used to affinity purify the antigen of interest from all other parasite components, making the native antigenic molecule available for testing its usefulness in an ELISA format.

A library of *A. marginale* specific MoAbs has identified several proteins. One of the MoAbs recognizes a protein that has the characteristics desired of a defined, subunit diagnostic antigen. The protein has a molecular weight of 86 kilodaltons, is common to all *A. marginale* isolates thus far tested, and the antigen induces high antibody titers from
cattle during infection with *A. marginale*. The protein is also another major component located on the surface of the *A. marginale* initial body and thus it has been named MSP-3. The MSP-3 has been affinity purified using the MoAb, and in western immunoblots carrier cattle, having recovered from an initial *A. marginale* infection from 2 months to 5 years without apparent reexposure, have antibodies recognizing MSP-3 with titers exceeding 1/1000, thus confirming the conclusion that MSP-3 is an immunodominant protein. Current studies include the identification and cloning of the gene expressing the peptide containing this immunodominant epitope so that a recombinant or synthetic peptide can be constructed, and comparing the native protein (and later the synthetic peptide) with the DNA probe under polymerase chain reaction amplification conditions, for their ability to identify all *A. marginale* carrier cattle.

CONCLUSIONS: The time may be rapidly approaching when simple-to-use kits containing bioengineered diagnostic tools can be used in laboratories and in the field to determine quickly and reliably the identity and prevalence of *Anaplasma* and other hemoparasites in livestock, wild ruminants and ticks. The use of a simple, quick, sensitive and specific defined antigen ELISA for determining accurate prevalence data would be of enormous benefit to the livestock industry and veterinary practitioners and regulatory officials. There is little doubt that DNA probes will prove invaluable in elucidating heretofore inaccessible epidemiological aspects of anaplasmosis. For example, the ability of such probes to detect and quantify *Anaplasma* in the blood of chronically infected carrier animals and in individual vector ticks should readily permit, in many cases for the first time, precise estimates of tick infection rates and parasite prevalence in the invertebrate host, and correlation of tick infection rates with different parasitemia levels in acutely and chronically infected host animals. Such information is essential to construct more accurate epidemiological models of the disease and thus in formulating more effective control strategies.

When the diagnostic techniques are fully developed, they should be used to reassess the epidemiology of anaplasmosis in the U.S., including determining infections in livestock, wildlife and tick vectors. This step should be followed by continued surveillance to provide accurate and reliable information on disease status and on changes due to natural conditions and the intervention of control measures, such as vaccines. Finally, strategies for tick and disease control should be modified based on the results obtained from continued monitoring.

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REPORT OF THE COMMITTEE ON HEMOPARASITIC DISEASES

Chairman: Dr. Rube Harrington, Jr., Arlington, TX
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; G.M. Buening, MO; D.B. Childs, FL; A.A. Cuthbertson, NV; W.C. Davis, WA; C.A. Gipson, FL; T.J. Holt, NY; J.D. Huber, TX; O.James, MT; D. Kimbrell, AR; M. Lee, Jr., LA; W.G. Nelson, ID; D.L. Notter, KY; J.O. Pearce, Jr., FL; M. Ristic, IL; G.P. Shibley, MD; C.E. Starkey, AR; J.E. Stickland, GA; N.R. Swanson, WY; G.G. Wagner, TX; J.M. Williams, CO.

The Hemoparasitic Diseases Committee met on Thursday, October 31, 1991 at 1:30 p.m. in the chamber room. There were 13 members and 8 guests present.

Dr. Gale Wagner of Texas A & M University reported on the development and use of non-immunologic methods of diagnosis of bovine babesiosis. DNA probes, specific for Babesia bovis DNA, were found to be able to detect as few as 1,000 parasites in a 10 ml. blood sample. Efforts are underway to apply the probe to field samples using a non-radioactive label. A fluorescent probe, acridine orange, has been used in the commercially available system. Using this method, as few as 10,000 infected red cells per 10 ml. sample could be detected. The method is simple, rapid and has been shown in limited field studies to detect infected cattle 2–3 days earlier than by conventional light microscopy.

Dr. Wagner also reported on a vaccine trial underway with 30 Sammental heifers. The heifers were vaccinated with 106 cell culture attenuated B. bovis. All vaccinated animals reacted, some with anemia. All recovered. These cattle have been shipped to Mexico, where they will be challenged by B. bovis infected ticks. Results will be reported.

Dr. Gerald Buening reported on research being conducted at the University of Missouri related to improvement of diagnostic tests. He stated that a PCR/non-radioactive probe assay was developed. The assay was highly sensitive and detects parasitemias of B. bigemina infected erythrocytes. Preliminary results indicate the assay has application in identifying carrier animals in infected herds. The advantages of this assay were: (a) procedure highly sensitive and (b) radioactive materials were not used. Dr. Buening also reported that a PCR probe assay was developed for the detection of Anaplasma marginale infected erythrocytes. The assay was evaluated on a total of 480 whole blood samples from six infected herds. Preliminary results indicate the assay was highly sensitive and has application in identifying carrier animals in infected herds.

Dr. Miodrag Ristic presented a paper on a series of laboratory and field experiments with cryopreserved modified-live A. marginale conducted
in the United States, Peru, Venezuela, Columbia and Mexico. This paper will be published in the proceedings.

Dr. Goss, ARS Hemoparasite Laboratory in Pullman Wa., reported on recent progress concerning anaplasmosis and babesiosis. The most notable progress during the past year being: (1) the demonstration that both *Boophilus microplus* and *Dermacentor variabilis* ticks are suitable vectors of *Babesia equi*; (2) the development of a sensitive monoclonal antibody-based competitive ELISA for diagnosis of *Babesia equi* carrier horses; and (3) the development of an immunofluorescence serology test for *Anaplasma marginale* and *Anaplasma ovis* applicable to wild ruminants. He also said that use of specific DNA probes, an experimental susceptibility study and actual field isolations, the fluorescence test established that there are areas of significant prevalence of *Anaplasma ovis* in bighorn sheep.
Modified-Live *Anaplasma marginale* Vaccine, ANAVIV™.


**SUMMARY**

An isolate of virulent *Anaplasma marginale* was obtained from a pool of blood samples collected from naturally-infected cattle in various regions of Florida. The basic methodology used for attenuation of the Florida isolate was: (1) induction of an accelerated rate of mutation of the organism by exposure to irradiation; (2) selection of an avirulent *A. marginale* strain by serial passage of irradiated organisms in splenectomized deer (2 passages) and sheep (138 passages). In a series of laboratory and field experiments with cryopreserved modified-live *A. marginale* conducted in the United States, Peru, Venezuela, Colombia and Mexico, it was shown that the strain induced a protective immune response in susceptible adult cattle against challenge with virulent endemic strains. Failure of the organism to revert to virulence after a series of consecutive passages (5 to 10) in highly susceptible mature cattle, pregnant cattle, and splenectomized calves, attest to the stability of the attenuated strain. The major difficulty in making this product commercially available was the inability to preserve the viability of the modified organism by means other than cryopreservation. Recently, this aspect has been successfully resolved. A method has been developed whereby the organism can be preserved and its viability retained by lyophilization. The efficacy of the vaccine in its lyophilized form was confirmed in a vaccination and challenge experiment involving a large number of susceptible cattle.

**Introduction**

Anaplasmosis is an arthropod-borne disease of cattle and some wild ruminants caused by the rickettsia *Anaplasma marginale*, or the relatively less virulent *Anaplasma centrale*. [*Anaplasma marginale* is host-specific and the mature erythrocyte is the only cell known to support growth and development of the organism in an infected animal.][1] Some 600 million cattle in tropical and sub-tropical regions of the world are being threatened by anaplasmosis. The only available means of immunoprophylaxis in these regions is premunization, which consists of infection with *A. centrale*, or a virulent strain of *A. marginale*, to establish a carrier state. However, this procedure is hazardous, time consuming and costly.[2] Moreover, the majority of animals being premunized are not maintained in confined areas, making it extremely difficult to observe the post-premunization reactions and to initiate treatment, which often becomes necessary. Hence, there is a great need for a safe, practical and economical vaccine delivery system which induces solid, long-lasting protection following administration of a single dose. To meet this demand, ProtaTek International, Inc. has

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[1]: cited reference
[2]: cited reference
developed a modified-live A. marginale vaccine, "ANAVIV™", which satisfies all these criteria.

A detailed description of the development of the attenuated A. marginale from the virulent Florida isolate has been published.\(^3\) The basic methodology used for attenuation of the Florida isolate has been (1) induction of an apparently accelerated rate of mutation of the organism by exposure to irradiation; and (2) selection of an avirulent A. marginale strain by serial passage of irradiated organisms in splenectomized deer (2 passages) and sheep (138 passages).

A series of laboratory and field vaccination trials conducted by several groups in the United States\(^{3,4,5}\), Peru\(^{6,7,8}\), Venezuela\(^6\), Colombia\(^{10,11,12}\), and Mexico\(^{13,14}\), have shown that the immune response induced by this vaccine protects adult susceptible cattle against challenge with virulent endemic strains. These studies were conducted with the organism maintained in the frozen state prior to vaccination. Although the cryopreserved organism induced excellent protection in vaccinated animals, the delivery system was impractical and laborious. This aspect has now been resolved, and we report here the development of a method whereby the viability of the organism can be preserved by lyophilization.

**Materials and Methods**

**Vaccine Production**

Stabilates of sheep blood-derived modified-live A. marginale seed of established immunogenicity, growth pattern and safety were preserved in liquid nitrogen. For vaccine production, the seed material was reactivated in susceptible, splenectomized, disease-free cattle by injecting 2 ml of the frozen stabilate intravenously and another 4 ml subcutaneously into each animal. Animals were monitored at regular intervals for the percent parasitized erythrocytes (PPE) and packed cell volume (PCV). When the PPE reached 35–40%, they were sedated with Ketamine–Rompun and aseptically exsanguinated from the jugular vein into sterile blood bags containing acid–citrate–dextrose (ACD) anticoagulant. The erythrocytes were then washed in sterile phosphate-buffered saline, 0.15M, pH 7.2, in a Millipore Pellicon cassette system through a 0.1 \(\mu\)m filter. Determination of the PPE, PCV and total number of erythrocytes was made on washed erythrocytes. The washed erythrocytes were then suspended in a special lyophilization medium (proprietary) to contain \(1.0 \times 10^8\), \(1.0 \times 10^7\) and \(1.0 \times 10^6\) respectively, of A. marginale–infected cells in a 0.5 ml volume. Five ml volumes were aliquoted into lyophilization vials and loaded into a lyophilizer. Following lyophilization, the vaccine was stored at 4°C until use.

**Viability and Stability Studies**

Viability of the organism following lyophilization and reconstitution
RI STIC, WANDURAGALA

was ascertained by an *in vitro* staining technique utilizing fluorescein diacetate (FDA) stain (15). Lyophilized material, reconstituted and maintained at 23°C, was stained by FDA at 5, 10, 20, 30 and 45 minutes respectively, in order to determine whether the viability of the organism could be maintained over a reasonable period of time between reconstitution and injection into an animal. Viability of the lyophilized vaccine stored at 4°C for varying periods of time up to 250 days, or at 37°C for 7 days in an accelerated aging test, was also assayed by this technique.

Determination of viability of the organism in the lyophilized state was also done by calf inoculation. Two 6-month-old calves were each inoculated intramuscularly with a 2 ml dose of lyophilized vaccine containing $1.0 \times 10^6$ parasitized erythrocytes at 15 and 30 minutes respectively, following reconstitution. Two other calves received a similar dose of vaccine from vials incubated at 37°C for 7 days.

**Vaccination Trial**

A total of 26 Holstein yearlings determined to be free of *A. marginale* by the indirect fluorescent antibody (IFA) test and DOT-enzyme immunooassay (DOT-ELISA), and by microscopic examination of Giemsa/acridine orange stained blood smears were used in this experiment. The animals were randomly divided into 4 groups. Three groups, each consisting of 6 animals, were vaccinated, and the remaining group of 8 animals served as controls. The lyophilized vaccine was reconstituted in a special diluent (proprietary) and a 2 ml dose consisting of a finite number of *A. marginale* infected erythrocytes was administered as follows:

- **Group I** - received $1.0 \times 10^6$ infected erythrocytes.
- **Group II** - received $1.0 \times 10^7$ infected erythrocytes.
- **Group III** - received $1.0 \times 10^8$ infected erythrocytes.

Blood for serological, parasitological and hematological examination was collected on the day of vaccination (Day 0) and on Days 7, 21, 28, 32, 36, 38, 40, 44, 48 and 56 post-vaccination. Additionally, the cattle were observed daily for clinical signs.

**Challenge**

Twelve weeks after vaccination, the 3 groups of vaccinates, together with the non–vaccinated controls, were subjected to needle–challenge exposure with a virulent isolate (Florida) of *A. marginale*. The challenge dose, consisting of $2.0 \times 10^8$ infected erythrocytes in a 2 ml volume of blood was administered intramuscularly.

Animals in all 4 groups were bled by jugular venipuncture on Days 0, 7, 21, 28, 32, 36, 38, 40, 44, 48 and 56 post–challenge for hematological and parasitological evaluation.
Results
Viability and Stability Studies

Anaplasma marginale inclusion bodies contained in erythrocytes of the reconstituted material did not exhibit the same high intensity of FDA fluorescence observed with organisms in fresh infected cells. Hence, any marginal body showing even a pale fluorescence was recorded as live. Lyophilized material stored at 4°C for up to 250 days, and that incubated at 37°C for 7 days, contained viable A. marginale at levels comparable to that observed in material examined immediately after lyophilization; i.e., between 55–63% of the viable organismal count in samples assayed on erythrocytes prior to lyophilization.

In animal inoculation studies, the two calves that received the vaccine developed a patent rickettsemia at 34 and 37 days respectively, following immunization. The vaccine stored at 37°C for 7 days produced a patent infection in the 2 calves on Day 32 and Day 38 respectively, after inoculation.

Vaccination Experiment

Table 1 depicts the maximum percentage PCV decrease (group mean +/- SE) and the average maximum PPE of the principals following vaccination and of all groups of animals following challenge exposure. The vaccines did not exhibit any clinical signs of anaplasmosis following vaccination or challenge. Upon challenge exposure, all the non-vaccinated controls showed clinical signs typical of anaplasmosis; namely, anemia, depression and anorexia. Three of the controls died while the other five animals required treatment for anaplasmosis.

<table>
<thead>
<tr>
<th>Vaccinated Groups*</th>
<th>Parasitized RBCs per dose</th>
<th>PCV Decrease</th>
<th>PPE</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 x 10^7</td>
<td>3.00 ± 1.39</td>
<td>1.17</td>
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<tr>
<td>2</td>
<td>1.0 x 10^7</td>
<td>3.33 ± 1.50</td>
<td>0.47</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>1.0 x 10^6</td>
<td>2.33 ± 1.06</td>
<td>0.26</td>
<td>None</td>
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<tr>
<td>Control Groups*</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>17.00 ± 1.56</td>
</tr>
</tbody>
</table>

* Animals = Holstein yearlings.
** Challenge dose = 2.0 x 10^7 erythrocytes infected with virulent A. marginale in 2.0 ml of blood administered I.M.
+ Group mean ± standard error.
Table 2 depicts the kinetics of the antibody response in the three groups of vaccinates following immunization. Although no statistical analysis was done, the antibody titers of the animals that received the lesser vaccine dose, namely $1.0 \times 10^6$ parasitized erythrocytes were markedly lower than in the other two groups.

**Table 2. Kinetics of the antibody response in animals vaccinated with modified-live A. marginale vaccine (AnaVIM™) as measured by DOT-ELISA**

<table>
<thead>
<tr>
<th>Animal #</th>
<th>0</th>
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<th>30</th>
<th>40</th>
<th>44</th>
<th>48</th>
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<td>361</td>
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<td><strong>Group II - n=6, dose=$1 \times 10^7$</strong></td>
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<td><strong>Group III - n=6, dose=$1 \times 10^6$</strong></td>
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<td>80</td>
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</table>

* Antibody levels expressed as the reciprocal of the serum dilution at which the test was still positive, i.e., end-point serum dilution factor.
** Parasitized erythrocytes in a 2.0 ml volume administered I.M.
*** No measurable antibody response.
Discussion

In order for a live vaccine to be suitable for worldwide use, it has to be safe, effective, easy to handle and above all be able to retain the viability of the organism for an extended period of time under normal storage conditions, namely 4°C. Results reported in this study demonstrate that the viability of the organism can be preserved by lyophilization, and according to the results of the accelerated aging study, the viability of A. marginale could be maintained for at least 2 years under refrigeration.

Following reconstitution, the lyophilized vaccine contained viable organisms for at least 30 minutes, as evidenced by animal inoculation studies and the FDA staining technique. This provides sufficient time for a veterinarian working under field conditions to inject into animals the contents of a 10 dose vaccine vial. The decreased intensity of fluorescence of A. marginale in lyophilized material was determined to be due to the interference of lyophilization media ingredients with the staining process.

Post-vaccination PPE values observed in this study were lower than those reported in previous studies conducted with the frozen vaccine(2,11). The average maximum PCV decrease was also low for all groups. A previous study reported an average PCV decrease of 10%(2). These differences in post-vaccination reaction may be attributed to the lower infectious dose we used in the present study. However, all three dosage levels induced measurable serological responses and good protective immunity. The higher antibody levels observed in animals receiving $1.0 \times 10^8$ parasites obviously was due to the high parasite mass observed in this group following vaccination.

For routine vaccination we recommend a dosage of 2 ml containing $1.0 \times 10^8$ parasitized erythrocytes. This dose is not too high to evoke an exaggerated post-vaccination response, while at the same time it contains sufficient number of organisms to compensate for any loss of viability during storage and reconstitution.

At present, no data is available on the differences at the molecular level between the modified-live A. marginale and the parent virulent Florida isolate. With the use of the vaccine, it will become necessary in the future, for disease surveillance purposes, to differentiate between naturally-infected animals and vaccinates. Work is currently underway in our laboratory to ascertain if there are any differences at the molecular level between the two strains. If so, isolation of the putative marker antigen would facilitate development of a diagnostic test which could be used in satisfying the above requirement.

REFERENCES

EQUINE INFECTIOUS ANEMIA (EIA)—A DISEASE CONTROL PROGRAM IN THE UNITED STATES OF AMERICA (USA)

Ralph C. Knowles, D.V.M.³
J. E. Pearson, D.V.M., M.S.⁴
Manuel A. Thomas, Jr., D.V.M.⁵

INTRODUCTION

Equine infectious anemia (EIA), or swamp fever, is an acute or chronic infectious disease of horses and other equidae. It is characterized principally by intermittent fever, marked depression, progressive weakness, loss of weight, edema, congestion and icterus of the mucous membranes, and anemia of a transitory or progressive type. The causative agent of EIA is considered to be a virus. This disease is present in nearly every part of the world where there are sufficient numbers of equidae and vector populations.

A practical means of controlling EIA was not available until the agar gel immunodiffusion (AGID) test was perfected. The horse inoculation test was used as a diagnostic aid in certain outbreaks; however, the cost and delay in confirming EIA by this method made horse inoculation impractical and unpopular.

CHANGING LIFESTYLES IN THE USA

Just after World War II lifestyles notably changed in the USA. Leisure time was available to more people, hobbies were developed, and many persons became involved in activities with horses, such as competitive events (endurance rides, gymkhanas, and rodeos), exhibitions, pleasure and trail riding. Many new horse riding and training stables were established. Horse racing was expanded to include more racing days and new race tracks that included competition Thoroughbreds, Standardbreds, Quarterhorses and Arabian horses.

These horse activities caused many horses to be transported and

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EQUINE INFECTIOUS ANEMIA (EIA)

congregated. As with other infectious diseases, EIA outbreaks increased. Another factor that helped spread EIA was the common practice of applying antibiotics and other injectable "medicines" to horses. Too often the same hypodermic needle was used among individual horses in a stable.

NOTABLE EIA OUTBREAKS – RENEWED SCIENTIFIC INQUIRIES

In 1965 and 1966 several outbreaks of EIA at horse race tracks and breeding farms in the Northeastern part of the USA focused attention on the need to develop more knowledge concerning certain scientific aspects of EIA, especially the need for a confirmatory test.

Investigations into the characteristics of EIA, the methods of transmission, and aids to diagnosis were carried out at several universities and in government laboratories.

The agar gel immunodiffusion (AGID) test, otherwise known as the Coggins test, was developed at the New York State Veterinary College, Cornell University. This tool was evaluated by several other university scientists. The American Association of Veterinary Diagnosticians reviewed the AGID test and endorsed its use.

In August 1972 the AGID test was declared as an official test for EIA by the U.S. Department of Agriculture (USDA). The AGID test antigen distribution is controlled by USDA and can be sold to and used only in approved laboratories. All AGID tests are official and USDA and State regulatory actions are taken based on official test results.

EIA CONTROL PROGRAMS WERE INITIATED

Animal Health officials in many states issued regulations requiring that equidae entering their state, entered in racing events or exhibitions, and being sold or otherwise changing ownership be AGID tested and found negative for EIA. The USDA issued regulations that prohibited the interstate movement of AGID test positive or horse inoculation test positive equidae. There are three exceptions to this prohibition: 1) EIA affected animals can move to slaughter; 2) they can move to approved research or diagnostic institutions; or 3) in the case of valuable purebred animals, they can move once, back to their "home farm" to be quarantined if the receiving state officials approve of this quarantine arrangement.
In 1976 USDA decreed that all equidae offered for importation into the USA must be AGID test negative prior to entry.

THE SOCIOLOGICAL IMPACT OF EIA CONTROL PROGRAMS

Land for raising horses is less expensive in the Mid-south such as Arkansas, Louisiana, Texas and Tennessee than in the Northeastern US such as New York, Massachusetts, New Hampshire, and New Jersey. Many horses are raised in the Mid-south and sold to persons in the Northeastern US. This "flow" of horses to the Northeastern US coupled with the fact that many equidae after an initial EIA "acute attack" remain carriers of the EIA virus and are subclinical or inapparent disease carriers created a sociological problem among horse owners in the eastern USA.
Frequently these inapparent carriers would find their way to locations in the Northeastern states; here they would be AGID tested and found positive. To such horse owners, their animals had shown no evident signs of sickness. Groups of these horse owners would band together in an attempt to repeal State EIA regulations; however, few modifications to ease the EIA restrictions on positive horses were enacted. Only temporary modifications in State regulations took place—thus, a solid front to control EIA remained.

Even though the USDA and most state animal health officials carry out regulatory actions to control EIA, small "pockets" of EIA affected horses and other equidae remain in the equine population of the USA. These are animals that do not move from their home farm, and only occasionally does EIA now occur that is traced to these "pockets" of infection.

Eradication of EIA has been considered by USDA. The cost benefit study concerning eradication revealed that with the low prevalence of EIA (believed to be less than 0.5%) coupled with the expense of AGID testing made it cost prohibitive to attempt eradication.

**RECOMMENDED METHODS THAT CAN BE USED TO CLEAN UP OR ERADICATE EIA**

When clinical or inapparent EIA is found on a farm, ranch, or in a stable, certain measures can be taken to eliminate infection and free the premises from EIA. The following actions are essential to obtain an EIA free equine population:

1. Apply the AGID test to all animals on the premises
2. Remove all AGID positive animals
3. Control the use of hypodermic needles and other equipment used on horses that may contact blood or blood serum
4. Apply pesticides to reduce flying, biting insects
5. Apply the AGID test in a retesting pattern with the last test to be at least 90 days after the removal of infected animals
6. Introduce only animals that are AGID negative into the clean herd.

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SUGGESTED PROCEDURES FOR ELIMINATING EIA FROM INFECTED HERDS

A. Herd is defined as:
   1. On a ranch, farm, or stable: all equidae on one premises or where the owner has several units with interchange of equine animals among all of units.
   2. At a horse racetrack: all animals handled by the same trainer who is in charge of the reactor or, if animal health officials so deem it necessary (based on vector pressures), all animals in the same shed row or the whole stable area.

B. AGID Testing Schedule and Vector Control
   1. Official test on all horse stock in herd. Identify and remove all reactors.
   2. Repeat "1" above (does not apply to horse racetracks) until all horses are negative on three consecutive tests, the first two to be conducted at 30–40 day intervals with the final test to be conducted 60–90 days following the preceding test.
   3. All introduction of horses into herd shall have a negative test conducted within 90 days prior to entry.
   4. Vector control practices should be followed in herds to reduce exposure in these herds. These practices should include insect and human vector control such as periodic application of insecticides to horses and facilities occupied by these animals, and cleaning and disinfection of equipment used among animals in the herd.

THE CELISA TEST CAN BE USED

Several indirect ELISA procedures have been described for the detection of EIA antibody and antigen. The procedure described by Matsushita et.al. was evaluated and a commercial kit adopted for the serologic diagnosis of EIA. It was accepted as an official test by the USDA in 1986.

The test uses p21 antigen which is purified from the Wyoming strain of EIA propagated in cell culture. The antigen is used to coat the wells of microtiter plates. The test serum, 0.025 ml, and 0.025 ml of monoclonal against the p21 EIA antigen are added to the wells after 30 minutes
incubation. The plates are washed and goat antimouse antibody conjugated
to horseradish peroxidase is added, incubated for 30 minutes, and ARTS is
used as an indicator. The results are determined by comparison to a weak
positive control serum supplied with the kit. The test can be read visually or
confirmed using an ELISA reader at an OD of 410 nm.

Before USDA approval, this test was evaluated using 225 negative
and 12 positive field samples supplied by diagnostic laboratories and 193
samples supplied by the National Veterinary Services Laboratories (NVSL).
The NVSL samples included 150 positive and 43 negative samples. At least
10 of the positive samples produced very weak reaction on the AGID test.
This means 100% correlation between the AGID and competitive ELISA
(CELISA)
test.

Since 1986 either the CELISA or AGID test could be used in the
United States. There have been less than 10 discrepancies between the two
tests. Due to the higher cost of the CELISA and additional technician time
needed to perform the CELISA test, the AGID is still the primary test used
in the United States. A total of about 800,100 tests per year have been
performed. The CELISA has proven to be a sensitive and specific test for the
identification of EIA antibody.

SUMMARY
1. Outbreaks of EIA occurred at horse racetracks in the mid 1960's
   in the USA.
2. Intensified research helped to: further characterize EIA; better
   outline methods of disease spread; and, provided a practical aid
to diagnostic confirmation of EIA
3. State and Federal Animal Health regulatory bodies acted to
   prevent the spread of EIA. Control of EIA has been successful
   in the USA.
4. Methods are now available to eliminate EIA from a farm, ranch
   or a stable.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. R.C. Knowles, Rehoboth Beach, DE

J.B. Anderson, TN; C.C. Black, GA; S.A. Blackburn, RI; C.L. Campbell, FL; L. Coggins, NC; L.H. Greene, CT; R.D. Hobbs, AZ; F.M. Jones, TX; D.H. Lein, NY; P.L. McDonough, NY; C.W. McGinnis, NH; R. Mead, WA; W.D. Miller, VA; M.A. Owen, MA; L. Schlater, IA; C.E. Starkey, AR; M.A. Thomas, MD; C.D. Vail, CO; H.A. Virts, MD; A. Wallop, DC; R.L. Whitlock, PA

Fourteen committee members were in attendance providing a quorum.

EQUINE ENCEPHALITIS UPDATE

Dr. A. D. Alstad (NVSL) reported for USDA on the recognition of encephalitis in equids; his report is appended to this report to provide details on encephalitis.

The committee considered the problems associated with the continued practice of vaccinating horses with VEE vaccine. In view of the absence of confirmed cases of Venezuelan Equine Encephalitis within the United States or in any other countries in North or South America within the last eighteen years, as well as recent problems associated with the acceptance of VEE vaccinated horses in some foreign countries. The committee questions the continued manufacture and usage of polycvalent vaccines that included the VEE fraction, and requests that the USAM Committee on Veterinary Biologics immediately review this matter and comment on our committee's concern.

EQUINE INFLUENZA UPDATE

NVSL reported a new equine influenza isolate; an influenza virus of the H3N8 subtype was isolated from an equine submission from Alaska. It was associated with a respiratory disease with a high morbidity and low mortality. It was determined to be antigenically distinct from the prototype of equine A2, A/Elq/MI/2/63 (H3N8). Dr. Robert Webster's laboratory at St. Jude Childrens Research Hospital in Memphis, Tn., has confirmed these observations. Dr. Webster's group also did molecular studies and identified that Equine Alaska influenza virus is closely related to the equine A2 isolate, especially the most recent prototype isolate from Kentucky, and the virus is not related to the avian H3 viruses. This virus has been identified as A/Elq/AK/29759/91 (H3N8).

Since its original isolation, antigenically similar viruses have been recovered from horses in Idaho and Texas. There has also been serologic
REPORT OF THE COMMITTEE

evidence of its presence in horses in Indiana and Illinois. Clinical histories accompanying these submissions have reported a respiratory disease compatible with that described for equine influenza.

SUMMARY OF EIA TESTING IN THE UNITED STATES

Dr. M. A. Thomas reviewed recent serological testing in the U.S., revealing 966,788 tests and 2,728 positive tests, and a map representing this testing is appended to this report.

Equine Infectious Anemia

The committee noted that there is a lack of uniformity of EIA testing requirements for entry of horses into states. The committee recommends that USDA review and critique the present EIA state regulations and the EIA prospectus and guidelines and propose a model EIA control program for states to follow.

SUMMARY OF USDA'S AFRICAN HORSE SICKNESS AHS TEST EXERCISE

In late July 1991, USDA and Florida animal health officials carried out an AHS test exercise involving a simulated outbreak of African Horse Sickness in Florida horses.

This test exercise pinpointed the importance of: (1) prompt state quarantines to control the movement of exposed horses; (2) the problems of
INFECTIOUS DISEASES OF HORSES

disposing of dead horses; (3) the logistics of dealing with the tracing of horses that moved from the "affected" area before quarantines were issued. USDA and the State of Florida are to be commended for the comprehensive effort that they put into this test exercise.

Dr. J. A. House provided an update on studies of an inactivated commercial AHS vaccine (Type 4). These studies are being carried out at USDA's Plum Island facility. These preliminary studies show promise that such an inactivated vaccine may be of value and Dr. House and his colleagues will continue their work.

EQUINE VIRAL ARTERITIS (EVA)

Recent reports indicate that EVA virus shedding stallions and semen from such stallions are found in interstate and international commerce.

After a lengthy discussion, the committee recommends that USDA and the American Horse Council bring to the attention of horse breed associations the dimensions of EVA and international commerce. They should solicit feedback from these breed groups as to the need to regulate EVA in international commerce. This committee requests that the USAHA Import/Export committee review this EVA situation.
Report to Infectious Diseases of Horses Committee
A. D. Alstad, National Veterinary Services Laboratories

Summary of Venezuelan Equine Encephalitis Surveillance
January 1, 1990 – October 1, 1991

Since the 1971 epizootic of Venezuelan equine encephalitis (VEE), the National Veterinary Services Laboratories (NVSL) has been testing samples for the equine encephalitides as part of the VEE surveillance program. The majority of the samples are submitted by state veterinary diagnostic laboratories. Samples were also submitted by Veterinary Service (VS), U. S. Department of Agriculture (USDA) veterinarians, and veterinarians in private practice.

Virus isolation and serologic procedures were the same as described in previous reports. Most of the positive cases are based on the results of tests on a single serum sample. A single sample was reported positive if it had a neutralizing antibody titer of >1:10 and hemagglutination inhibition antibody titer of >1:40 against only eastern equine encephalitis (EEE) or western equine encephalitis (VEE). Some of the EEE and WEE results have been confirmed by a diagnostic increase in antibody titer or virus isolation, and most EEE serology results have been confirmed by EEE I&M capture enzyme-linked immunosorbent assay (ELISA). The results shown in tables 1–4 are composites of reports submitted to the Centers for Disease Control (CDC), Fort Collins, Colorado, from NVSL test results, and from several state veterinary diagnostic laboratories.

In 1990, 23 cases of EEE and 11 cases of WEE were diagnosed at the NVSL. The CDC reported two additional cases of WEE and 57 cases of EEE with one additional EEE case in pigeons. In 1991, there were more EEE cases over a wider geographic area than any other recent year. For the period of January 1 to October 1, 1991, 330 diagnostic submissions were received at the NVSL, and 126 of these were positive for EEE and seven for WEE. Eight of the EEE cases were from birds with four in pheasants, three in chukar partridge, and one from chickens. There was one EEE case from neonatal pigs. There were an additional 232 cases of EEE and one of WEE reported from Dr. Chet Moore of CDC as well as several state veterinary diagnostic laboratories. These included an additional porcine case of EEE as well as two canine and two bovine cases, all from Georgia.

There are an average of five sporadic cases of EEE reported in humans annually. In 1990, there were three in Massachusetts and one each in North Carolina and South Carolina. There have been seven confirmed cases in 1991 with five in Florida and one each in Mississippi and Georgia.

In 1990, 25 horses in 11 state had antibody against VEE, and 12 horses in nine states were positive in 1991. Many of the horses had stable antibody titers; vaccination histories were vague or not available in most horses, and in most cases there was no apparent explanation for the VEE
antibody titers.

The U. S. Army Medical Research and Developmental Command, University of Wisconsin, and the NVSL have continued virus isolation and serologic surveillance for evidence of VEE activity in Central America; however, there have been no confirmed cases of epizootic VEE since 1973.

In summary, there were more cases of EEE in 1991 than in any recent year with 358 cases from 17 states. The isolation of EEE from Ohio represents the first isolation of this virus in this state. The number of cases of WEE in 1990 and 1991 is similar to other years. There has been limited WEE activity for the two years reported.

Equine Encephalitis Export Testing

In the past few years there has been a moderate decline in export testing for equine encephalitis in spite of continued interest of this disease for export requirements. In fiscal year 1988, the NVSL performed 1286 export tests, 2431 tests in fiscal year 1989, 2267 tests in fiscal year 1990, and 2035 in fiscal year 1991.

Table 1. Eastern equine encephalitis positive cases for calendar year 1990

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
<th>State</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>1</td>
<td>Mississippi</td>
<td>1</td>
</tr>
<tr>
<td>Connectic</td>
<td>1</td>
<td>New Jersey</td>
<td>6</td>
</tr>
<tr>
<td>Florida</td>
<td>30</td>
<td>New York</td>
<td>5</td>
</tr>
<tr>
<td>Georgia</td>
<td>11</td>
<td>North Carolina</td>
<td>4</td>
</tr>
<tr>
<td>Louisiana</td>
<td>2</td>
<td>South Carolina</td>
<td>12</td>
</tr>
<tr>
<td>Maryland</td>
<td>3</td>
<td>Texas</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>80</td>
</tr>
</tbody>
</table>

Human Cases 5: Massachusetts – 3, North Carolina – 1, South Carolina – 1
### Table 2. Western Equine encephalitis positive cases for calendar year 1990

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
<th>State</th>
<th>Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montana</td>
<td>2</td>
<td>North Dakota</td>
<td>3</td>
</tr>
<tr>
<td>Nebraska</td>
<td>4</td>
<td>Texas</td>
<td>1</td>
</tr>
<tr>
<td>New Mexico</td>
<td>1</td>
<td>Wyoming</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>

### Table 3. Eastern equine encephalitis positive cases for January 1 – October 1, 1991

<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>25</td>
<td>New Jersey</td>
<td>2</td>
</tr>
<tr>
<td>Arkansas</td>
<td>1</td>
<td>New York</td>
<td>2</td>
</tr>
<tr>
<td>Florida</td>
<td>151</td>
<td>Carolina</td>
<td>7</td>
</tr>
<tr>
<td>Georgia</td>
<td>55</td>
<td>Ohio</td>
<td>11</td>
</tr>
<tr>
<td>Kentucky</td>
<td>1</td>
<td>Pennsylvania</td>
<td>1</td>
</tr>
<tr>
<td>Louisiana</td>
<td>15</td>
<td>South Carolina</td>
<td>55</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>2</td>
<td>Tennessee</td>
<td>2</td>
</tr>
<tr>
<td>Michigan</td>
<td>16</td>
<td>Virginia</td>
<td>1</td>
</tr>
<tr>
<td>Mississippi</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>358</strong>*</td>
</tr>
</tbody>
</table>

Human Cases: Florida – 5  Mississippi – 1  Georgia – 1

*Includes 7 avian, 2 porcine, 2 bovine, and 2 canine cases*
REPORT TO INFECTIOUS DISEASES OF HORSES COMMITTEE

Table 4. Western equine encephalitis positive cases January 1 - October 1, 1991

<table>
<thead>
<tr>
<th>State</th>
<th>Positive Cases</th>
<th>Positive State</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorado</td>
<td>1</td>
<td>North Dakota</td>
<td>2</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1</td>
<td>South Dakota</td>
<td>2</td>
</tr>
<tr>
<td>Montana</td>
<td>1</td>
<td>Texas</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Equine encephalitis cases 1980–1990

<table>
<thead>
<tr>
<th>Year</th>
<th>WEE</th>
<th>EEE</th>
<th>Cases Submitted To NVSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<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>
<td>47</td>
<td>519</td>
</tr>
<tr>
<td>1983</td>
<td>106*</td>
<td>131</td>
<td>426</td>
</tr>
<tr>
<td>1984</td>
<td>19*</td>
<td>121</td>
<td>274</td>
</tr>
<tr>
<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>
</tr>
<tr>
<td>1987</td>
<td>177*</td>
<td>88*</td>
<td>440</td>
</tr>
<tr>
<td>1988</td>
<td>92*</td>
<td>61*</td>
<td>290</td>
</tr>
<tr>
<td>1989</td>
<td>13*</td>
<td>195*</td>
<td>379</td>
</tr>
<tr>
<td>1990</td>
<td>13*</td>
<td>80*</td>
<td>265</td>
</tr>
</tbody>
</table>

*Includes the positive cases reported by the Centers for Disease Control and state veterinary diagnostic laboratories.
The purpose of this document is to contribute to assessment of the risk of introducing FMD virus through imports of livestock, meat, semen, and embryos, taking into account health guarantees provided by the exporting countries. It is based on:

- examination of incidents of FMD occurring through imports,
- statistical evidence of imports of animals and products which may act as sources of infection to countries or territories free from FMD, and
- review of precautions imposed by importing countries to protect against the disease.

Sanitary or veterinary officials of some countries also believe, as a result of experimentation or actual experiences, that animals vaccinated against FMD, or coming from countries where vaccination is practiced, may be sources of infection, even though the country may be free from the disease as defined by the Code. The same countries also treat semen, embryos, fresh meat, milk products, biological products, and products for the pharmaceutical industry in the same way.

Over the last 30 years the worldwide incidence of FMD in many countries has dropped gradually. In those countries which still experience outbreaks the incidence of infection, number of animals infected, as well as the severity of infection have also declined due to a number of factors, including systematic application of better vaccines, more rigid control measures especially in relation to recovered animals, slaughter and disposal of infected animals, and decontamination of premises. For example, FMD has not occurred in the European Communities (EC) since 1989. In view of these changes it is considered timely to assess the risk of introducing FMD virus through imports, taking into account the sanitary guarantees provided by the exporting countries.

I. Incidents of FMD occurring through imports

Table I is taken from a working document presented to the 24th
INTERNATIONAL TRADE AND FOOT AND MOUTH DISEASE

Session of the FAO European Commission for the Control of FMD, and notifications of FMD outbreaks received by OIE concerning the sources of infection in primary outbreaks in certain countries of Europe and the Mediterranean Basin, from 1968 to 1990.

This table shows that, out of 86 outbreaks, 19 were attributed to imports or to movement of animals across frontiers, 15 to imports of meat (including 11 due to meat from South America), and 18 following vaccination, or escape of the virus from vaccine production laboratories.

Table II shows the origins attributed by the Ministry of Agriculture of the UK to FMD outbreaks from 1954 to September 1967.

As shown in Table I, infected meat seems to have been responsible for a large number of outbreaks including the 1967–1968 incident in the United Kingdom. There is no doubt that the UK, which has been greatly dependent upon imports of meat, has made the biggest contribution to epidemiological investigations.

The report of the Commission of Inquiry into the FMD outbreaks of 1967–68 (referred to as the Northumberland Report) is a particular rich source of information.

For this reason it would be useful to summarize very briefly the facts concerning the potential role of imports in the occurrence of primary outbreaks of FMD.

The Northumberland Report, in a retrospective study of the period 1929 to September 1967, recalls that the UK had experienced outbreaks of FMD every year between 1929 and 1962. Then between 1962 and 1966 there was a long period free from outbreaks, interrupted only by a single outbreak caused by virus of type C in April 1965.

Between 1954 and September 1967 there were 179 primary outbreaks, the origins of which are shown in Table II. An outbreak in a farm adjoining the Animal Virus Research Institute at Pirbright in January 1960, and another in Sussex in September 1966 were caused by virus of types SAT2 and A22 (respectively), both of which were absent from the Americas and Europe. All other epizootics were caused by types of virus common to South America and Europe. The epizootic which lasted from October 1967 into 1968 was caused by type O1 virus. The authors of the Report cite the conclusions of the Chief Veterinary Officer of the UK concerning the origin of the outbreak that: "It was not possible to establish conclusively that imported frozen lamb carried FMD virus to the farm, but as all other generally recognized sources of the infection had been eliminated, it remained the most probable vector".

After the prohibition of import of sheep meat from infected areas in South America into the UK for 23 years, no other cases from that source occurred. Two outbreaks occurred on the island of Jersey (close to the French coast) and the Isle of Wight in 1981. The virus had probably spread by aerosol from an epizootic in pigs which was prevalent at the same time.
in Brittany.

Semen and embryos have never been shown to be responsible for the occurrence of FMD.

Imported milk products, bones, horns and hooves for industrial purposes, hides, wool, hair and bristles, were not recorded as a source of infection. Biological products, and straw have been reported to be responsible for primary outbreaks.

II. Statistics on products imported by a certain number of countries.

The countries concerned are listed in Table III, together with their area, susceptible livestock population and the date of the last outbreak of FMD.

These countries have been chosen because of their prominence as importing countries in livestock and livestock products (excepting Canada):
- the European Communities (EC), with 12 countries, are the biggest importers of livestock and meat in the World,
- the USA are in second position.

However, for the purpose of this study USA statistics are of limited value, because this country applies a policy of zero risk, and permits imports only from disease-free countries which do not vaccinate. Exceptions to this rule are animals for breeding, and semen, which may be imported under strict precautions from infected countries or disease-free countries which practice vaccination. USA has permitted imports many years after Canada, and for this reason Canada is included in the list of countries.

As far as the EC are concerned, the situation is interesting because there are two groups of countries with differing disease control policies:
- Denmark, Ireland and UK prohibit vaccination within their territory, as well as Greece where vaccination which was carried out into the Turkey/Greece buffer zone is no longer practiced since 1989
- the 8 other EC countries are encouraging, so far, systematic vaccination of cattle, while Greece vaccinated in the Turkey/Greece buffer zone until 1989.

The vaccination policy still leaves a large proportion of the susceptible animal population unvaccinated, as shown in Table III. It should be added that a proportion of cattle in countries with a vaccination policy remains unvaccinated. During annual vaccinations, large numbers of young cattle are susceptible to FMD after disappearance of maternal antibody.

First imports of non vaccinated breeding stock in recent times from continental Europe into Canada took place in 1965, with 110 Charolais cattle from France. Other European countries, including Switzerland, Federal Republic of Germany, Austria and Italy also exported livestock to North America. Table IV shows the number of head of cattle and sheep imported from 1965 to 1985. Table V shows importation of livestock into the USA from
INTERNATIONAL TRADE AND FOOT AND MOUTH DISEASE

FMD infected or vaccinating countries, while Table VI shows semen importations into the USA from FMD infected or vaccinating countries.

Table VII shows the importance of trade in livestock within EC and from other European countries. The equivalence between 1 ton and the number of heads being estimated as 5 for cattle, 15 for pigs and 26 for sheep and goats, trade in Europe would involve approximately 4 million cattle, 7.5 million pigs, 4 million sheep and goats.

Imports into three of non-vaccinating countries – Denmark, Ireland, the UK – from vaccinating countries are limited approximately to 1,600 purebred cattle and 20,000 small ruminants. In contrast, Greece which also applies a non-vaccination policy, imported approximately 75,000 cattle, 2,000 pigs and 80,000 small ruminants from vaccinating countries.

Table VII also shows that EC trade in meats of cattle, swine, sheep and goats, as a whole, reaches approximately 3.5 million tons. Imports of bone-in beef from EC vaccinating countries into the group of non-vaccinating countries are approximately 150,000 tons. This quantity is largely exceeded by the group of vaccinating countries which import more than 620,000 tons.

Statistics for imports of boneless beef from infected countries are of particular interest. EC imported in 1989 from South America and Southern Africa, more than 100,000 tons out of which, 31,000 tons were imported by non-vaccinating countries. In this respect, it is also to be noted that, from 1968 to 1990, exports of beef from Argentina to the EEC reached more than 1 million tons, of which more than 500,000 tons were for the UK.

Imports of pork into Denmark, Ireland, the UK and Greece from EC vaccinating countries exceeded 100,000 tons, while trade within the latter group of countries involved approximately 800,000 tons.

Non-vaccinating countries imported 1,500 tons of bone-in meats of sheep and goats from EC vaccinating countries. EC vaccinating countries and Greece imported 6,330 tons and 633 tons respectively of this category of meats from infected countries in South America. EC vaccinating countries imported also from these countries 1,516 tons of boneless cuts of sheep.

III. Import policies of selected countries to prevent the introduction of FMD virus

Specific policies for imports of countries considered in the previous chapter, namely USA and EC, are discussed below.

Examination of these import policies is confined to animals susceptible to FMD, including their meat, semen and embryos.

1. Regulations of the USA

In applying the Tariff Act, adopted by Congress in 1930, it is
prohibited to import into the USA ruminants and pigs and their meat except that treated by heat and other methods which destroy FMD virus, from countries which are not free from FMD.

Countries may be considered to be free from the disease if it has not occurred for at least 1 year, vaccination is not practiced, and importations of animals and their products from infected countries are strictly regulated.

All countries of North and Central America have regulations similar to those of USA.

Since the 1960's, special permits have been granted, first by Canada and then by USA, for imports of breeding stock and semen.

**Breeding stock**

Cattle and sheep may be imported for breeding only if they have not been vaccinated against FMD, and have given negative results to the probang test for FMD virus, and serological tests for FMD antibodies. They have to be confined to their farm of origin during testing, followed by quarantine pending export, and retesting for FMD.

The animals also have to undergo quarantine in the importing country, and are tested for FMD before being released to their final destination.

Exports of unvaccinated cattle from European countries which practice systematic vaccination to countries as strict as North American countries provide valuable information on the potential danger of FMD vaccination. It is known that immunized cattle may become virus carriers after exposure to virus or infected animals, and some authorities have concluded that vaccination could conceal the presence of the virus in a country.

During these exportations mentioned above, several thousand young unvaccinated cattle were exported from vaccinated herds over a 25 year period without a single positive probang test. These results demonstrate that imports from vaccinated countries can be safe under conditions of systematic application of well controlled vaccines and other measures.

**Semen**

The US regulations until 1985 stipulated the following conditions:

1. The donor animal is inspected on the farm of origin by inspectors who determine by examination of the animal but more importantly animal records that the animal was never infected with FMD and that the infection had not existed on that farm the previous year.
2. The donor animal is permanently identified and is not a carrier of FMDV, and is free of antibodies to FMDV.
3. The donor animal is kept under surveillance for 30 days, and is then moved to a collection facility where it is kept for the collection period.
and 60 days afterwards, at which time it is again examined for FMD viral antibody.

4. Ten percent of each ejaculate is retained, non-diluted for examination for FMDV in susceptible cattle and tissue culture systems.

5. The entire operation must be under the supervision of a USDA veterinarian.

Using this system, more than 1.7 million doses of semen were safely imported and used over a 10–year period. It was costly and time consuming, but was proven to be safe.

In more recent years, the system has been extended to include semen from bulls which have been vaccinated against FMD and thus have neutralizing antibody to FMDV. They must be negative to virus infecting antigen (VIA) antibody and must not be carriers of infection, as demonstrated by negative oesophageal–pharyngeal samples. Using this procedure, several hundred thousand doses of semen have been imported and safely used. Perhaps the best demonstration of the safety of semen from vaccinated bulls is provided by the example of France since 1961. In that year the French authorities decided to implement a programme of compulsory immunization against FMD. The decision was made to vaccinate all cattle in France except those in the Finistere Department (more than 600 000). These were left unvaccinated for several reasons, including the need for cattle free from FMD antibody for testing FMD vaccines. Also, the official quarantine export facility for livestock is located in Finistere at Brest, and no outbreak of the disease had occurred in that Department since 1957. The general movement of livestock was away from the Department, and not into it. During the past 30 years, only a few cattle in Finistere had been immunized against FMD, and then only because they were taken for exhibition to other parts of France. Semen from bulls throughout France has been used routinely in Finistere without a single occurrence of FMD in the Department. This 30 years of experience would be difficult to repeat. The situation during the 7 years between 1963 and 1971 has been summarized by Parez. By extending the observations of Parez to 1990, and incorporating other reports, the following conclusions may be drawn.

a) FMD can be transmitted readily by artificial insemination with semen from an infected bull.

b) It is possible to devise safe procedures for transferring bull semen from an infected to a disease–free country.

c) Semen from bulls which have been vaccinated several times with an efficacious product, and which are not virus carriers, is safe to use.

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in animals which have not been immunized.

Embryos

Regulations for importing bovine embryos into the US from countries which are considered to be infected with FMD by the USA were published after this paper was prepared. They are fully described in the U.S. Federal Register Vol. 58, No. 210. Wednesday, October 31, 1991 – Rules and Regulations, pages 55804–55812.

2. Regulations in EC countries

The European Commission adopted in 1964 veterinary regulations concerning intracommunity trade, followed in 1972 by regulations for imports from third countries. Note that only cattle and pigs are covered by these regulations. Member countries are free to make their own decisions concerning other species of animals, however a sheep and goat directive has recently been adopted and will be implemented before 1993.

It must be noted that, from December 31, 1991, Directive 85/511/EC, modified by Directive 90/423/EC of 26 June 1990, prohibits the use of vaccination in the European Community, however these countries are allowed to cease vaccination prior to this date.

Current regulation of intracommunity trade

The initial Directive 64/432/EC of June 1964 involved the first six member countries of the Community: Belgium, France, Italy, Luxembourg, Netherlands and German Federal Republic. Since then it has been modified on several occasions to include Denmark, Spain, UK, Greece, Ireland and Portugal. The modifications have taken into account the different animal health policies, particularly with regard to FMD, between the original group of members and the later arrivals.

Member countries are placed in two categories:

- those free from FMD for at least 2 years, which do not vaccinate, and do not accept vaccinated animals;
- those which practice vaccination and/or admit vaccinated animals.

Cattle and pigs

Those countries of the first category (Denmark, UK, Ireland) permit the entry of cattle and pigs provided that they have not been vaccinated against FMD. Cattle and pigs coming from countries of the second category
INTERNATIONAL TRADE AND FOOT AND MOUTH DISEASE

have to be certified that they are:
- negative to the probang test,
- negative to serological tests for FMD antibodies,
- kept in isolation in the country of origin, whether on a farm or in a quarantine station, for 14 days under the supervision of an official veterinarian.

This is followed by 21 days of quarantine in the importing country.

Countries of the second category require that cattle coming from another country of the same category be certified as:
- vaccinated within at least 15 days and not more than 4 months against FMD virus of types A, O and C, in the case of cattle for breeding or for food production, over 4 months old,
- vaccinated within at least 15 days and not more than 4 months against FMD virus of types A, O and C, in the case of cattle intended for slaughter over 4 months old. The duration of validity of vaccination is 12 months for animals revaccinated in those member countries which practice annual vaccination, and which operate a slaughter policy for animals with FMD.

No conditions are placed on cattle coming from a country of the first category, except the option of vaccination against FMD before admission to the herd of destination.

Meat

Except in the event of an epizootic of FMD in a member country of the EC, intracommunity trade in meat is not subject to any restriction on account of FMD.

Member countries formerly had the right to prohibit the entry of fresh meat from countries which vaccinate against FMD, but this no longer applied after 1985.

Semen


Semen has to be collected and processed at a collection centre approved from the aspect of animal health. The donor animals have to come from isolated premises, either situated in the centre of a zone or region of 10 km which has been free from FMD for at least 30 days, or free from FMD for at least 3 months.

Member countries which do not vaccinate against FMD may not
prohibit the import of semen from vaccinated bulls.

In such a case, not more than 10% of each ejaculate, with a minimum of 5 semen straws, may be tested for virus isolation. If the result is positive, entry of the semen may be refused.

Embryos

Intracommunity trade of bovine embryos is regulated by Directive 89/556/EC of 25 September 1989. The embryos are to be obtained following artificial insemination with semen which conforms to the requirements of Directive 88/407/EC, mentioned above.

Embryos have to be collected, processed and stored by an approved team, and they have to be washed at least ten times in ten fold dilutions in a special fluid for embryos.

Until 31 December 1992, member countries which do not vaccinate against FMD are entitled to:

- prohibit the import of fresh embryos from member countries which vaccinate;
- impose the following requirements on frozen embryos from member countries which vaccinate: the donor comes from a herd in which no animals has been vaccinated during the 30 days before collection, and is free from restrictions or quarantine for animal health reasons; the embryos have to be stored under approved conditions for at least 30 days before dispatch.

Regulations governing imports from third countries

Importation into the EC of cattle and pigs and also fresh meat from cattle, pigs, sheep and goats or meat products from third countries is subject to the provisions of Directive 72/462/EC of 12 December 1972, in force since 1 January 1973. As in the case of intracommunity trade, this Directive has been modified on several occasions. It entails the establishment of a list of countries or parts of countries from which member countries are authorized to import animals and animal products. Admission to this lists depends on, among other stipulations:

- the state of health of livestock and the animal health status of the neighbourhood of the third country,
- the regularity and rapidity of supplying animal disease information, particularly the occurrence of diseases of lists A and B of OIE,
INTERNATIONAL TRADE AND FOOT AND MOUTH DISEASE

- regulations concerning the control of diseases of animals,
- the structure of the veterinary service and its powers,
- the organization and execution of control measures against diseases of animals.

Conditions to be met for imports are specified, for each approved country, by decisions of the Commission of the EC. The decisions concerning certain countries of South America and southern Africa are of particular interest, because they enable infected countries to establish zones or regions free from FMD.

In this way the decisions of the Commission apply to all member countries in the case of beef from Argentina, permitting the import of carcasses or cuts with bones from the southern part of the country. The same decision completely prohibits imports of meat from the provinces of Chaco and Formosa. It authorizes imports of deboned meat (excluding fresh offal) from the rest of Argentina, provided that there is no FMD in the area of origin of the cattle, that the cattle have been vaccinated against FMD and that the meat is left to mature at a temperature above 2°C for at least 24 h and the pH of the meat has to be less than 6.

The decisions of the Commission concerning certain countries of southern Africa (Botswana, Namibia, Swaziland, Zimbabwe) permit the import of meat provided that bones are removed and the meat treated as stated above. In contrast to Argentina and because prophylactic vaccination against SAT Types of FMD virus is not carried out in Europe, the meat animals have to come from a disease-free zone in which no animals are vaccinated against FMD.

Semen


Semen has to come from an approved collection centre situated in a country cited in the Commission’s list of countries from which imports may be made.

Conditions under which semen may be imported are fixed for each approved country, by Commission decision.

Embryos

Importation of embryos from third countries is subject to the provisions of Directive 89/556/EC of 25 September 1989.

Embryos may be imported only from a country cited in the Commission’s list of approved countries. They have to be collected, processed and stored by a collection team approved by decision of the
Commission. The Commission stipulates for each approved country the health conditions governing imports.

Regulations governing the import of sheep and goats

A Community Directive applying to import of sheep and goats is in the process of implementation. So far, Member countries are free to make their own decisions, and taking into account the importance of international trade in sheep, in particular, and their potential role in the spread of FMD, it is of interest to examine the health regulations applied by two large importing countries, France and Italy.

These two countries import about 3.7 million sheep a year, accounting for 92% of EC imports. These imports, which come mostly from eastern Europe and EC countries which practice vaccination, are subject to:

- in France, certification of the absence of FMD from the farm and region of origin,
- in Italy, the same guarantee, and also vaccination against FMD in the case of sheep which are not slaughtered immediately.

IV. Conclusions

The world-wide incidence of FMD in many countries has dropped gradually over the last 30 years. In those countries which still experience sporadic outbreaks, the incidence of infection, number of animals infected, as well as the severity of infection have declined due to a number of factors, including systematic applications of better vaccines, more rigid control measures, especially in relation to recovered animals, slaughter of and disposal of infected animals and decontamination of premises.

The European Community is a notable example, for FMD has not occurred since 1989. These changes are cause for reassessment of risks of introducing FMD virus through imports of livestock and livestock products, taking into account many of the trade practices which have been shown to be safe in EC and other countries.

Semen and embryos have never been shown to be responsible for transmitting FMDV providing recommended protocols are followed.

The report of the Northumberland Commission, appointed following the 1967–68 FMD outbreaks in the United Kingdom, contained important recommendations with reference to the movement of meat from FMD infected to FMD free countries. In the 23 years following issuance, FMDV has not been transmitted by meat from infected countries to the UK and this is attributed to the recommendation that only deboned beef be imported from infected areas and that offal, unless cooked, pork and sheep meat be banned.
Experience has demonstrated that it is possible to devise protocols for the safe movement of non vaccinated cattle from countries with a vaccination policy, or from infected countries.

It has been demonstrated that livestock, especially sheep, originating in East Europe where cattle are vaccinated can be safely moved to other countries where FMD vaccination is practiced in cattle, but with a significant animal population which is susceptible to the disease.

It also appears that very large quantities of bone–in meats of cattle, swine, sheep and goats have been safely imported from vaccinating countries into FMD free countries without vaccination.

Imported milk products have not been recorded as a source of primary infections.

Hormones, insulin, extracts and other products are obtained from animal glands for use in man. Traditionally, glands have been imported from several FMD infected to FMD free countries for processing. These experiences have shown that the procedures for movements of such materials are practical and safe.

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</tr>
<tr>
<td>Movements</td>
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<td>A</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>1973</td>
<td>Turkey</td>
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</tr>
<tr>
<td></td>
<td>1975</td>
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</tr>
<tr>
<td></td>
<td>1977</td>
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<tr>
<td></td>
<td>1977</td>
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<td>A</td>
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<td>A</td>
</tr>
<tr>
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<td>1978</td>
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<td>A</td>
</tr>
<tr>
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<td>1978</td>
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</tr>
<tr>
<td></td>
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<td>Portugal</td>
<td>C</td>
</tr>
<tr>
<td></td>
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<td>O</td>
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<tr>
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<td>O</td>
</tr>
<tr>
<td></td>
<td>1990</td>
<td>Morocco</td>
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Sub-Total 19

| Meat                     | 1968 | Switzerland  | A             |
|                         | 1969 | Belgium      | A             |
|                         | 1969 | Greece       | A             |
|                         | 1969 | Switzerland  | A             |
|                         | 1970 | Greece       | A             |
|                         | 1971 | Belgium      | A             |
|                         | 1972 | Greece       | A             |
|                         | 1972 | Italy        | A             |
|                         | 1976 | Federal Republic | A |
|                          | 1977 | Italy        | A             |
|                          | 1977 | Greece       | A             |
|                          | 1978 | Italy        | A             |
|                          | 1979 | Italy        | A             |

Sub-Total 15
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<tr>
<td></td>
<td>1972</td>
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<td></td>
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<td>Hungary</td>
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<td>TOTAL 86</td>
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### Table II (contd)

Origin Attributed by the Ministry of Agriculture of the UK to Foot and Mouth Disease Outbreaks from 1954 to September, 1967

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<th>Year</th>
<th>Primary outbreaks</th>
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<th>From unidentified countries</th>
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<td></td>
<td>Imported Meat and Meat Wrappings</td>
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<td>1966</td>
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<td>1967*</td>
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<td>Total</td>
<td>179</td>
<td>74</td>
<td>4</td>
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* January to September
Table III

Areas and populations of FMD susceptible livestock in selected countries
(thousands heads, 1989)
Sources: FAO/WHO/OIE Animal Health Yearbook

<table>
<thead>
<tr>
<th>Country</th>
<th>Area (km²)</th>
<th>Cattle</th>
<th>Pigs</th>
<th>Sheep &amp; Goats</th>
<th>Total population</th>
<th>Susceptible population</th>
<th>Date of last outbreak</th>
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<td>12,195</td>
<td>10,625</td>
<td>755</td>
<td>23,585</td>
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<td>99,180</td>
<td>35,499</td>
<td>12,608</td>
<td>167,287</td>
<td>167,287</td>
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<td>86</td>
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<td>5,637</td>
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<td>5,000</td>
<td>11,598</td>
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<td>11,902</td>
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<td>25,107</td>
<td>48,635</td>
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<td></td>
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<td>Bel/Lu</td>
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<td>2,967</td>
<td>5,950</td>
<td>198</td>
<td>9,115</td>
<td>6,148</td>
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<td>14,659</td>
<td>22,589</td>
<td>1,516</td>
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<td>24,105</td>
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<td>21,780</td>
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<td>13,104</td>
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<td>8,737</td>
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<td>12,837</td>
<td>30,993</td>
<td>22,196</td>
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<td>13,020</td>
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<td>43,087</td>
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INTERNATIONAL TRADE AND FOOT AND MOUTH DISEASE

Table IV
Imports of breeding stock into Canada and USA from continental Europe 1965-1985

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<th>YEAR</th>
<th>CANADA</th>
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<tr>
<td>1967</td>
<td>220</td>
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</tr>
<tr>
<td>1968</td>
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</tr>
<tr>
<td>1969</td>
<td>419</td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>497</td>
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<tr>
<td>1972</td>
<td>590</td>
<td></td>
</tr>
<tr>
<td>1973</td>
<td>522</td>
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<tr>
<td>1974</td>
<td>590</td>
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<td>1975</td>
<td>458</td>
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<tr>
<td>1976</td>
<td>162</td>
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<td>1977</td>
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<td>1979</td>
<td>228</td>
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<td>1980</td>
<td>90</td>
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<tr>
<td>TOTAL</td>
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<td>401</td>
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Table V
Imports of breeding stock into the US from infected or vaccinating countries
Source: USDA Figures

<table>
<thead>
<tr>
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<th>TYPE OF ANIMALS</th>
<th>NUMBER OF ANIMALS</th>
<th>RELEASE DATE</th>
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<tr>
<td>Brazil</td>
<td>Cattle</td>
<td>52</td>
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<td>Brazil</td>
<td>Cattle</td>
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<td>Brazil</td>
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<td>124</td>
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<tr>
<td>Bolivia</td>
<td>Camelids</td>
<td>167</td>
<td>July 1987</td>
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<td>Chile/Brazil</td>
<td>Camelids</td>
<td>472</td>
<td>December 1988</td>
</tr>
<tr>
<td>China</td>
<td>Swine</td>
<td>140</td>
<td>July 1989</td>
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Table VI
Semen importations into the US
Source: USDA Figures

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<th>DOSES</th>
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<td>1985</td>
<td>4 bulls</td>
<td>3,359 doses</td>
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<td>Brazil</td>
<td>1986</td>
<td>9 bulls</td>
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<td>Brazil</td>
<td>1987</td>
<td>8 bulls</td>
<td>9,964 doses</td>
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<td>Brazil</td>
<td>1988</td>
<td>12 bulls</td>
<td>12,346 doses</td>
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<tr>
<td>Brazil</td>
<td>1989</td>
<td>9 bulls</td>
<td>13,771 doses</td>
</tr>
<tr>
<td>China</td>
<td>1989</td>
<td>16 boars</td>
<td>12,000 doses (approx.)</td>
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### Table VII

**EC Trade in livestock and meat (in tons)**

**Source:** EC Statistics 1992-93

<table>
<thead>
<tr>
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<th>Eur. 12</th>
<th>Denmark</th>
<th>Ireland</th>
<th>UK</th>
<th>Greece</th>
<th>EC Vaccinating Countries</th>
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<td>1,644</td>
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<td></td>
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<tr>
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<td>591,700</td>
<td>(112)*</td>
<td>(30)*</td>
<td>(172)*</td>
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<td></td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>173,335</td>
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<th>Ireland</th>
<th>UK</th>
<th>Greece</th>
<th>EC Vaccinating Countries</th>
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<tr>
<td><strong>Swine</strong></td>
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<td>210</td>
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<tr>
<td></td>
<td>(1,839)*</td>
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<tr>
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<td>11</td>
<td>10,950</td>
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<td></td>
<td>(37)*</td>
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<tr>
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* Pure bred animals
### Table VII

EC Trade in livestock and meat (in tons)

*Source: EC Statistics 1989*

#### Sheep and Goats

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<th>UK</th>
<th>Greece</th>
<th>EC Vaccinating Countries</th>
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<td>2,984</td>
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<td>(180)*</td>
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<td>-</td>
<td>19</td>
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<td>-</td>
<td>14</td>
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<td>50,486</td>
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#### Bone-in chilled beef

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<th>UK</th>
<th>Greece</th>
<th>EC Vaccinating Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra EC</td>
<td>1,003,809</td>
<td>25,046</td>
<td>11,516</td>
<td>50,492</td>
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* Pure bred animals
### Table VII (contd)

**EC Trade in livestock and meat (in tons)**

*Source: EC Statistics 1989*

#### Boneless chilled beef

<table>
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<tr>
<th></th>
<th>Eur. 12</th>
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<th>UK</th>
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<th>EC Vaccinating Countries</th>
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<tr>
<td>Intra EC</td>
<td>136,211</td>
<td>1,814</td>
<td>439</td>
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<td>26,794</td>
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<td>Southern Africa</td>
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<td>-</td>
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#### Bone-in frozen beef

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<th>Greece</th>
<th>EC Vaccinating Countries</th>
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<tbody>
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<td>Intra EC</td>
<td>54,976</td>
<td>121</td>
<td>5,424</td>
<td>8,284</td>
<td>2,106</td>
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### Table VII

EC Trade in livestock and meat (in tons)

*Source: EC Statistics 1989*

<table>
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<th>Ireland</th>
<th>UK</th>
<th>Greece</th>
<th>EC Vaccinating Countries</th>
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<tr>
<td><strong>Boeef, frozen beef</strong></td>
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<td></td>
<td></td>
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<tr>
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<td>898</td>
<td>963</td>
<td>40,467</td>
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<td>51</td>
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<td>-</td>
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<td><strong>Fresh, chilled or frozen pork</strong></td>
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<td>11,997</td>
<td>84,466</td>
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<td>1,085</td>
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Table VII (contd)

EC Trade in livestock and meat (in tonnes)
Source: EC Statistics 1989

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<tr>
<th>Fresh, chilled or frozen bone-in meat of sheep or goats</th>
<th>Eur. 12</th>
<th>Denmark</th>
<th>Ireland</th>
<th>UK</th>
<th>Greece</th>
<th>EC Vaccinating Countries</th>
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<table>
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<th>Fresh or chilled boneless cuts of sheep</th>
<th>Eur. 12</th>
<th>Denmark</th>
<th>Ireland</th>
<th>UK</th>
<th>Greece</th>
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<tr>
<td>Intra EC</td>
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<td>South America</td>
<td>1,516</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1,516</td>
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</table>
REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: Mr. Dan B. Childs, Lake Placid, FL

J.A. Acree, MD; W.L. Adams, GA; J.N. Armstrong, NV; L.T. Benson, NY; C. Booth, TX; R.B. Caffey, MD; R.A. Carmichael, IA; T.M. Cook, DC; L.A. Detwiler, MD; D.A. Dunn, IL; P.M. Eppele, SD; W.H. Fales, MO; R. Fetzer, VA; W.C. Foote, UT; R. Frost, CA; H.J. Hanson, MA; F.H. Harding, IL; R. Harrington, TX; J.B. Healy, NM; R.D. Heilman, VA; W.P. Heuschele, CA; E. Hoffman, CA; J.L. Hounigan, VA; T.H. Howard, WI; T. Hunt, MI; R.C. Knowles, DE; N. Konnerup, WA; H.A. Kryder, Jr., MD; J. Lemmermen, FL; D.W. Luchsinger, VA; C.A. Mebus, NY; W.D. Prichard, OR; D. Raths, MT; G.B. Rea, OR; T.C. Schooher, TX; J.R. Stafford, VT; P. Sutmoller, VA; P.J. Taylor, MT; S. Telez, TX; S.V. Timberlake, NY; M.C. Turner, TX; W. Utterback, CA; C.D. Vail, CO; W.H. Waldo, NE; J.S. Walker, DC; A. Wallop, DC; C.R. Weston, NH; R.D. Whiting, MD; G.O. Winegar, MD.

The I-E Committee met Wednesday at 1:30 p.m. with 20 members and 18 guests present. In the absence of Chairman Dan Childs the committee was chaired by Mr. Lemmermen.

The Committee received the Export Animal and Products Report FY 1991 from Dr. R. D. Whiting, USDA/APHIS.

Some of the highlights include:

USDA finalized a regulation extending the allowable time period from 72 hours to 30 days for the temporary export of horses to Canada. USDA developed procedures for importation of feeder sheep and slaughter goats from Mexico.

Dr. Bob Goode presented the USDA/APHIS Plant Protection and Quarantine Import Report. Items of interest include:

- There are 42 x-ray baggage inspection sires in operation.
- There are presently 23 trained dog teams at major airports and post offices. This program is slated to expand to 50 teams in the next 2 years.
- Automated Commercial Systems have been installed at 46 PPQ units to facilitate a paperless commercial manifest scan system.

Dr. Jerry Callis spoke to the committee about "International Trade and Foot and Mouth".

Citing data about the sources of FMD outbreaks in the UK and Europe since 1954, Dr. Callis stressed that with the precautions presently being taken, importation of animal products from FMD affected countries can be accomplished with a minimum of risk.
REPORT OF THE COMMITTEE

He also stated that the regulation allowing importation of Bovine Embryos from FMD countries was published Wednesday.

A short discussion about the added costs of washing these embryos ensued and led into the next presenter.

Mr. S. V. Timberlake, Chairman of the Embryo Movement Sub-Committee, reported on the meeting held Monday. The full text of which will be in the Proceedings.

The USDA/APHIS staff including R. D. Whiting, Dr. Henry Kryder, Dr. John Acree, Dr. Sam Richardson and Dr. Lisa Ferguson, discussed a number of topics of interest to members of the committee including:

Quantitative and Qualitative Risk Assessment vs. Just Risk Assessment.
User Fee Proposal.
Requirements for U.S. horses competing in the upcoming Olympics.
Mystery Swine Disease.
Restrictions on U.S. animals exports to the Far East.
Mexican approved facilities for export of U.S. animals.
New CEM requirements for imported horses.
Update - Imported llamas from Bolivia.
Export of bovine embryos and semen to EEC.
Import restrictions on animals and products from known BSE affected countries.
New processing methods for imported meats.
Smuggled bovine fetal serum.
Update on revision of regulations on animal by-products – Part 95.

A complete printed report of all the USDA/APHIS discussions will be in the proceedings.

Two resolutions were passed unanimously by the committee.

One from the Sheep and Goat Committee, involved amending Rule 98, to include Ovine and Caprine embryos as soon as the scrapie histopathology study is completed.

The other passed through the Embryo Movement sub-committee and involves developing a project to determine if embryos after washing can transmit TB either to recipients of resulting offspring.

In other business:

Dr. Knowles, Chairman Infectious Diseases of Horses Committee asked that the following be read into this report:

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IMPORT-EXPORT

EQUINE VIRAL ARTERITIS (EVA)

Recent reports indicate that EVA virus shedding stallions and semen from such stallions are found in interstate and international commerce.

After a lengthy discussion, the committee recommends that USDA and the American Horse Council bring to the attention of horse breed associations the dimensions of EVA and international commerce. They should solicit feedback from these breed groups as to the need to regulate EVA in international commerce. This committee requests that the USAHA Import/Export committee review this EVA situation.

The meeting was adjourned at 4:30 p.m.
The meeting was called to order by Chairman Shelby V. Timberlake at 1:30 p.m. There were (45) in attendance including seven members of the subcommittee.

Dr. Michael Thibier – Director of The Laboratory for the Control of Reproduction, Maison Alfot France reported on EEC movement of genetics. Embryo Transfer is a growing industry in Europe. 32,237 donors were collected in 1990 which is a 26% increase. France had 8,034; USSR 4,800; W. Germany and The Netherlands 2,521.

Transfer of embryos totaled 170,000, a 73% increase. France had 39,944; W. Germany 23,350; USSR 27,000; The Netherlands 14,642; and Czechoslovakia 16,500.

Fresh embryo transfer amounted to 67% in W. Germany; 55% France; 46% The Netherlands; 52% USSR; 81% Czechoslovakia.

Dr. Thibier advised that all semen bilateral agreements are not terminated within the common market and into the U. S. The semen collection center must be inspected and approved by federal authorities. All bulls which qualify must be tested yearly to maintain acceptance. Dr. Thibier added that all EEC countries have stopped FMD vaccination this year. Dr. Thibier reviewed the disease categorization by the Research Committee of The International Embryo Transfer Society. Dr. Thibier discussed the presentation of Appendices to OIE with respect to Porcine, Invitro embryos, including ovine and caprine proposals. In process are the "invitro" fertilized embryos and micro-manipulated embryo appendices. There is some risk of disease transmission in "invitro" transfer.

EEC requirements for embryos are in process with Spain and The Netherlands operating under the new regulations. The United Kingdom has removed the requirement for outside observation by a veterinarian and all other countries require negotiated bilateral agreements.

Dr. David Wilson – Head Development and Review Animal Quarantine & Exports Branch, Australian Quarantine & Inspection Service, Australia discussed embryo collection and imports. He considers as low risk countries: The U.S., Canada, Europe, Japan, Norway, Sweden with respect to FMD. Concern is less testing with an improved embryo collection team. High risk countries – still awaiting recipients on offshore "cocos" islands. Main concern and interest is in Bovine embryos at 10 ET centers in Australia.
EMBRYO MOVEMENT SUB-COMMITTEE

Dr. Wilson reported that about 2000 llamas had been imported to Australia and are interested in deer from Canada.

Dr. Robert Whiting – USDA/APHIS

Dr. Lisa Ferguson – USDA/APHIS (Exports)

Team of vets from EEC to come to U.S. to approve ET facilities for embryo collection. Most with mobile units.

Dr. Whiting – discussed Part 98, Code of Federal Regulations – change in Part 98 occurred in August 1990. Part 92 was rearranged to afford changes. Part 98 first addressed the import of green plum to the U.S. attempt underway to rewrite the laws which affect imports.

TB testing of donor cows. Imports require a permit with specific test requirements. U.S. require one year without an outbreak to receive an FMD free status. Europe requires 6 months after last outbreak or variations. Dr. Sam Richardson – USDA-APHIS spoke with respect to publication of 9 CFR, Part 98 and its changes to become effective November 29, 1991.

Dr. Tony Wrathall – Head Diseases of Breeding Department Control Vet Lab, Weybridge, England – reported there are 10–15 ET teams in the U.K. Half of them do ET work full time, however, most work for individual farms.

Dr. Wrathall reported on the horse embryo work done with imported polo ponies. The horse embryo is more difficult to handle which has resulted in very few pregnancies in these early tests. More work needs to be done to determine if the embryos suffer damage during the washing process.

Dr. Wrathall gave a detailed report on the very serious BSE (Bovine Spongiform Encephalopathy) epidemic in Great Britain which has seriously cutoff export of animals which has had an enormous impact on the agricultural economy of the country. Dr. Wrathall believes that the epidemic is levelling off and will decline in the coming months due to drastic curtailment of certain food supplements fed to the cattle in that country.

Dr. Wrathall described the current research program now underway in Northern England involving imported New Zealand bovines. It is hoped that this seven year research project will show that embryos can be collected from BSE positive donors with good results.

Dr. Claude Lavigne – AG Canada reported that embryo exports from that country totalled 11,600 and were shipped to nineteen different countries. 240 embryos were imported from Belgium, France, and other countries.

In 1992 there will be an inspection tour to Canada. 14 teams have been approved and anticipate 22 by 1992.

Discussion and negotiations for U.S. Exports with respect to collections and qualifying donor animal ongoing. May soon have agreement for movement to the U.S.

Dr. John Acree – Argentina Embryo Transfer/FMD Field Trials

Dr. Acree gave a short detailed review of the results of this project which will be published by the end of 1991.

Chairman Timberlake – Reviewed the contents of Dr. W. C. Hares
communication with respect to the new work being done at the University of Utrecjt for invitro fertilization of oocytes from genetically superior animals using transvaginal ultrasound guided follicular aspiration from naturally cycling cows. Dr. Hare advised that it is most important that regulatory officials be aware of this work in order to prevent the destruction and/or loss of genetically superior animals because of too restrictive health requirements. Officials must know the risks of disease transmission using these procedures.

Dr. Paul Sutmoller – discussed the testing of collection fluids and washing fluids of Bovine Embryos.

Dr. Warren Foote – presented an update on the "ongoing" sheep scrapie research project. He reported no transfer of scrapie through embryo transfer.

The "open" meeting of the committee was adjourned at 4:45 p.m.

There followed a meeting of the following members of the committee:
Chairman Timberlake
Dr. Konnerup
Dr. Inebus
Dr. Luchsinger
Dr. Carmichael
Mr. Hansen
Dr. Jerry Walker

The committee discussed the following matters:
1. The purpose of the committee as requested by the Board of the USAHA. Our written statement is attached and was approved by the committee unanimously.
2. Resolution on The Transmission of Bovine Tuberculosis via Bovine Embryos. The resolution was passed unanimously for presentation at the Import–Export meeting by Dr. Jerry Walker (See attached).

There being no further business to discuss, the committee adjourned the meeting at 5 p.m.
APPLICATION OF MULTIPLE DIAGNOSTIC TESTS TO
THE DIAGNOSIS AND PROFILING OF M.
PARATUBERCULOSIS INFECTED HERDS.

Anderson, P.R., Seymour, C.L., P.R. Wood +, Sackett D.C.*, Collins, M.T.*
IDEXX Laboratories, Portland ME 04101, + CSIRO Division of Animal Health,
Parkville, Australia 3052, *Department of Pathobiological Sciences, University
of Wisconsin, Madison, WI 53760

ABSTRACT

To obtain a better understanding of disease progression and provide
a means of profiling the disease within a herd, three types of diagnostic tests
were applied to study herds. Humoral immune response to M.
paratuberculosis was evaluated using an absorbed antibody ELISA Cellular
immune response to M. paratuberculosis was evaluated by measuring the
release of bovine γ-IFN from whole blood cell cultures stimulated with
mycobacterial antigens. Cattle shedding M. paratuberculosis were identified
using either culture or the M. paratuberculosis specific DNA probe. Utilization of the two tests to measure immune response gave results which
exhibited good sensitivity and specificity and allowed detection of
immunologically sensitized animals at both early and late stages of infection.
Information from the γ-IFN assay can be used in conjunction with the
absorbed antibody ELISA and the DNA probe to create a herd profile in less
than a week- Monitoring both disease progression and eradication on a herd
basis and in individual animals can be performed using a herd profile and
repeated testing.

INTRODUCTION

Mycobacterium paratuberculosis is the causative agent of Johne's
disease, an infectious enteritis of cattle. Infection can occur in animals at a
very young age. Intrauterine transmission is known to occur in about 25%
of the calves born to infected and critically ill cows. Calves can also be
infected by ingesting Colostrum and milk from infected cows. Despite the
likelihood of early infection, clinical manifestations of the disease may not be
apparent in cattle until the animal is 2 to 5 yews of age. Adult transmission
can occur by the ingestion of feed and water which has been contaminated
with manure harboring M. paratuberculosis.

An infected herd will consist of:

1. Animals with clinical disease actively shedding mycobacteria.
2. Infected animals exhibiting no clinical disease, but actively shedding
mycobacteria.

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MULTIPLE DIAGNOSTIC TESTS

3. Infected animals which are apparently healthy and not shedding detectable levels of mycobacteria.

4. Animals exposed and immunologically sensitized to mycobacterial whose infection status is unknown.

5. Uninfected cattle.

Successful control and eradication of this disease from infected herds will require diagnostic tests which can identify animals in each of these five categories.

IDEXX has developed and received a USDA license for the Mycobacterium paratuberculosis DNA test kit. This Johne’s DNA Probe test is used for the identification of cattle that are actively shedding Mycobacterium paratuberculosis as in cases 1 and 2 above.

A pro herd management system requires a testing procedure that can identify infected animals prior to fecal shedding of M. paratuberculosis as in crises 3 and 4, as well as uninfected animals showing no evidence of exposure (case 5.). Two new test systems have been developed to address this need: a serological test kit for the detection of serum antibody response, and the γ-IFN test kit for the measurement of an animal’s T-cell mediated immune response. The aim of these two products is to develop a flexible three pronged approach for the management of herds. The γ-IFN test kit was designed to screen and aid in the management of cattle herds thought to be infected with Mycobacterium paratuberculosis. This screening tool together with serology would be used to identify and segregate Mycobacterium paratuberculosis exposed animals. Final diagnosis of Mycobacterium paratuberculosis infection would be made by identification of the organism using microbiological culture or the IDEXX Mycobacterium paratuberculosis DNA test kit.

Measurement of immunological responses in the diagnosis of Mycobacterium paratuberculosis is limited by the fact that M. avium is antigenically related to Mycobacterium paratuberculosis (Camphausen, et al., 1988). Despite this fact distinct advantages to monitoring both serological and cellular immune responses to these shared antigens of Mycobacterium paratuberculosis we indicated by the results presented in this paper.

The Mycobacterium paratuberculosis γ-IFN test kit detects animals in the early stages of infection, so they can be identified and separated from animals which show no evidence of exposure to mycobacteria. The test kit utilizes a ratio of bovis to avium PPD response that can be used to rule out infection with Mycobacterium bovis, and indicate a preferential response to Mycobacterium paratuberculosis/M. avium related antigens.

When animals have progressed to the stage of disease where fecal shedding occurs, the Mycobacterium paratuberculosis organism can be positively identified by culture or DNA probe.

The ability to identify a cell-mediated immune response (CMI) to Mycobacterium paratuberculosis in the early post-infection stage of Johne's disease prior to
fecal shedding or antibody response, demonstrates the value of detecting cell-mediated responses in herds as a screening tool and in the management of known infected herds. This is shown in Table 1. The rationale for this approach has been evaluated in two published articles which discuss the utilization of lymphocyte transformation in the diagnosis of *M. paratuberculosis* infection. Both papers found lymphocyte transformation assays to be efficacious in the diagnosis of paratuberculosis (Buergelt et al., 1977, and Milner, et al., 1981). Lymphocyte transformation assays are impractical for conventional screening since they require the use of radioactive isotopes and a three day incubation period. The γ-IFN assay described in this paper measures the same parameter as the lymphocyte transformation assay, with an easy non-radioactive format and makes this type of assay practical for use in any laboratory that performs ELISAs.

The antibody test kit detects animals in the intermediate and later stages of infection, so they can be identified and separated if possible from animals which show no evidence of exposure to mycobacteria. The test kit utilizes a preabsorption step which is designed to remove antibodies which are not specific for *M. paratuberculosis*.

The development of an antibody response in cattle to *M. paratuberculosis* during the progression of the disease is illustrated in Table 1. A number of serological tests are currently in use to measure this antibody response: the complement fixation test an agarose gel immunodiffusion test and the enzyme-linked immunosorbent assay (ELISA). ELISA technology has evolved over the years. With the addition of a preabsorption step, significant improvements in specificity were obtained (Yokomizo, 1986). In the preabsorption step, test sera are incubated with Mycobacterium phlei antigens to reduce non-specific reactions. This approach has been further refined by investigators at the Commonwealth Serum Laboratories (CSL) in Victoria, Australia. This test kit which CSL has developed and for which IDEXX is making an application for a USDA license has been extensively evaluated both in Australia and in the United States. Two recently published articles describe the performance of the test kit (Collins, et al., 1991; and Cox et al., 1991).

**MATERIALS AND METHODS**

**SAMPLE COLLECTION**

The *M. paratuberculosis* antibody test kit will detect antibody to *M. paratuberculosis* in bovine serum or plasma. A sample of blood is collected into a sterile evacuated collection tube. A standard serum collection tube may be used. If a herd is to be evaluated for both humoral and cellular immune response to *M. paratuberculosis* (the later utilizes the *M. paratuberculosis* gamma interferon test kit) a tube containing sodium heparin
MULTIPLE DIAGNOSTIC TESTS

can be used to collect the sample and then gently mixed. The sample may then be split for use in both tests.

For the herds surveyed in this study fecal samples were collected and then examined by the M. paratuberculosis DNA probe test kit. For a number of the herd studies described, samples were split and subjected to radiometric culture (Collins, et al., 1990) and conventional culture (Whipple, et al., 1991).

M. PARATUBERCULOSIS ANTIBODY ASSAY

The plasma or serum samples to be assayed for antibody to M. paratuberculosis were incubated with sample diluent containing M. phlei antigen for a minimum of 30 minutes at room temperature. Test samples and controls were then added to microwells coated with M. paratuberculosis antigen. Following a 30 minute incubation step and a wash step, an horseradish peroxidase antibody conjugate specific for bovine immunoglobulin was added. A second incubation step (30 min.) and awash step was followed by addition of substrate/chromogen solution. Following the last incubation step (10 min.), optical density of each well was measured spectrophotometrically and recorded.

The positive cutoff value for a valid assay was the mean of the negative controls plus 0.100, e.g., if the two negative controls were 0.055 and 0.085, the cutoff would be 0.070 + 0.100 = 0.170. A positive result indicates the animal has been immunologically sensitized to M. paratuberculosis. A negative result for a single animal can only be interpreted when the paratuberculosis history and test results of the entire herd are known.

M. PARATUBERCULOSIS γ–IFN ASSAY

Within 12 hours of blood collection, three heparinized whole blood cultures were prepared, one without antigen, a control culture, one with M. avium PPD, and one with M. bovis PPD. These samples were incubated at 370 C for 24 hours. The cells were then removed by centrifugation and the plasma was collected and stored frozen or refrigerated for assay the following day.

The plasma samples to be assayed for bovine γ–IFN were incubated in microwells coated with mouse monoclonal antibody to bovine γ–IFN. Following a 1 hour sample incubation and a wash step, a mouse monoclonal:HRPO conjugate specific for bovine γ–IFN was added. A second incubation step (30 min) and awash step was followed by addition of substrate/chromogen solution (30 min.) After this final incubation step, the optical density of each sample was measured and recorded.

An avium culture OD that was 0.10 units above the control culture's
OD value, indicates that the T-cells present in the M. avium PPD culture have recognized the avium antigen and have released γ-IFN into the plasma. Preferential response to M. avium was indicated by calculating the ratio of ELISA OD values for the bovis PPD culture to the M. avium PPD culture. If this ratio was less than 0.7, the animal was preferentially responsive to M. avium PPD. This preferential response was indicative of exposure to mycobacteria of the M. paratuberculosis/M. avium group.

M. PARATUBERCULOSIS DNA PROBE

Fecal samples were processed, PCR amplification performed, and products analyzed according to the IDEXX DNA probe test kit product insert.

RESULTS

The results from the M. paratuberculosis γ-IFN studies are presented as the ratio of the levels of γ-IFN produced in response to M. bovis PPD and M. avium PPD. Samples were determined to be γ-IFN positive if the ELISA OD obtained in the γ-IFN assay for the avium whole blood culture exceeded that for the control whole blood culture by 0.10 OD units (avium−control value) and the ratio of ELISA OD for the bovis whole blood culture to the ELISA OD for the avium whole blood culture was less than 0.7 (bovis/avium ratio). The results for the M. paratuberculosis antibody test kit for each herd were also presented. In this assay, a sample is called positive if its ELISA OD value was greater than the negative control mean OD plus 0.10 units.

An example of the application of these tests is illustrated by the M. paratuberculosis infected herd SD. Fresh whole heparinized blood and fecal samples were collected from a beef herd with a previous history of Johne's. The herd consisted of 219 adult cattle, 206 cows and 13 bulls. Fifty-two yearling calves were also tested (27 heifers and 25 bulls). All adult animals were tested using the DNA probe, γ-IFN, and antibody tests. All calves were tested using the antibody and the γ-IFN test kits.

The frequency distributions obtained in the γ-IFN test for herd SD we presented in Figures 1 and 2. The mean of the A−C values for the herd was 0.055 with a standard deviation of .192. The mean of the bovis/avium ratio was 0.902 with a standard deviation of 0.273. These values are not very different than those seen for a Johne's free herd. This suggests that the level of infection was low in this beef cattle herd. Indeed only six shedders were found in the 219 adult animals tested by the DNA probe. Only 29 of the 219 adult cattle (13.2%) tested positive by at least one of the 3 methods (DNA probe, antibody, and γ-IFN). A frequency distribution of antibody ELISA OD values is shown in Figure 3, the cutoff value was 0.244. As shown in Figure 4, of the 6 DNA probe positive animals, 5 were antibody positive, and only one was γ-IFN positive. Of the 29 animals positive by any
test 21 were positive by the γ-IFN test while 11 were positive utilizing the antibody test. The 17 DNA probe negative, antibody negative, and γ-IFN positive animals (−+) were segregated from the remainder of the herd. Four months later, as these cows were calving, they were retested by all three methods. One cow (823) remained γ-IFN positive, became positive on the antibody ELISA and was shedding *M. paratuberculosis* as identified by the DNA probe. Another cow (647) did not change its status. It remained γ-IFN positive and had an elevated antibody ELISA OD which was still below the cutoff. The remainder of these cows went from a γ-IFN positive status to γ-IFN negative. During the eight month period following this testing, no clinical cases of Johne's disease were observed in this herd.

**DISCUSSION**

Four major advantages of the combined use of the *M. paratuberculosis* antibody and γ-IFN tests have been demonstrated by this and other studies. They are:

1. Good specificity (97%) and sensitivity (90%).
2. Ability to detect immunologically sensitized animals at an early age (as early as 14 months of age).
3. Information from the γ-IFN assay can be used in conjunction with antibody ELISA and DNA probe to create a herd profile in less than a week.
4. Monitoring both disease progression and eradication on a herd basis, and in individual animals, can be performed using a herd profile and repeated testing of the herd.

We believe that the use of the assays described provide producers with the additional tools required for the control and management of Johne's disease.

**LITERATURE CITED**

ANDERSEN,SEYMOUR,WOOD,SOCKETT,COLLINS

29:2514–2519.


TABLE 1


<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>Early Post Infection</th>
<th>Intermediate (incub. period)</th>
<th>Advanced Clinical (Disease)</th>
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<tr>
<td>Fecal Shedding</td>
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<td>+++</td>
</tr>
<tr>
<td>Antibody Response</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Skin Test</td>
<td>+(-)</td>
<td>+(-)</td>
<td>+(-)</td>
</tr>
<tr>
<td>Cell–mediated Response</td>
<td>+++</td>
<td>+++</td>
<td>+(-)</td>
</tr>
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MULTIPLE DIAGNOSTIC TESTS

HERD SD
N = 271

Figure 1

HERD SD
N = 271

Figure 2

274
**DNA TEST POSITIVE**

<table>
<thead>
<tr>
<th>Result</th>
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<tr>
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<td>830(68.35)</td>
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<td>+++</td>
<td>806(82.84)</td>
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<td>++</td>
<td>521(60.96)</td>
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**DNA TEST NEGATIVE**

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<tr>
<td>++</td>
<td>695(19.55)</td>
</tr>
<tr>
<td>+++</td>
<td>6(05.27)</td>
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</table>

CATTLE

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<tbody>
<tr>
<td>911(32.82)</td>
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<tr>
<td>827(1.6.58)</td>
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NOT

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<thead>
<tr>
<th>O.D. Value</th>
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<tr>
<td>917(50.60)</td>
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<tr>
<td>908(80.5)</td>
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LISTED

<table>
<thead>
<tr>
<th>O.D. Value</th>
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</thead>
<tbody>
<tr>
<td>647(16.69)</td>
</tr>
<tr>
<td>510(04.35)</td>
</tr>
</tbody>
</table>

Results presented as (probe, antibody, γ-IFN)

**Figure 4**

---

*ANDERSEN, SEYMOUR, WOOD, SOCKETT, COLLINS*

**JOHNE'S INFECTED HERD S.D.**

*N = 242*

![Graph showing optical density data](image)
ENVIRONMENTAL SURVEY FOR MYCOBACTERIUM PARATUBERCULOSIS ON DAIRY FARMS WITH A KNOWN HISTORY OF JOHNE'S DISEASE*

By
Dr. Robert H. Whitlock, Ms. Anne E. Rosenberger, Mr. Mark Siebert, Dr. Ray Sweeney

New Bolton Center
Department of Clinical Studies
School of Veterinary Medicine
University of Pennsylvania
Kennett Square, PA 19348
215–444–5800

*Funded in part by the American Guernsey Association and the Pennsylvania Department of Agriculture

The extent and persistence of Mycobacterium paratuberculosis environmental contamination on farms with a known history of Johne's disease is a commonly asked question by the agricultural community. Prospective buyers of farms following a dispersal sale often inquire about the possibility of newly acquired cattle becoming infected with M. paratuberculosis from the environment where Johne's cattle had previously been located. Thus, based on continuing inquiries from cattlemen, we developed a prospective research proposal to assess the extent of environmental contamination with M. paratuberculosis associated with known levels of infection in the herds as determined with whole herd fecal culture tests of adult cattle using a sensitive technique utilizing centrifugation (Whitlock 1989).

Experimental Design:

Eleven herds previously known to have had multiple animals infected with M. paratuberculosis were selected because of the willingness of the herd owners to cooperate by allowing the investigators to take the environmental samples and the farms were located within 150 miles of New Bolton Center, Kennett Square, PA. The herds were believed to represent a cross-section of dairy management practices in Pennsylvania. The herd size ranged from 40 to 125 milking cows in a stanchion barn or free-stall design barn. The herds were either of the holstein or guernsey breed.

Approximately 50–60 individual predetermined sites were selected from which to obtain the environmental sample specimens. Samples from sites inside the barn were scraped with a clean plastic cup and the placed in a four ounce specimen container. Soil samples from outside the barn were
SURVEY FOR MYCOBACTERIUM PARATUBERCULOSIS

collected with a clean metal trowel and then placed in a covered specimen container. Obvious manure samples were avoided. Maternity stalls, calf hutches, mangers, feed bunk areas and heifer pens provided sites for 20–25 samples from inside the barn. The cow pasture, exercise lots, heifer pastures, silage feeding areas both moist and dry represented the predetermined sites outside the barns. All environmental samples were collected between June and August, 1990.

Culture procedures:

A two gram aliquot of each environmental sample was processed using the double incubation described by Shin. (Ref) Each of the four tubes containing mycobactin and pyruvate (4.1 gm/liter) was inoculated with 0.20 ml of resuspended pellet. The tubes of Herrold’s egg yolk media was incubated at 37°C for 16 weeks, with the tubes usually being evaluated every two weeks for colonies compatible with M. paratuberculosis and contaminants. Colonies morphologically compatible with M. paratuberculosis were sub-cultured onto two tubes, one with and one without mycobactin. The organisms were stained with Ziehl–Neelsen stain and evaluated microscopically to be acid–fast and club or dumbbell shaped.

Results:

Five of the eleven farms sampled had samples positive for M. paratuberculosis. Four of the five farms had two or fewer positive samples. Only one farm had extensive environmental contamination with 13 of 59 samples positive, many with high colony counts. Of the total of 672 environmental samples obtained, 20 or 3% were culture positive for M. paratuberculosis. Except for the heavily contaminated farm all positive cultures were from the pastures and exercise lots and not inside the barn.

Discussion:

Previous attempts to isolate M. paratuberculosis from soil utilizing the techniques reported to be adequate for fecal samples resulted in extensive contamination primarily from fungi but also included bacteria. (Whipple, Whitlock) The contamination rate often ranged between 70–100% of the tubes inoculated. Therefore, until techniques were developed to control the excessive contamination of the culture tubes the project was delayed. The development of the double incubation technique by shin and co–workers at Cornell represented a major advance in the culture methodology since this technique virtually eliminates both bacterial and fungal contamination in fecal cultures for mycobacteria. The contamination rate is further enhanced by centrifugation which concentrates the fungal elements and bacterial spores along with the mycobacteria.

The frequency of occurrence of Mycobacterium paratuberculosis in the environment of farms with known infection of cattle with M.
paratuberculosis has not been reported. However, the survival of *M. paratuberculosis* in water, slurry, urine and other media has been reported. (Jorgenson, Larsen, Lovell, McCaskey). Previous authors often utilized laboratory grown isolates then inoculated a variety of materials and monitored the recovery rate over time.

Larsen first reported that bovine urine inhibited growth of *M. paratuberculosis* in the laboratory with decreased growth directly related to increasing concentrations of urine. With 2% urine in the media no growth occurred. The critical value seemed to be between 2% with no effect and at 5% urine in the media with complete cessation of growth. The addition of bovine feces also inhibited growth in a proportional manner. Thus, it would seem that recovery of *M. paratuberculosis* in a slurry media would be inhibited, however, the slurry storage experiments would suggest otherwise.

Larsen found that *M. paratuberculosis* survived in tap water or saline for up to 17 months, with the pH adjusted to either be more acid at 5.5 or more alkaline up to 8.5; with added 1% gelatin the survival time was still 14 to 17 months. Suspension of organisms in bovine urine marked reduced survival to less than a month. The constituent in urine inhibiting growth or survival of *M. paratuberculosis* was not identified.

The survival of *M. paratuberculosis* in both swine and bovine slurry was studied experimentally by inoculating 0.1 mg (3.3 X 10^6 viable units) per ml then storing the slurry in sealed vials at 5^\circ C or 15^\circ C under anaerobic conditions. (Jorgensen 1977) Viable organisms were detectable up to 252 days in both swine and cattle slurry at 5^\circ C while survival was reduced to 182 days for swine slurry and 98 days for cattle slurry when stored at 15^\circ C. The pH of the slurry from both swine and cattle was quite alkaline (8.3 to 8.5) which may favor survival in as much as *M. paratuberculosis* grows better in an alkaline environment. The initial dose of viable organism used per ml of slurry was likely in excess of the concentration in even the environment from a farm with many infected cattle. The concentration used was nearly 10,000 fold greater than present in the manure of a cow with an advanced stage of infection, but not yet showing clinical signs. Jorgensen's results were similar to those of Lovell, et. al. (1944) who found that naturally infected cattle feces contained viable organisms after exposure to atmosphere conditions for 246 days.

Test cultures of *M. paratuberculosis* placed in bovine manure silage and corn silage became negative by the fifth day after ensiling. The pH of the silages were less than 4.2 while media above 4.6 supported growth of *M. paratuberculosis*. (McCaskey 1981) The recovery of mycobacteria from environmental samples is a function of several independent processes including sample size, decontamination procedures, sample preparation procedures, inoculum size per tube and recovery medium composition. We believe the process utilized in this current study to be state-of-the-art but not necessarily optimal. The double incubation step nearly eliminated both
bacterial and fungal contamination yet did not impair growth of *M. paratuberculosis*. (unpublished data) However, it is possible and perhaps likely that the environmental contamination is higher and more extensive than the data described. Although *M. paratuberculosis* was not detected in the samples, it is not proof the organism was not present. Similarly infected cattle likely shed the organism in the feces for many months prior to detection by standard methods. In all likelihood the organism was present but the culture techniques did not have the sensitivity to detect *M. paratuberculosis*.

**Conclusions:**
Although previous published data suggested that *M. paratuberculosis* can survive for prolonged time periods (12–16 months) under a variety of experimental condition, the authors are unaware of any previously published information determining the prevalence of positive cultures from known infected herds. Therefore, we believe this study to represent the first report describing the extent of environmental contamination on dairy farms with cattle infected with *M. paratuberculosis*. The environmental contamination was very closely correlated to the herd prevalence of Johne's disease and the number of colony forming units of *M. paratuberculosis* present in the manure of infected cattle.

**References:**


REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

Chairman: Dr. Robert H. Whitlock, Kennett Square, PA  
Vice Chairman: Ms. Diana L. Whipple, Ames, IA

R.D. Angus, IA; M. Chaddock, MI; M.T. Collins, WI; T. Conger, AR;  
R.W. Dellers, WI; R.J. Eisner, NJ; C.F. Emerick, WA; C.Y. Erbel,  
NC; M.A. Essex, MD; W.H. Fales, MO; J.L. Funk, IL; A. Graybeal,  
ME; J.P. Huntley, NY; S.B.S. Hurley, WI; R.J. Jacobsen, NY; J.L.  
Jamagin, IA; R.L. Jones, CO; 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.A. Rossiter, NY; S.J. Shin, NY; D. Sockett, WI;  
C.O. Thoen, IA; R. Velure, ND; H.A. Virts, MD; H.W. Whitford, TX;  
B. Widger, NY; R.E. Yoxheimer, PA.

The committee meeting began at 1:15 PM included 15 presentations,  
5 subcommittee reports and concluded at 7:00 PM. Attendance included 21  
of the 36 committee members and 38 guests (many did not sign the  
register).

Dr. Jacobson discussed the various serological tests for Johne's  
including the LAM, D, and protoplasmic antigens and the Australian CSL test  
kits. None are ideal, but the partially purified protoplasmic antigen seems to  
hold the most promise to detect cattle at earlier stages of infection. Dr.  
Anderson presented encouraging information about the gamma interferon  
test which should detect cattle in their earlier stages of infection and when  
used in conjunction with the DNA probe and the elisa test will give much  
more information about the infection status of the herd as well as individual  
cattle. The reported specificity for each of these tests was high with an  
overall specificity nearly 98%.

Dr Collins reported an excellent interlaboratory correlation test  
performance among 8 laboratories to evaluate the Australian CSL test kit for  
Johne's disease. This test kit is currently under USDA review. Greater than  
99% specificity and reasonable sensitivity has been reported for the test  
which utilizes an absorbed elisa format. Dr. Lein reported on a 14 month old  
bull that was fecal culture positive for M. Paratuberculosis. Tissues  
collected at slaughter at 18 months of age were heavily infected including the  
seminal vesicals and epididymis, but with minimal histologic lesions in the  
accessory sex glands.

Drs. Shin, Whitlock and Whipple reported on a collaborative study  
to critically evaluate constituents of HEY media. Initial evaluation indicates  
that the current media constituent concentrations may not be optimal for both  
recovery of M. paratuberculosis and rate of growth. Each laboratory will  
investigate three constituents at 5 different concentrations using fecal  
samples from 25 cattle previously culture positive originating from 7 different  
herds. Preliminary results suggest it is possible to markedly improve the  
sensitivity of the fecal culture technique and to reduce bacterial and fungal
contamination to a minimum level ie less than 5%. In an effort to enhance the quality control of fecal culture techniques used among the state, private and other diagnostic laboratories, up to 70 individual samples with more than 50% from previously culture positive animals were distributed to 20 laboratories to process. The tests will be complete soon after the first of the year. Dr. Whitlock will tabulate the results for next years meeting.

Mr. Nelson presented resolutions from the Purebred Dairy cattle Association, the Holstein Association and the Livestock Conservation Institute all of which supported the development of criteria for a “National Certification Program for Paratuberculosis” (NCPP). Several states including Pennsylvania, Wisconsin, New York and Maryland either have certification programs or will in the near future. Drs. Olson, Sackett, Whitlock and Chaddock reviewed programs for their states. Dr. Essey provided insight about the significance of a federal program disease while reviewing the tuberculosis eradication program.

SUBCOMMITTEE REPORTS:

Standardized culture method – D. Whipple reported on a study comparing three fecal culture techniques: sedimentation; centrifugation and the double incubation utilizing 145 fecal samples; 35 were positive on at least one technique; while 29 (82%) were positive with centrifugation; 18 (51%) were positive on the double incubation technique and 10 (29%) were positive with the sedimentation technique. A major drawback with the centrifugation technique was the high rate of contamination nearly 80% of the tubes. Current research work in progress should lead to marked improvements in the culture techniques in the next 1 – 3 years. A publication detailing a standard technique has been submitted and will be published in the October issue of the J.Veterinary Diagnostic Investigation, 1991. This report was moved for acceptance – Approved.

Serological test standardization – R. Jacobson reported that a detailed proposal will be forthcoming next year. The elements of a serologic reference center were outlined.

Johne’s management recommendations – C. Rossiter A draft of these recommendations were outlined and will be provided in full detail to the committee next year.

Quality control for M. Paratuberculosis culture – M. Collins reported that NVSL could not provide any quality control for Johne’s fecal culture unless it was a national program disease.

Herd certification criteria – D. Sackett (for A. McLaughlin) indicated a willingness to help establish the criteria but with the retirement of McLaughlin, a full report was not available.

The chair appointed D. Whipple to head a new subcommittee to establish criteria for a national certification paratuberculosis program (NCPP). This was seconded and approved. This effort would be conducted in concert with LCI and several other organizations.
IMPLANTABLE ELECTRONIC IDENTIFICATION, AN UPDATE OF GLOBAL FIELD TRIALS AND ITS APPLICATION IN ANIMAL DISEASE CONTROL AND ERADICATION PROGRAMS.

By Kevin D. Maher 10
10–1–91

The activities around the world in field testing applications of electronic identification (EID) in livestock have accelerated in the past two years. Many government agencies and animal industry groups have implemented field testing of the technology for the following reasons:

1. Security of EID in specific anatomical sites
2. Automation of data collection
3. Reduction in the number of man hours required to process animals, laboratory samples, and reports.
4. Increased accuracy of animal ID recording and tracking due to less hand transcribing of numbers.

The following summarizes numerous trials around the world involving over 20,414 animals of the Destron/IDI identification system:

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>Ovine/caprine</th>
<th>Porcine</th>
<th>Bovine</th>
<th>Equine</th>
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<tr>
<td>United States</td>
<td>252</td>
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<td>Canada</td>
<td>100</td>
<td>2000</td>
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<tr>
<td>Other</td>
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<td>250</td>
<td>150</td>
<td>150</td>
</tr>
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</table>

Total          | 4477          | 5983    | 1605   | 8350   

10 Manager, Field Support Destron/IDI, 2545 Central Ave., Boulder, CO 80301
IMPLANTABLE ELECTRONIC IDENTIFICATION

The animal disease control and eradication aspects of these trials are underscored by countries in need of a secure and automated solution to the massive task of tracking millions of animals. The additional compliment of software linking EID to barcode technology and the microchip reader, allows increased automation to test and track a high volume of animals.

SPECIES GROUP DISCUSSION

Ovine and Caprine

In the U.S. much effort has been accomplished in locating a suitable anatomical site for the EID transponder to be used in the sheep industry scrapie control program. Over 252 sheep (half adult animals) have been implanted to verify a suitable anatomical site in the ear. A site has been discovered which is not readily palpable externally. We are conducting trials at a sheep slaughter facility to verify that the transponder will be removed with the ear and pelt during slaughter.

The "Scrapie Negotiated Rulemaking Advisory Rulemaking Committee" has issued the "Scrapie Flock Certification and Animal Identification Procedures" in the Federal Register including electronic ID as part of the certification program. Much effort has been expended in trials and keeping the U.S. sheep industry informed on our progress in the utilization of EID in this important program.

Southern European interest in EID is for disease eradication of Brucellosis in cattle, sheep, and goats as well as other disease concerns. The country of Malta has chosen to implement EID as their method of tracking animals and information in the eradication of Brucellosis, Leucosis and T.B. testing. A software program has been developed in conjunction with the program requirements. The software will accept barcode entered data as well as ID's downloaded from the reader/scanner. Reactive animals may easily be sorted by provisions made in the hardware and software.

Many European countries presently utilize tattoo and neck tags for ID of animals. They want a more secure and automated method of handling the task of eradication of disease in millions of sheep, goats, cattle and swine.

Animal breed registries are interested in the incorporation of EID into their procedures and are looking for added security in maintaining purity in genetic lines.

BOVINE

The U.S. and Canadian trials have been directed toward the dairy industry. In Canada, 690 animals were implanted with the large transponder (3.5mmx29mm). in the mid-ear site. Holstein Canada has voted to begin introduction of the Destron/IDI system via Anitech Identification Systems;
exclusive Canadian distributor for Destron/IDI. The implantation of transponders in dairy animals is expected to commence in 20 pioneer herds beginning fall 1991. The decision by Agriculture Canada to allow the introduction follows their evaluation of the previously mentioned trial.

In the U.S., Anitech Identification Systems has been granted approval from F.D.A. to proceed with trials in lactating dairy cows with 0 hours milk withholding. Cull dairy cows are also involved in the slaughter portion of the study for removal of the transponder. Over 660 animals are involved in various studies involving the large transponder in the mid–ear site.

The U.K. has led the European countries in the evaluation of EID in cattle. The Meat and Livestock Commission (MLC) has conducted trials involving four vendors of EID. The trial began in the fall of 1990. Destron/IDI results to date on 249 cattle have revealed a success rate of 97.2%. The transponder is placed vertically in the area of the ear attachment to the head. The slaughter phase of the trial is underway and results are pending.

**PORCINE**

The Dutch trials involving EID in swine has been well published in journals over the past two years. In January, 1991, the EID trial entered the field test (practical phase) involving 5,000 pigs per vendor. The swine producers themselves are implanting the pigs in this phase. Thus far, over 1200 animals have been implanted in the practical phase (delayed by the outbreak of MSD) and 1121 pigs from an earlier phase. The site selected by the PVV remains vertical to the base of the ear; in the neck of the animal.

Destron/IDI has sponsored a trial in conjunction with the Dutch research institute IVO to evaluate the medium transponder (3.0mmx19mm) in three anatomical ear sites. We have discovered one site, which with further investigation, indicates high retention (over 99%) and transponders are more easily removed at the slaughter plants. Similar ear implanting trials are underway in three additional countries.

During the FDA filing process, an eartag containing the transponder is being researched.

**EQUINE**

A recent trial in Colorado of 50 mares was conducted (in conjunction with Colorado State University). The 90 day results indicate no rejection and no abnormal inflammatory response. X–Ray indicated there was no migration and transponder implantation occurred in the desired location. The site of transponder implantation was in the ligament nuchae.

An additional 250 foals have been implanted with the small transponder (2.1mmx11mm) at a Standardbred farm in Kentucky from the
IMPLANTABLE ELECTRONIC IDENTIFICATION

spring of 1989 to the spring of 1991. No rejections or failures were reported in repeated readings over the two year period.

SLAUGHTER PLANTS

Currently five plants have been integrated in North America with EID. More work is underway to evaluate the needs of the slaughter industry and provide them a reliable ID system that has the potential to integrate with producer animal ID. EID in the slaughter industry is gaining momentum in the U.S. as more high tech. methods of carcass evaluation are incorporated as well as offering automated evaluation and reporting advantages in disease surveillance programs.

F.D.A.

Currently we are cooperating with the F.D.A. request to oversee implantable EID under the provisions of an I.N.A.D. for a food additive petition. If ears are not rendered and become landfill, it appears EID would be ruled as a Veterinary device and the food additive petition would not apply. This would require support in various segments of industry and government. It appears the food additive petition process has the potential to turn into a lengthy one thus slowing the progress and potential integration of a superior form of animal identification that has been needed for decades. EID presents vast benefits for potential improvement of the health of the animals in the U.S. and around the world.
 REPORT OF THE COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: Dr. E.R. Hinshaw, Phoenix, AZ
Vice Chairman: Dr. Donald R. Bridgewater, Northglenn, CO

J. M. Alumbaugh, IA; J.B. Ashcraft, CO; J.S. Cargile, TX; J.P. H.F. Embry, IL; M.A. Essey, MD; J. Fraley, IL; R. Gadd, SD; B. Gallagher, SD; L. Graham, MT; T.V. Haas, KY; R.D. Heilman, VA; 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, NY; G.W. Roberts, CA; N. Robinson, MO; E.C. Roukema, VA; G.L. Seawright, NM; J. Selz, WI; J.G. Shoun, CO; S.L. Spahr, IL; W.E. Stemler, IL; J.E. Thomas, ID; J.F. Wortman, NM.

The committee met Wednesday, October 30, 1991 at 1:30 PM in the Senate/Forum room, Town and Country Hotel, San Diego, California.

There were 12 members and 19 guests present. Five topics were addressed. The topics covered were:

1. Mr. Richard Nelson gave an update from the Livestock Conservation Institute Committee on Electronic Identification. He reviewed the protocol for the implementation of electronic identification of dairy cattle through field trials, and noted that the confirmational phase of the field trial program had been started by one producer of electronic identification equipment. The complete text of this presentation will be included with the committee report as it appears in the proceedings.

2. Dr. Ralph Knowles, Destron/IDI, discussed Injectable Electronic Identification with an update of global field trials and its application in disease control and eradication programs. Field trials were summarized for ten countries involving five species (bovine, caprine, porcine, bovine and equine) or over 20,414 animals. Canada is a leader in electronic identification involving all species. The "Scrapie Negotiated Rulemaking Advisory Committee" has issued the "Scrapie Flock Certification and Animal Identification Procedures" in the Federal Register including electronic ID as part of the certification program.

3. Dr. James Davis, USDA, APHIS, VS, gave an evaluation of swine identification (tattoos, backtags and ear tags) for all classes of swine. He reported on a survey of 1645 livestock markets and buying stations, involving 30,000,000 swine, located throughout the United States. Four fifths of the swine traveled less than 24 hours enroute to slaughter. The majority of the swine traveled 11 to 300 miles to slaughter. Many markets and buying stations were not identifying all classes of swine. Thirty one violations were submitted.
REPORT OF THE COMMITTEE

by APHIS last year. The Animal and Plant Health Inspection Service has maintained a policy of informing the swine industry that swine in interstate commerce must be identified. If markets are unwilling to apply identification or not trying to comply, APHIS may consider issuing notice of violations.

4. Mr. Al McCooey, Texas Instruments, discussed advances in Radio Frequency Identification in Livestock. He introduced TIRIS – Texas Instruments Registration and Identification System. Controlled trials are being conducted in countries located in North America, Europe and the Asia Pacific. TIRIS will provide an "Integrated Quality Control" system furnishing feedback to the producers and breeders to allow them to improve their process of production. A prototype, programmable, handheld interrogation unit is projected to be marketed in mid 1992.

5. The Economics of Cattle Identification was discussed by Mr. Bill Gallagher, representing the National Cattlemens Association. He stated that the NCA is against mandatory identification. He advised, however, that the NCA is interested in alternative methods of identification.

Dr. Thomas Neuzil, Iowa Veterinary Medical Association, and Mr. John Caspers, Immediate Past President of the Iowa Pork Producers Association (IPPA), recommended to the committee that the Administrator of USADA, APHIS, approve the "official feeder swine tattoo" for the identification of swine moving in interstate commerce in accordance with the following requirements:

1. The person has applied to the AVIC or State animal health official and received an assigned tattoo unique to their specific herd.
2. The tattoo shall be applied to either ear of all feeder pigs so that all digits are legible.
3. The official ear tattoo shall be applied to the feeder swine on the premises of origin.
4. Feeder swine moved in interstate commerce may also require additional unique identification according to applicable state importation regulations or applicable identification requirements of feeder swine moving through concentration points.

Dr. Neuzil made a motion which was seconded and passed that the USAHA Committee on Livestock Identification strongly recommend to the Administrator, USDA, APHIS, that an ear tattoo following the guidelines formulated by the Iowa Pork Producers be approved for the interstate shipment of feeder pigs.

There being no further business to come before the Committee, the meeting was adjourned at 5:10 PM.
PROGRESS TOWARD IMPLEMENTATION OF ELECTRONIC IDENTIFICATION IN THE DAIRY INDUSTRY

Richard E. Nelson
Holstein–Friesian Association

Last year this committee was shown the Electronic Identification Guidelines for Implantable Transponders prepared by National DHIA/Holstein Association. These have remained substantially the same with minor adjustments and the last revision is dated March 30, 1991. The objectives have been revised only to add a fourth and they now state;

1. To verify the adherence to specifications for the numbering system.
2. To establish minimum operating characteristics for use with various Holstein Association, DHIA and other industry programs.
3. To harness the enhanced management capacity and benefits provided by automated identification systems.
4. To select a single appropriate transmission protocol within the DHIA and Holstein Association systems.

As outlined by Mr. Philip Dukas of National Dairy Herd Improvement Association at the meeting of this committee last year, the first phase is that of the electronic equipment producer submitting a formal application to participate in the field trials. This application will request preliminary data and certify that the equipment meets the specifications set forth in the guidelines in the areas of numbering capacity, accuracy, sensitivity, range, orientation ratio, speed, visual readout, selectivity, rejection of pairs, hermetic sealing and thermoshock, electric isolation, capability and safety precautions.

Phase two is described as confirmational testing. In this, an independent laboratory or research facility will conduct preliminary testing in a controlled situation in order to evaluate performance claims before respective equipment is cleared for phase three, the actual operations at a dairy. At this time, Dr. S. L. Spahr at the University of Illinois is conducting confirmational testing.

It was expected that three or more producers of electronic equipment would have submitted applications to participate in the field trials soon after the application forms were sent in late February or early March, 1991. Only one producer of equipment has submitted an application and has started the confirmational testing process.

Confirmational testing is in two parts. In drawing from protocol developed by Dr. Spahr, the manufacturer is to supply 100 implantable ID units, an implant tool, a walk through antenna of the design proposed for commercial use and a portable hand held reader. The portal antenna will be
IMPLEMENTATION OF ELECTRONIC IDENTIFICATION

mounted in the return alley of the milking parlor or other suitable location to allow automatic identification of the animals twice daily as they pass through the milking parlor or at such time as they take water or feed. In addition, each type of equipment will be monitored upon implantation and once weekly thereafter for four weeks with a hand held reader. The effect of direction of the reader relative to the plane of the ID unit on the distance that the tags can be read will be determined. Migration of the unit, expulsion of the units, and units which cease to operate will be noted. Data gathered through the walk through readers will be recorded on a personal computer for twenty eight days and in order to proceed to step three testing, 98% of the tags must be readable by both styles of readers. In addition, 98% of the readable tags must be read automatically through the walk through antenna (56 opportunities per ID unit) during the 28 day test period.

A second phase of confirmational testing involves implanting electronic ID devices as a permanent method of identification from birth until the initiation of lactation. This trial is designed to be conducted in conjunction with "confirmational" testing of implantable electronic ID devices for lactating cows.

Initially, 50 replacement heifers from two to twenty months of age will be implanted. The goal of this trial will be to determine in a short period, the affect of different stage of heifer development on the performance of the system. Specifically, responses to be studied include:

>- Rate of rejection or loss of ID devices; cause for loss.
>- Percent of devices that remain operational
>- Reasons (breakage, infections, device failure) for failures in devices retained
>- Range of interrogation achieved before and after implantation
>- Affect of orientation of device on range of interrogation achieved after implantation.
>- Ease and success of the implantation process.

A second group of 50 implantable devices will be implanted within the first three days of birth. The responses studied for this group will be the same as the responses for the group implanted at different ages.

In the first attempt to start confirmational testing using non lactating dairy animals, the site of implantation was about one third of the way out the ear from the base of the left ear. The implantation tunnel was parallel to the length of the ear. From this experience, it was determined that this implant site was not suitable and all of those implanted devices were surgically removed. After this initial effort, fifty yearling heifers were implanted in the triangular cartilage area near the base of the ear on or about July 10 and at this time, they are all working.
NELSON

Devices have been implanted in fifty baby calves, five of which came back out but were found to be still operational and two cannot be read but the reason has not been determined. It has been found to be more difficult to implant the devices in baby calves than in yearling heifers because the ears are small and the cartridge area is soft.

Texas Instruments recently completed the design of portal or walk through identification equipment. A unit has recently been installed at the University of Illinois site. Because this device has been installed to identify non lactating animals, it is near the source of water in order that each animal is identified as it approaches the source of water. This antenna or interrogating device is 1,000 feet from the computer recording the identification. The time it is identified and the animal's identification is recorded and it is working very satisfactorily. This suggests that identification of milking animals in the parlor should meet expectations as well.

Following completion of phase two, the supplier and DHIA/Holstein Association representatives will schedule and conduct field testing which will extend over a six month period of time. The plan calls for a minimum of 300 tags in at least two dairies with stanchions or tie stall barns requiring the official use of hand held identifying equipment and a minimum of 1,000 tags will be used in at least two locations using milking parlors in which case walk through identification/interrogating equipment will be used with daily readings recorded. Stanchion and tie stall barns will also be fitted with walk through equipment in order that daily readings can be taken. Technical evaluations will be made during this six month time.

After each producer's device has completed a six month field trial, a single protocol will be selected for general distribution and companies have verbally agreed that licensing arrangements will be available in order that all companies can produce and distribute devices that conform to the protocol selected for distribution. The approval for distribution is intended to extend for period of three years after which there will be reevaluation and a determination made to determine if this equipment using the pre approved protocol will continue to be distributed.

Field trial assignments and evaluations are under the jurisdiction of a joint technical committee whose mission statement and role is as follows:

*The mission of this Committee is to implement electronic identification of dairy cattle with the use of implantable devices for permanent non-duplicative identification and develop procedures that make such electronic identification effective.

The role of this Committee is to -

1. Act on applications from electronic identification producers/vendors
2. Determine if product of respective vendor qualifies
IMPLEMENTATION OF ELECTRONIC IDENTIFICATION

to enter field trials
3. Determine/approve field trial locations
4. Develop/approve format for data collection during field trial
5. Monitors progress at field trial and measures progress and performance against protocol/guidelines
6. Makes the decision called for in the guidelines at the end of the field trial on whether device can/will be distributed
7. Develop procedures whereby the encoded series of digits translates into effective records.

In a session with representatives of the packing industry, the renderers, and USDA/FSIS, it was determined that it would be necessary to develop procedures for the movement of animals with electronic implants in the field trials to slaughter. This procedure will call for an agreement between the field trial herd owner and the packing plant to which his animals will be sent. In these initial stages it is suggested that identification was necessary but not a problem as such animals could be handled as rejects. All packing plants are equipped to handle such animals with the special attention they require.

Recognizing that each company producing electronic identifying devices must apply to FDA for approval of a new feed additive, with this approval required before milk can be sold from implanted animals or before implanted animals can be sent to slaughter, it was also recognized that in the next step, each company must petition FDA for approval of their units to be included in the meat and bone meal and to be classified as a product that was Generally Regarded As Safe*. Reasons that can justify this classification include:

1. Human safety would not be an issue since cattle ears are all rendered.
2. The materials used to manufacture the units are not toxic to animals.
3. There would be no physical danger since the material would be ground fine enough to pass through a 10 mesh screen during the rendering process.
4. The components would be present in such small quantities that no distinguishable change would occur in the chemical and nutrient composition of the resulting meat and bone meal.
5. There would be no way to assay for the presence of the
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units in meat and bone meals since the units would be destroyed in the rendering process.

6. No leakage of toxic material would occur into the meat or milk since 1) no toxic materials are used in manufacturing (Must be ascertained from ingredient list.) and 2) surgical glass is leak-proof.

It seems pertinent to recognize that International Standards Organization has established a working group to address the matter of standardizing electronic identification of livestock on a world wide basis. This group has been meeting in Europe and they are working toward the standardization of the identifying units and reading units in order that an international system can emerge.

As an aside, this Identification Committee and the US Animal Health Association approved a universal identification system last year that could be adaptable to electronic identification but not necessarily contingent upon it. The International Standards Organization is proposing twelve digits in the numbering code rather than the ten in our proposal with three digits for the country code using the ISO country coding system with six digits available as free code to be assigned by each national body. This does not necessarily mean that this will emerge as the final product but a device requiring at least 64 bits of memory will undoubtedly prevail.
THE FLUOROQUINOLONES IN ANIMAL HEALTH

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ABSTRACT

The 6-fluoroquinolones are a series of synthetic antibacterial agents that are undergoing extensive investigation for both human and veterinary use in the treatment of a variety of bacterial infections. These agents work through the inhibition of DNA gyrase, interfering with the supercoiling of bacterial chromosomal material. As a result, these agents are rapidly bactericidal primarily against gram-negative bacteria, mycoplasma, and some gram-positive bacteria, with little to no activity against group D streptococci and obligate anaerobic bacteria. Resistance develops slowly and almost always is chromosomal and not plasmid-mediated. From a pharmacokinetic perspective, these agents are variably but well absorbed from the gastrointestinal tract and nearly completely absorbed from parenteral injection sites, and they are well distributed to various tissues in the body due to their high lipid-solubility. The fluoroquinolones are metabolized and renally excreted, with many of them having approximately equal contributions by the hepatic and renal excretory systems. The primary toxicity observed at therapeutic doses involves the gastrointestinal system, although at higher doses central nervous system toxicity and ocular cataracts are observed. Administration to immature animals may result in erosive arthropathies at weight-bearing joints, and administration of high doses to pregnant animals results in maternotoxicity and occasionally embryonic death. The fluoroquinolones are approved for indications such as urinary tract infections, and approval for bovine respiratory disease is being sought for other fluoroquinolones. Other indications for which the fluoroquinolones have been used in animal health include deep-seated infections, prostatitis, and other bacterial infections resistant to standard antimicrobial therapy.

The 6-fluoroquinolones (also known as 4-quinolones or quinolones) are a series of synthetic antibacterial agents with many physicochemical, microbiological, and pharmacokinetic properties that make them exciting new compounds for the 1990's. Older compounds that were also quinolone antibacterial agents (although not fluoroquinolones) include nalidixic acid and oxolinic acid. The 1 position is a nitrogen on the bicyclic aromatic ring structure, with an alkyl group (ethyl or perhaps cyclopropyl) often attached there. A carboxylic acid at position 3 is required for antimicrobial activity, as is a keto group at position 4. Many improvements on these first quinolone carboxylic acids have since been made based on systematic structure-
activity studies. A fluorine atom at the 6 position on the quinolone carboxylic acid nucleus enhances the efficacy of these compounds against gram-negative pathogens and broadens the spectrum of activity against gram-positive pathogens; a basic nitrogen-containing moiety at position 7 enhances tissue penetration and reduces central nervous system toxicity. Because of both a carboxylic acid and one or more basic amine-functional groups, these antibacterial agents are amphoteric; however, between the pKₐ of the acidic and the basic functional groups (between pH 6 and 8), these compounds are sufficiently lipid soluble to be able to penetrate tissues.

**Mechanism of Action**

Quinolones bind to and inhibit the A subunit of DNA gyrase, abolishing its ability to supercoil bacterial chromosomal material, possibly by interfering with the DNA-rejoining reaction. The result is rapid bactericidal activity at relatively low concentrations. Furthermore, post-antibiotic effects (decreased or abnormal growth of bacteria after exposure to an antibacterial agent) of 4 to 8 hours have been seen. The fluoroquinolones are considerably less effective at concentrations much higher than as well as lower than their minimum inhibitory concentrations against bacterial pathogens.

**Bacterial Resistance**

Although low frequency chromosomal mutations are the primary source of bacterial resistance encountered to date, plasmid-mediated resistance has not been demonstrated to the fluoroquinolones to date. Bacteria that contain R-plasmids that carry resistance to other antibacterial agents remain sensitive to many of the fluoroquinolones. Cross-resistance with β-lactam antibiotics, aminoglycosides, tetracyclines, macrolides and polypeptide antibiotics, sulfonamides, diaminopyrimidines, and nitrofurans does not occur. Single-step resistance occurs in 10⁻⁹ to 10⁻¹⁰ bacteria. When resistance does occur, cross-resistance between fluoroquinolones may be observed to a certain extent, occurring with a higher frequency for the older and less potent quinolones such as nalidixic acid and flumequine.

Resistance occurs primarily by alterations in bacterial cell wall penetration, with mutant forms of DNA gyrase occurring only rarely. Permeability changes occur either via decreased permeability of the hydrophilic pores (OMP) or through alteration in the active transport pump, thereby decreasing the intracellular content of the fluoroquinolones. Enzymes that degrade the quinolone antibacterial agents have not been observed.
Spectrum of Activity

In general, the fluoroquinolones have excellent activity against enterobacteriaceae, fastidious gram-negative bacteria, and \textit{Ps. aeruginosa}; good to moderate activity against staphylococci, mycobacteria, chlamydia, mycoplasma, and ureaplasma; and little or no activity against streptococci (particularly group D streptococci), enterococci, and anaerobic bacteria. The post-antibiotic effect of the fluoroquinolones has been shown to be 4–8 hours against \textit{Escherichia coli, Klebsiella, Serratia,} and \textit{Pseudomonas aeruginosa}. Comparison of ciprofloxacin, norfloxacin, pefloxacin, pipemidic acid, and a variety of nonquinolone antibacterial agents (nitrofurantoin, sulfamethoxazole, trimethoprim, cephradine, and amoxicillin) demonstrated that ciprofloxacin the broadest spectrum of activity against all gram-negative bacteria and streptococci tested with the exception of \textit{Str. faecalis} and \textit{Str. pneumoniae}. Although enrofloxacin was not tested, its structural similarity and similar antibacterial spectrum to ciprofloxacin may allow similar conclusions to be made for it as well. The minimum inhibitory concentration of danofloxacin against 90\% of field isolates of \textit{Pasteurella hemolytica, P. multocida,} and \textit{Hemophilus somnus} was found to be $<0.125 \mu g/ml$. Many gram-negative bacteria that have become resistant to other classes of antibacterial agents, including aminoglycosides, anti-pseudomonal penicillins, and third-generation cephalosporins, remain susceptible to the fluoroquinolones.

Pharmacokinetics

Oral absorption of the fluoroquinolones depends on the specific agent administered, with ofloxacin absorbed better than ciprofloxacin, pefloxacin, or enoxacin; all of these were more readily absorbed than norfloxacin, with an absolute oral bioavailability of norfloxacin in dogs of approximately 35\%. Bioavailability is lower in ruminating animals. Bioavailability from parenteral injection sites is nearly 100\% for all fluoroquinolones. Food generally inhibits oral absorption of the fluoroquinolones.

Distribution of fluoroquinolones is very good to tissues, owing to the lipid-solubility of these agents. Plasma protein binding of the quinolones varies, with the newer quinolones less bound to plasma proteins than nalidixic acid. The steady-state volume of distribution of the fluoroquinolones is large, ranging from 0.75 $l/kg$ (flumequine) to 2.46 $l/kg$ (danofloxacin). Blister-fluid concentrations (indicative of interstitial fluid concentrations) equal serum concentrations within 2 hours of oral administration. Furthermore, tissue cage fluid concentrations were not substantially higher than concurrent plasma concentrations after six hours post-oral administration, and they were lower than concurrent plasma concentrations from zero to six hours.
FLUOROQUINOLONES IN ANIMAL HEALTH

post-dosing in normal dogs. High concentrations are achieved in saliva, nasal secretions and nasal mucosa, and bronchial epithelium, although not substantially higher than concurrent plasma concentrations. Enrofloxacin concentrations up to 3 times serum concentrations were observed in tissue homogenates from calves taken 1 hour after dosing, with 12-hour concentrations in tissue homogenates exceeding concurrent serum concentrations. Enrofloxacin concentrations in bile and urine exceeded serum concentrations by 10–20 fold; tissue homogenate concentrations were in the following order: liver > kidney > heart > lung > spleen > intestinal wall > serum = muscle = lymph nodes. In dogs, uterine and prostatic fluid concentrations were 2.2 and 1.4 µg/ml 1 hour after an oral dose of 2.5 mg enrofloxacin/kg, whereas 1 hour serum concentrations were 1.2 µg/ml after an oral dose of 5 mg enrofloxacin/kg. Penetration into the CNS is relatively good.

Metabolism of the fluoroquinolones is highly variable but can be extensive. In general, phase I metabolism occurs, primarily through hydroxylation and oxidation to oxoquinolones. Quite often, glucuronidation occurs, primarily at the carboxylic acid on position 3. Enrofloxacin and pefloxacin are N-dealkylated to form ciprofloxacin and norfloxacin, respectively. The glucuronide conjugates of the fluoroquinolones may be excreted through the urine or bile, depending on the fluoroquinolone and the species to which it was administered. There are indications that enterohepatic circulation of fluoroquinolones may occur.

Renal excretion of the fluoroquinolones is also variable, although glomerular filtration occurs for the unbound fraction of all fluoroquinolones. Active tubular secretion by the organic anion transport system also occurs to a more variable degree. Probenecid blocks the renal tubular secretion of norfloxacin and ciprofloxacin, but because of the other routes of excretion drug accumulation does not occur to a large extent. In normal animals, the biological half-life (t1/2) of most fluoroquinolones ranges from 3–6 hours.

Alterations in disposition are noted upon multiple dosing and with larger doses of some fluoroquinolones, potentially due to altered metabolism and/or decreased absorption from orally administered dosage forms.

Toxicity

Toxicity of the fluoroquinolones is mild at therapeutic doses, and generally consist of gastrointestinal disturbances such as nausea, vomiting, and diarrhea. At slightly higher doses, CNS signs may be seen. High serum concentrations may produce immediate but transient toxic reactions, principally CNS in nature, within 2–3 minutes of rapid intravenous injection of norfloxacin solution. Other fluoroquinolones may not be as likely to produce these CNS effects. Crystalluria can occur in dogs and humans at high doses of norfloxacin, although the occurrence is rare in human beings.
treated with ciprofloxacin and has not been reported with either danofloxacin or enrofloxacin. Ocular cataracts are seen with prolonged use in humans. Non-inflammatory, erosive arthropathies preferentially located at weight-bearing joints can be observed in growing animals treated with fluoroquinolones. Photosensitization occurs with all marketed fluoroquinolones, although it is rare for norfloxacin and ciprofloxacin. Enrofloxacin and several other fluoroquinolones have been shown not to be mutagenic. Very large doses of fluoroquinolones, maternotoxicity and some embryonic deaths have been reported in pregnant laboratory animals; such observations have not been observed in the target species treated with fluoroquinolones at therapeutic doses.

Drug Interactions

Oral absorption of the fluoroquinolones is decreased by antacids containing magnesium and aluminum, and other agents such as sucralfate also decrease the absorption of the fluoroquinolones. Some fluoroquinolones (particularly ciprofloxacin) after repeated administration have been shown to decrease the hepatic clearance and increase the elimination half-life of theophylline, caffeine, and antipyrine. However, others have shown that oral doses of ofloxacin, enoxacin and norfloxacin showed no significant effect on several drug–metabolizing enzymes. Enoxacin decreases the hepatic clearance of the R–enantiomer of warfarin but not the S–enantiomer, and the anticoagulant effects of warfarin are increased with concurrent administration of ofloxacin. No drug–drug interaction studies have been published for either enrofloxacin or danofloxacin.

Therapeutic uses

In animals, enrofloxacin is currently approved in the United States (as of 1991) for use in dogs and cats for complicated and uncomplicated urinary tract and respiratory infections, although it is used in an extralabel manner for a variety of other infections caused by susceptible bacteria. In dogs, a therapeutically equivalent dose of ciprofloxacin is 4–5 times the dose (on a mg/kg basis) of enrofloxacin, which is 2.5 mg/kg twice a day. Studies indicate that enrofloxacin may be effective in the treatment of acute salmonella infections in calves and in eradicating salmonella–positive fecal cultures both 5 and 12 days post–treatment in Salmonella carrier calves. In swine, enrofloxacin is reported to eliminate the carrier state for Salmonella at an oral dose of 200 ppm in the feed for 10 days. Clinical field studies have been conducted with enrofloxacin in swine colibacillosis, poultry colibacillosis, and other poultry bacterial and mycobacterial diseases, with therapeutic success. Danofloxacin has undergone extensive field efficacy studies in bovine respiratory disease, indicating that a dose of 1.25 mg/kg every day for 3–5 days may be effective under a variety of management systems.
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Other unapproved indications for the fluoroquinolones in animal health that have been proposed include deep-seated infections, prostatitis, CNS infections, skin and soft tissue infections, bone and joint infections, and nosocomial infections resistant to other antibacterial agents. Other fluoroquinolones are being investigated for either human or veterinary use, although none are being investigated for use in both animals and man.

References available upon request.
REPORT OF THE COMMITTEE ON PHARMACEUTICALS

Chairman: Dr. D. O. Farrington, Rahway, NJ  
Vice Chairman: Dr. R. F. Taylor, Edgewood, NM

D.A. Armstrong, CO; L. G. Biehl, IL; R. E. Bohlender, NE; N. J. Corlett, OH; G. L. Cowman, CO; J. E. Fox, GA; D. L. Froe, MO; D. A. Gable, KS; R. A. Gessert, MI; D. D. Gingerich, IA; J. S. Gloyd, DE; C. H. Graham, KS; G. W. Hausman, IA; J. P. Honstead, CO; S. Hutchins, VT; L. Leach, VA; G. D. Lindsey, IN; K. B. Meyer, IN; G. A. Mitchell, MD; J. F. Mock, NJ; L. F. Moore, KS; T. A. Neuzil, IA; J. J. Rash, MO; R. A. Schultz, IA; M. G. Scroggs, TX; M. Sharar, MD; T. K. Shotwell, TX; J. Webb, Washington, D.C.

The Committee met at 1:30 p.m. on Tuesday, October 29, 1991 in the Committee Room at the Town & Country Hotel Convention Center in San Diego, CA. Sixteen Committee members and nine guests were in attendance.

The Committee has maintained a continuing emphasis on providing a forum to identify and address issues concerning the availability and safe use of pharmaceutical products in animals. Continuing education at all levels regarding proper and effective use of pharmaceuticals has been encouraged as a means of achieving these goals. Drs. John W. Paul, Scott A. Brown, Joseph C. Paige, Gary L. Cowman and Andrew J. Beaulieu were invited speakers and summaries of their presentations are included in this report.

Dr. Paul, Manager of Professional Services, Hoechst-Roussel AgriVet Co., presented an update on the activities of the United States Pharmacopeial Convention (USP). It was stated that the USP is now a triprofessional organization, with veterinary medicine having recently been added to the previously existing professional disciplines of human medicine and pharmacy. It was further suggested that the USP may be a resource to veterinarians for unbiased information regarding issues such as compounding of veterinary drugs, use of human drugs in companion animals and extra-label use of drugs. By 1995 the USP hopes to establish standards for all drugs used in veterinary medicine and develop official reference standards. Its drug indexing division also hopes to create informational monographs for all animal drugs and for human drugs used in veterinary medicine.

Dr. Brown, Clinical Research Scientist, The Upjohn Company, presented a review of the fluoroquinolone class of antibacterials. This important group of compounds has broadspectrum antimicrobial activity and the quinolones are being widely researched for their animal health applications. One of these compounds, enrofloxacin, has been introduced in the US animal health market for treatment of diseases in dogs and cats. This group of compounds has several interesting and useful features including a tissue storage tendency that is accumulative, enabling tissues in treated animals to consistently have higher antibiotic levels than body fluids.
REPORT OF THE COMMITTEE

Bacterial resistance to this class of antibiotics develops very slowly (based on their pharmacologic mechanism of action), and such resistance generally regresses when treatment is ceased. Ongoing research is oriented toward the ultimate goal of approval and clearance for these products in food animals.

Dr. Paige, Center for Veterinary Medicine FDA, presented a discussion of the Tissue Residue Reduction Program currently underway within the agency. This included a review of the structure of the Agency as involved in this project, a description of the objectives, public health approach, tissue residue databases being used, significant impact of the program, and objectives for the future. User education will be emphasized along with field investigations of problem farms identified by residue testing. The program may include greater involvement of state personnel, the staff of the Animal and Plant Health Inspection Service and Extension Service staff. Drug residues were found predominantly in culled dairy cows, veal calves, and market hogs. Antibiotic residues most frequently detected included penicillin and penicillin combination products, streptomycin, oxytetracycline and tetracycline, neomycin, gentamicin, and sulfamethazine and sulfamethazine combination products. Gentamicin sulfate residue violations showed an increase over previous years (in 16/64 of the violative cases, mastitis products had been administered by veterinarians). The most frequently cited cause for residue violations were failure to adhere to withdrawal time in culled dairy cows and drug carry over or crossover contamination in swine. In 35% of the investigations, the cause of the violation could not be determined. It was pointed out that this number is unacceptably high, and poses a negative impact on any preventive strategy. The Agency plans to implement an education program on violative residue prevention at the level of veterinary school curricula and extension services in the future.

Dr. Cowman, Associate Director for Science and Technology, National Cattlemen's Association (NCA), presented a summary of the NCA Injection Site Lesion Research Program. This program has been developed as an adjunct to the Beef Safety Assurance Program sponsored by the NCA. Surveys have shown that there is a significant economic loss at the retailer level due to blemishes resulting from intramuscular injection of drugs and biologicals. It was emphasized these are eliminated by trim and present no public health or human food safety concerns. NCA has implemented an educational program in conjunction with allied industry groups for producers and veterinarians in order to address this issue. This program suggests:

1. Using subcutaneous injections when possible,
2. When intramuscular injections must be given, that they be administered in low value areas (i.e. neck) and,
3. That injections not be administered to animals nearing slaughter.

The resultant decrease in blemish frequency as an outcome of these efforts is currently being closely monitored.

Dr. Beaulieu, Center For Veterinary Medicine, FDA, discussed the professional labelling concept. A recommendation was made to the FDA by the American Veterinary Medical Association (AVMA) and the Animal Health institute. The initiative is intended to resolve the need for extra-label use via broadening the approved indications for prescription animal drugs and by modifying the approval process to encourage manufacturers to pursue expanded drug labels. Formal consideration by the Agency will be underway soon. Professional (or flexible) labelling is based on the principles of establishing a minimal effective dose needed to treat a specific disease entity in a given animal species, with a maximum dose to be calculated from human safety parameters (residues) coupled with target animal safety maximum dosages when applicable. The resultant dosage range would theoretically enable a veterinarian to adjust his treatment dosage according to MIC information for a given bacterial agent, and adjust his withdrawal times for different dosages. Flexible labeling (as described above) would be strictly prescription labelling with no over the counter versions permitted. Physiological labeling for products used in a reproduction management capacity might also be considered under this flexible labeling concept.

Dr. Beaulieu also presented an overview of the CVM's activities in processing investigational and approval new drug submissions over the past several years. These activities continue at a relatively high level. While the number of significant approvals appear to have declined during 1991, it is expected to increase markedly in 1992 to approximately 40 such approvals.

A brief discussion ensued regarding the use of the "phasing" process of New Animal Drug Application review currently being employed by the FDA, with the comment that this seems to be a more expedient and efficient process for all parties involved. The Agency is emphasizing the issues of data integrity and manufacturing controls and there is a priority towards monitoring and auditing trials while underway whenever possible (based on manpower and financial constraints).

Dr. G. Dean Lindsey, Eli Lilly & Co., Manager, Animal Health Regulatory Affairs, presented an update on the status of the resolution regarding veterinary medicament compounding developed by the Committee on Pharmaceuticals and forwarded to the AVMA and FDA after the 1990 meeting. The FDA has serious concern about the issue of compounding, both from a legal and human safety aspect. There will be a compliance policy guide drafted soon; however, the concerns cited by the Committee and AVMA will be taken into consideration by the Agency during the policy establishment process.
The Delmarva peninsula is a body of land made up as its name implies of the three states of Delaware, Maryland and Virginia. The Delmarva is more than just a coincidence of geography, it is a viable economic entity, and the economic engine is the poultry industry. DPI, the Delmarva Poultry Industries Inc. is a model industry association formed to further the economic interests of its members and indeed the economic interest of agriculture in general on Delmarva. An important part of DPI is the Poultry Health Committee which meets regularly to discuss poultry health problems and solutions. The State Veterinarians of the three states are members of the committee, as are the poultry company veterinarians and agribusiness veterinarians (vaccine manufacturers). The committee also enjoys the membership and support of some of the production managers of the integrated companies.

As a result of the outbreak of Avian Influenza (AI) in Pennsylvania in 1983, DPI asked the poultry health committee to provide guidance to the industry on the shore (the Delmarva is often referred to particularly by Marylanders as the Eastern Shore or more often just "The Shore"). Specifically this guidance was to limit the spread of AI should it occur. They created the "DPI Emergency Poultry Disease Task Force" (EPDTF), made up of all segments of the integrated corporations and allied industries and state and federal disease control officials, both field and laboratory. The EPDTF set about to produce a training manual for the industry using by example everything that they could find that went wrong in Pennsylvania. It is fair to say that Murphy's law was in full operation in PA; i.e. everything that could go wrong did go wrong. To be fair, the APHIS PA Task Force was extraordinarily efficient in correcting mistakes and indeed in documenting these mistakes else the Task Force would have had a much more difficult time in producing its document. It is difficult to cite the final hour of the completion of their work but each of the procedural appendices is dated May 1985.

The author was only involved in the later stages of the completion of the manual, having been hired on in the fall of 1984 by the state of Maryland. On Christmas eve of 1984 Maryland had its own AI outbreak to manage and the author was asked by APHIS to supervise the 10 federal veterinarians and livestock inspectors assigned to help Maryland eradicate its outbreak. It is virtually certain that the reason for this assignment was twofold; the author is first, a former employee of Veterinary Services and
second, a Foreign Animal Disease Diagnostician. It most certainly was not because of any participation in the creation of this remarkable document.

The epidemiology of the MD outbreak involved over 200 tracebacks, and trace-forwards, 83 of which were in PA. It was during this episode that the germ of the idea for a "Mid Atlantic Poultry Health Agreement" was born. It became obvious that the border between our two states was indeed just an imaginary line on a map. The infamous MD "dealer", one could say "Purveyor" of AI had a market area that had nothing to do with state lines. What he did have was a few records, a remarkable memory and a total disregard for where he was when doing his business. At the corporate level on the shore there is an equal disregard for the borders of the three states except perhaps at income tax time. At the end of our brief flurry with AI it did seem strange that we would spend an enormous amount of time and effort to put together a single document as guidance for industry and yet each of the three states had a different set of regulations for industry to live with. It seemed logical that a single set of regulations could be developed to deal with those diseases that we could all agree are "emergencies". Because I had spent a good deal of time writing and rewriting regulations during my tour of duty in Washington I volunteered to draft the regulations but not before I got a commitment from the DPI poultry health committee to help.

At a very early stage of writing it became clear that we could get agreement on regulations to prevent the introduction of the diseases, but that actions taken to eradicate would be complicated by the issue of indemnity. Delaware had no provision for indemnity even though the Secretary did have the authority to destroy infected flocks. In order not to delay we decided to partition the task and to complete the Prevention phase as soon as possible. In addition when we looked at the issue of "market area" it was clear that Pennsylvania should be a part of the agreement. DPI decided that the best strategy would be to approach the poultry industry in Pennsylvania first, and then to go to the Secretary of Agriculture for Pennsylvania. This we did and were warmly received by all parties. In keeping with the axiom of "no surprises", I had also kept the Pennsylvania State Veterinarian's office informed. All of these contacts proved to have been worth the effort for when the time came for formal invitations to go out, the whole operation went smoothly. Indeed one of my most pleasant duties was to take the signature page of the document to each of the Governors of the other three states for them to sign.

The model poultry regulation is quite comprehensive with procedures as detailed as would be necessary to deal with an outbreak. Copies of the document are available to anyone who wishes to have it. It is probably useful in the context of this paper to repeat parts of the preamble of the regulation as follows:
Background.

Due to economics, the prevailing disease control procedures used by the poultry industry tend to emphasize medication and vaccination as the primary strategies and to reduce the importance of effective sanitation and security strategies. This reduced reliance on sanitation and security has caused the industry to become more vulnerable to the rapid dissemination of disruptive diseases such as Avian Influenza.

As to the current status of the document, Delaware has written an indemnity proposal which asks industry to bear the burden of the first 2,750,000 dollars at which time the state then picks up the next 5,000,000 dollars. After that is expended it is their intention to seek federal support. It is fair to say that if 5,000,000 dollars won't fix it, it will certainly be an extraordinary emergency. This proposal is slated to be considered at their next legislative session. In the meantime we all continue to monitor our poultry and pet bird populations. From time to time we test our biosecurity procedures. It is perhaps more correct to say that Laryngotracheitis tests our procedures. LT is a good model because it too is a disease where man plays an important part in the spread of the virus. The main trouble with this approach is that some years it can be a very expensive lesson.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY AND OTHER AVIAN SPECIES

Chairman: Dr. R.H. McCapes, Davis, CA
Vice Chairman: Dr. S.H. Kleven, Athens, GA

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The Committee met on October 30, 1991, with a total of 62 members and guests attending.

I. MICROBIOLOGIC CONTAMINATION OF RAW POULTRY MEAT AND EGGS

A. Salmonella negative contamination goal for animal feeds.

Dr. George A. Mitchell, FDA-CVM, gave several remarks on FDA's program for Salmonella negative feeds. He stated that the Center for Veterinary Medicine (CVM) has the responsibility for the regulatory options for controlling microbiological contamination of animal feed. In September 1990, CVM announced a goal of Salmonella-free animal feed and feed ingredients. In April 1991, CVM's program for zero Salmonella in animal feed was presented to their Veterinary Medicine Advisory Committee (VMAC). The proposed program was discussed by the committee and members of the audience. The committee recommended that CVM pursue the development of HACCP programs for the feed industry. Additionally, both the committee and members of the audience stated the need for a coordinated approach to Salmonella control that involved all elements of the industry from the feed ingredient manufacturer to the consumer. We have begun to implement these suggestions.

A week after the April meeting of the VMAC, representatives of CVM
REPORT OF THE COMMITTEE

met with approximately 50 members of the agri-business industry and presented and discussed our program for zero Salmonella in animal feed. We believe this meeting was very productive for both CVM and industry. A recurring theme in both the meeting with VMAC and with industry was the need to coordinate the goal of eliminating bacterial pathogens from feed with other programs and resources directed at controlling pathogens in the environment and on the carcasses of slaughtered food animals.

In response to the need for a new and highly coordinated approach involving producers, industry, State and Federal agencies in achieving Salmonella-negative feed, CVM led the effort of convening a Federal-State Steering Committee (SC) to consider the matter. The SC met on July 2, 1991. Senior officials from the Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), Agriculture Research Service (ARS), and the Food Safety and Inspection Service (FSIS); FDA's Center for Veterinary Medicine (CVM), Center for Food Safety and Applied Nutrition (CFSAN), and Office of Regulatory Affairs (ORA); the Department of Commerce's National Marine Fisheries Services (NMFS); the National Association of State Departments of Agriculture (NASDA); the Association of American Feed Control Officials (AAFCO); the Association of Food and Drug Officials (AFDO) and the United States Animal Health Association (USHA) attended the meeting. These officials agreed with the CVM proposal to request that the USAHA consider becoming a forum for the scientific debate on the means to best eliminate harmful microbiological contamination, including Salmonella, from the feed of food-producing animals. The executive body of USAHA agreed to the request and established an Ad Hoc steering committee on feed safety to consider how USAHA can fulfill this role and the scientific issues that it should consider. The first meeting of this Ad Hoc committee will be held tomorrow, October 31, 1991. Representatives of government, universities, producers, feed manufacturers, and equipment manufacturers and the transportation industry have been invited to attend. Specific meetings of this forum and discussion of the subject in scientific forums other than the USAHA are also probable.

On October 30, 1991, at the Symposium on the Diagnosis and Control of Salmonella we presented what we mean by Salmonella negative feed, the impact of sampling plans on the definition of zero, and other approaches beside HACCP that might assist in achieving the goal of Salmonella negative feed. As a summary, Salmonella negative feed means Salmonella are not detected in a sample using an agreed upon sampling plan and method of analysis. Salmonella is self-replicating and contagious. Therefore it makes little difference whether the prevalence in a production lot is 5% or 95%. It should be reduced to zero.

The protection offered by a sampling plan can be increased by collecting more samples. Compositing samples for analysis can be used to control workload. We are proposing that a negative Salmonella designation
be based on the testing of 30 samples per production run with none positive for Salmonella. Analysis of these samples would be carried out with 25 gram analytical samples using enrichment cultures and the procedures outlined in the Bacteriological Analytical Manual (BAM).

There are two basic approaches to achieving zero Salmonella in feed. 1) the development and implementation of quality assurance programs, either HACCP plans or GMPs, coupled with inspections and testing, and 2) endpoint testing with a specification of zero Salmonella based on a specific sampling plan and test procedure, coupled with methods (heat, chemical or radiation) that will assure the specification is met regardless of the bacterial load. We favor the quality assurance approach because it addresses the root causes of the contamination e.g. production, storage, or transportation. Endpoint testing while 'meeting the spec' provides a smaller margin of safety then the quality control approach and the potential for recontamination is greater.

In addition to our proposals for industry, we have suggested that feed blenders and on-farm feed mixers should add a Salmonella specification to their feed and feed ingredient purchase contracts. We recommend a zero level based on an accepted sampling plan and method of analysis. While we believe that this action is the most appropriate, recommending that ingredients and finished feeds be purchased only from manufacturers actively participating in industry approved HACCP programs would be a positive step towards our goal. If this last option is chosen, we have recommended that ingredient buyers should have the latest reports for use as references in their ingredient purchases and be certain that the specific plant that provides the product is the plant used by the supplier to supply samples to the HACCP program. We initially made the above recommendations at this year's National Broiler Council meeting and have subsequently recommended these industry wide. On October 31, 1991, at the Ad Hoc Steering Committee on Feed Safety, CVM will suggest to the Chairman and Vice-Chairman of the committee that discussion of the following topics would be helpful.

1. A discussion of the need for the Salmonella free designation.
2. A discussion of the need for a Salmonella analytical test that operates at a zero rate of false negative determinations.
3. A discussion of approaches, in addition to HACCP programs, for achieving zero Salmonella in feed.
4. A discussion of methods and approaches to obtain current information, from each segment of the feed industry, about the prevalence and incidence of contamination of raw and finished products with Salmonella.

Concern over contamination of human food and animal feed among consumers and government agencies are expected to continue to grow and
more stringent measures will be demanded to assure the safety of the food and feed supply. CVM believes that through a program for zero Salmonella in animal feeds and through a scientific forum provided by the USAHA we will be able to meet these expectations.

Dr. Patton L. Smith, President, USAHA, outlined his view of the USAHA role in the FDA program on Salmonella negative animal feeds. Dr. Smith has appointed an ad hoc committee to make recommendations regarding the role of USAHA in efforts to eliminate Salmonella in feeds. USAHA is a natural organization to do this because of its broad base of representation from industry producers as well as veterinarians, regulatory workers, and scientists.

B. Campylobacter

Dr. Maurice Potter, CDC, discussed the epidemiology of Campylobacter jejuni infections in the United States.

In the first eight years of Campylobacter surveillance, the annual number of isolates reported to CDC has increased, but the isolation rate has remained approximately 5 to 6 per 100,000 because the population under surveillance has also increased. Campylobacter isolates are still not reportable in all States. The reported isolation rates are close to that of Shigella (7-8 per 100,000), and considerably lower than that of Salmonella (1820 per 100,000). Of course, many people do not consult a physician for their illness, and these patients would not be reported to our system. An estimated 20 cases of campylobacteriosis occur for every patient who seeks medical care and is cultured.

A major source of under-reporting is the failure of many laboratories to routinely culture for Campylobacter in diarrheal stool specimens. In 1980, CDC conducted a study at 8 hospitals whose microbiology laboratories cultured Campylobacter routinely from diarrheal stool specimens, along with Salmonella and Shigella. Campylobacter was isolated twice as frequently as Salmonella and 4 1/2 times more frequently than Shigella. Since the incidence of Salmonella is at least 20/100,000, diagnosed Campylobacter infections should be 40–50/100,000, which is 10 times the reported rate. The isolation rate was 71/100,000 at a Health Maintenance Organization in Seattle Washington in the early 1980s. In a 1985 survey, it was 50/100,000 in the same population. So, when laboratories routinely look for Campylobacter, they find isolation rates which are approximately 10 times the national reported isolation rate, or roughly 50/100,000 population.

With these two figures, the estimated isolation rate if all laboratories looked for Campylobacter whenever they looked for Salmonella and Shigella, and an estimate for the number of milder infections that occur for every culture–proven case, we can calculate an infection rate for the United States for Campylobacter jejuni infections of approximately 10/1,000 population.
TRANSMISSIBLE DISEASES OF POULTRY

CDC also collects from state health departments reports of foodborne and waterborne disease outbreaks, including those caused by Campylobacter. Between 1978 and 1986, 57 outbreaks of campylobacteriosis were reported, including 45 foodborne outbreaks, 11 waterborne outbreaks, and one outbreak in a tourist group traveling overseas. The classic outbreak occurs after a school trip to a dairy farm, during which the school children get a glass of raw milk. However, it is important to remember that most Campylobacter infections occur as sporadic cases that are not associated with large recognized outbreaks.

Major differences exist between the epidemiology of outbreaks and that of the sporadic cases. Outbreaks have a marked bimodal distribution, with peaks in May and October, and the low point during the summer. Sporadic cases peak in the summer, suggesting that the circumstances of outbreaks are quite different from those of sporadic cases. This means that universal pasteurization of milk and proper treatment of all drinking water could prevent 90% of the outbreaks in the United States, but may have only a limited effect on sporadic cases.

Large case–control studies of sporadic Campylobacter jejuni infections have examined a number of different possible sources of infection. A study in Seattle identified poultry consumption as the dominant source of infection, and also associated infection with not washing down the kitchen cutting-board with soap. Several studies in Colorado found that infection was specifically associated with handling the raw chicken meat and with eating undercooked chicken. The person who prepared the chicken in the kitchen was the one most likely to become infected. In studies at the University of Georgia, we noted three ways in which poultry can cause Campylobacter jejuni infections. The first was eating chicken raw. Second, eating chicken undercooked. Finally, using a knife or cutting board for cutting up a raw chicken and then for something else without washing the knife and cutting board first also emerged as a risk factor.

Dr. Hans Riemann, University of California, gave a review of Campylobacter jejuni infections in animals.

Within the past decade Campylobacter jejuni has become recognized as the leading cause of acute bacterial gastro-enteritis in humans in many developed countries. The infection may be of even greater importance as a cause of diarrheal disease in developing countries. Unpasteurized milk and polluted water have been reported as vehicles in outbreaks while poultry seems to be the most important source in sporadic cases of campylobacteriosis. Sporadic cases are more numerous than cases associated with outbreaks, but exact figures are not known. In spite of the fact that clinical cases of campylobacteriosis outnumber cases of salmonellosis, it is the latter that dominates the news. Among approximately 750 news items published in News-O-Gram (Newsletter of the American Association of Food Hygiene Veterinarians) from 1987 through May, 1991,
only 11 (1.5%) dealt with Campylobacter. The majority of news items concerned Salmonella and Listeria. The reasons may be that Campylobacteriosis has a low case fatality rate, 3 per 10000, in outbreak associated cases. Also *C. jejuni* is a fragile organism that will be destroyed by any food processing condition that kills Salmonella. Norcross has stated that Salmonella has been the number one priority among USDA research projects since 1981. Efforts to eliminate Salmonella should also be useful in controlling *C. jejuni*.

Prevalence of *C. jejuni* Infection in animals.

*C. jejuni* is very common in wild and domestic mammals and birds and that makes it difficult to associate infection with disease although cases of sporadic and induced disease have been documented. The magnitude of prevalence is illustrated by the following fecal shedding rates:

- Turkeys: 100%
- Chicken: 83%
- Ducks: 88%
- Migrating Birds: 35%
- Town Dwelling Pigeons: 50%
- Sea Gulls: 20–70%
- Sheep: 11% (gall bladder infection)

The prevalence of infection in dogs and cats has been reported as 49% and 45%. Campylobacter is frequently isolated from the intestinal tract of swine, however, the isolates tend to be *Campylobacter coli* rather than *Campylobacter jejuni*, and if they are *C. jejuni* the serotypes are rarely identical with the ones that cause human disease. With the preponderance of *C. jejuni* in animals used for food it is no surprise that insufficient heating of such food or inadequate kitchen hygiene result in a large number of human cases campylobacteriosis. The disease can also be contracted by contact with pets, such as puppies and cats. Human to human transmission is rare.

Clinical Disease in Animals

The high prevalence of *C. jejuni* infection is apparently healthy animals makes it difficult to associate infection with disease. Sporadic disease has been described. In one study *C. jejuni* was isolated from 40% of diarrheic calves.

However, it is possible that the diarrhea could have been caused by another agent. Studies have also shown that *C. jejuni* can frequently be isolated from aborted lambs. *C. jejuni* has also been associated with cases
of diarrhea in puppies, monkeys and horses but rarely in cats. The incidence of diarrhea caused by *C. jejuni* in animals is unknown.

Experimental studies have provided some information about the susceptibility of animals to infection and disease. When $9 \times 10^7$ (ninety million) *C. jejuni* cells were fed to three day old chickens, 88% of the chickens developed diarrhea and there was 32% mortality. When the challenge dose was reduced to 9 (nine) *C. jejuni* cells, 20% of the exposed chickens became infected. In another study, 1 $1/2$ – 3 day old chickens fed $10^5$–$10^6$ live cells of strains of *C. jejuni* developed diarrhea; 25% of the exposed chickens had diarrhea on day 2, 49% on day 4 and 81% on day 5. *C. jejuni* multiplied 3–4 logs in all parts of the intestinal tract and systemic infection occurred frequently. In a different study, three day old chicks did not develop diarrhea after being fed *C. jejuni* but chicks 12 hours old did; 30 live cells of *C. jejuni* induced diarrhea in 50% of the 12 hours old chicks 4 days after feeding. The shedding rate for infected chicks was $10^7$–$10^8$ per gram of feces and shedding continued through the whole experiment (14 days). *C. jejuni* could be recovered from sites throughout the intestine with highest numbers in cecum and large intestine.

In the chickens that develop clinical disease, *C. jejuni* could be seen infiltrating mononuclear cells in ileum and cecum; there was no disruption of the intestinal mucosa but *C. jejuni* was located within epithelial cells and phagocytized in mononuclear cells in lamina propria; sometimes a submucosal edema was present. Challenge experiments in calves and lambs have likewise shown that *C. jejuni* can cause irritation and pathological changes in the intestinal tract of young animals. It has been suggested that infected animals remain in a life-long carrier state, the animal becoming immune to *C. jejuni*.

**Prevention of *C. jejuni* Infections**

Pasteurization of milk and treatment of water may prevent 80% of the outbreaks of campylobacteriosis in humans, but would have little effect on the sporadic cases. The sporadic cases can be prevented only by teaching people better kitchen hygiene or by eliminating *C. jejuni* from food animals.

It is obvious that *C. jejuni* is very infectious for young animals and that animals either become re-infected or do not live long enough to clear the infection. The density of animal population probably has an impact on the prevalence of infection. The prevalence can be expected to be high in livestock populations and in flocks of pigeons, sea gulls and migratory birds. The higher body temperature of birds may make them a most favored host since *C. jejuni* grows best at 42–45°C. *C. jejuni* is a fragile organism and probably not transmitted by commercial feed. The spread among animals is by contact (contaminated environment). In the case of poultry, newly
hatched chicks are Campylobacter free since C. jejuni is not egg transmitted. In poultry therefore, the introduction of infection most likely is from wildlife (rodents, birds) either direct or through contaminated water (like in human outbreaks). In other food animals, which are less important sources for human cases, there is direct contact between mother and offspring which represents an opportunity for transmission of infection. There is a need for well designed epidemiological studies to determine: length of shedding period, factors influencing length of shedding period, how infection is introduced into poultry flocks, role of water, development of immunity in poultry, longevity of C. jejuni, etc. After such studies, a control strategy can be designed based on tactics such as (1) biohazard control (2) competitive exclusion by adding inhibitory bacteria to feed or water (3) immunization, etc. It would be most logical to begin these studies with poultry because it is believed that poultry is the most important source for sporadic human cases. At the same time a program for poultry is likely to be successful.

A research update was presented by Dr. Roy Blankenship, USDA, ARS.

Campylobacter contamination of poultry and its correlation with human illness is becoming clearer as epidemiological data continues to accumulate. An understanding of the epidemiology of chicken colonization and the technological means to intervene are being increasingly widely studied, but as yet no technology has reached the point where it is ready for transfer to the poultry industry. We are making progress in several areas, but much remains to be done and learned before we can control the colonization of commercial chickens by Campylobacter. The general areas we are currently researching include the following: 1) competitive exclusion, 2) colonization and vaccine development, 3) environmental intervention, 4) host genetic factors influencing colonization resistance and susceptibility and 5) improved detection methods.

Dr. Norman Stern is developing competitive exclusion cultures from scrapings of the cecal mucosa of healthy adult chickens that show promise for preventing or limiting colonization of Campylobacter in chicks. Further studies are needed to extend the protective effect to older chickens. Studies are in progress to more clearly define the characteristics of the epidemiological spread of the organism throughout the growout period for broilers.

Dr. Rick Meinersmann is identifying unique outer membrane proteins of colonizing strains of Campylobacter and cloning the genes for these proteins. These proteins are being used to study colonization at the molecular level and for development of potentially effective immunogens that will stimulate the appropriate protective immune response. Congenic strain pairs are being used to identify unique differences between colonizing and noncolonizing Campylobacters.

Studies are being initiated to evaluate a variety of treatments
designed to prevent entry of Campylobacter into the growout house environment.

Dr. Stem is pursuing efforts to develop lines of chickens by classical genetic methods which will be enhanced in either resistance or susceptibility to colonization by Campylobacter. If successful, these lines will be used to study the mechanisms of colonization.

Dr. Stem has developed a new medium called "Campy-Cefex" that has some advantages over CCDA medium. He has also compared various combinations of enrichment media along with certain selective isolation media to obtain the most sensitive recovery procedure.

II. DISEASES OF IMPORTANCE AND RELATED ISSUES

A. Industry research priorities.

Dr. Morris Cover, Chairman of the SEPEA Research Committee, presented a summary of industry supported research.

The Southeastern Poultry & Egg Association continues to believe that research is one of the most important ways to improve the quality and wholesomeness of final product, to reduce cost of production, and to meet marketing needs.

This year, SOUTHEASTERN has allotted one million dollars to the research program. At the present time, there are 44 active projects with 9 more that will be added in the immediate future. The total cost of these projects is more than one and one-half million dollars. We screen 40–60 research proposals twice a year (July 1 and January 1) and fund about 15–20% of the proposals received. The following chart shows the distribution of these research projects within the various phases of the poultry industry activity.

<table>
<thead>
<tr>
<th>Area</th>
<th># Projects</th>
<th>% of Total</th>
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</thead>
<tbody>
<tr>
<td>Disease</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>Microbiology</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Processing</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Nutrition</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Management</td>
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<td>44</td>
<td>100</td>
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The average cost of these projects is $32,000 and a time required to complete them of 1.7 years per project.

With the declining availability of research funds, especially from national organizations and from federal and state sources, industry must
support the research area. The poultry research community must meet the needs of industry, must solve its problems, and must provide needed information. The future of the poultry industry and of the research community depends on the communications, coordination and cooperation of these two groups. Southeastern Poultry & Egg Association should be commended for its foresight in supporting research efforts and in doing, also helps keep university graduate programs alive.

Some of our active projects are:

In disease: laryngotracheitis, mycoplasmosis, reovirus, studies of the immune system, green liver—osteomyelitis of turkeys, bronchitis, pasteurellosis, avian influenza, bordetella, ulcerative dermatitis, turkey stunting syndrome.

In microbiology: new disinfectants, Salmonella, Listeria, Campylobacter, bacteriocins and pacifarin.

In processing: meat texture, yolk flavor, mechanical deboning.

In nutrition: heat stress, feather hydrolysis.

In management: hatchery sanitation, broiler harvesting, turkey broodiness.

In waste handling: phosphorous, water quality, litter as fertilizer.

In cholesterol: genetic importance, dietary factors.

B. Poultry Health Issues.

Dr. Tom Holder, Allen's Hatchery, Delaware, presented broiler industry concerns relatively to the control and treatment of broiler diseases. Topics of concern are:

1. – Vertically Transmitted Diseases
   a. – MG – MS
   b. – Salmonella and Arizona sp.
   c. – Chick Anemia Virus
   d. – Other bacteria that are either egg transmitted or egg shell contaminants

2. – Exotic Foreign Animal Diseases
   a. – TRT – Have we got it in the U.S. already?
      Shouldn't we educate people on recognition of this disease?
      Could this be done at the AI conference next year?
   b. – Avian Influenza – always a threat
   c. – VVND – Pet bird regulations need strengthening
   d. – Imported Hatching Eggs
      It is our understanding that imported eggs can be commingled with
domestic eggs and hatched. At one time, there was an isolation and quarantine period before chicks could be considered safe. We would like to see more regulation on importations of hatching eggs.

3. **Infectious Rutting – Stunting Syndrome – Spiking Mortality**
   a. Cause – Viral, bacterial, nutritional – interaction of some or all of the above
   b. Research needs to help With this problem

4. **Inflammatory Process**
   a. The number two cause of condemnations in many areas.
   b. Cause – Is it infectious?

5. **Colibacillosis**
   a. Ultimate cause of death of high percentage of broilers
   b. Predisposed by immunosuppressive agents
   c. Research on genetically altered vaccine is needed

6. **Marek’s Disease**
   a. Continue research at East Lansing
   b. Only a matter of time before new strain emerges

7. **Respiratory Viruses**
   a. Newcastle
   b. Infectious Bronchitis
   c. Infectious Laryngotracheitis

8. **Reoviruses**
   a. Problems that this virus causes other than leg problems

9. **Antibiotic Removal**
   a. FDA has removed antibacterials recently
   b. Are they justified scientifically?
   c. Was the removal due to special interest pressure?

10. **Slow Response of New Drug and Combination Clearances by FDA.**
    a. Two plus years since new drug or combination has been approved
    b. New drug clearance protocol is a moving target
    c. Bird density and NRC dietary requirements are problems with the reviewers
    d. Reviewers are not familiar with our industry – we will help familiarize them with our industry

11. **Food Safety**
    a. Always of concern to our industry
    b. Regulations must be practical and improve the overall problem
    c. Will the consumer pay the price?

I leave you with one thought. Wholesome chicken comes from healthy chickens.

Dr. John Brown, DeKalb Research, IL., presented the perspective of the table egg industry.
REPORT OF THE COMMITTEE

The major health issue facing the egg industry today is *Salmonella enteritidis*. This infection along with the legal and regulatory ramifications has the potential to drive egg production companies out of business. There are vaccines currently in development that may prove beneficial, but with their use regulations regarding blood testing will need to be re-examined. Marek's Disease has re-emerged as a problem both to the industry and the diagnostic laboratories. The disease has evolved into an adult bird as well as pullet disease. Clinical signs and gross lesions have moved away from the classical description of Marek's to more vague lesions. Research in the development of new and better vaccines, vaccination techniques, and management practices should continue at the university level.

Mycoplasmosis continues to be a regulatory problem. On the one hand the breeders are virtually 100% negative, the commercial egg industry is well on its way to being 100% positive. Diagnostic serology still continues to be a challenge, with false positive plate test reactions and some HI activity occurring as a result of the widespread use of the inactivated viral vaccines. Lastly, infectious laryngotracheitis is an issue of concern. The USAHA has a proposal before it to recommend eradication. While the egg industry agrees with the intent of this proposal, we are concerned with the methods of enactment. Provisions need to be made to account for the pockets of endemic areas which need special consideration. Vaccines need to be available in case of outbreaks in these areas.

The view of health issue concerns of the turkey industry was presented by Dr. Yan Ghazikhanian, Nicholas Turkeys, CA.

The following concerns were expressed:

1. Sporadic fowl cholera outbreaks have occurred in breeder and meat turkeys. The extent of outbreaks have been less than in the past.
2. Sporadic MG and MS in some breeders and meat flocks been diagnosed. Extent of MM in meat turkeys is not known.
3. A couple of outbreaks of PMV-2 or at least suspicious.
4. Some outbreaks of PMV-3 been detected. Vaccination for PMV-3 is being done in California, Virginia and NC prior to production.
5. Enteritis associated with, or at least in the mind of diagnosticians, enteroviruses in commercial meat flocks.
6. Mild field vaccine associated NC titers detected in meat turkeys around the country.
7. Non-spreading colibacillosis still remains the major cause of turkey mortality associated with bordetellosis, NC (Vaccine/mild field strains)/ME heat.
8. Bacterial osteomyelitis is still being detected in the plants and the controversy surrounding the diagnostic technics on the processing line on whole body still exit.
9. *S. arizonae* recovered from commercial breeder replacements hatched in the same hatchery where perhaps positive commercial
meat poults hatched.

10. Vaccination for Salmonella is being done in commercial and primary breeders for PMV-3. More intensive vaccination is being done in West and East.

11. Breast blisters incidence in 1991 has taken place of the breast button incidence which held as a major downgrading in the past 7-10 years in 13-16 wk. turkeys.

12. Drop in egg production of unknown nature simulating viral infection has occurred in some breeders flocks.

13. Drop in egg production related to heat has been experienced (non-pathogenic related).

14. Non-bacterial leg abnormalities have also occurred in 1991.

15. Quarantine is still in effect for egg/poults imported from Europe (UK).

16. Avian influenza outbreaks will be reported by Dr. Pomeroy.

C. Status reports.

The report of NVSL Diagnostic Bacteriology Laboratory Activities was given by Dr. Lee Ann Thomas, APHIS-NVSL. A detailed report can be found in the report of the Salmonella Committee, which is contained in this proceeding.

The velogenic viscerotropic Newcastle disease report was given by Dr. James E. Pearson, APHIS-VS/NVSL.

On March 29, 1991, a veterinary practitioner in Las Vegas, Nevada, reported a suspected case of exotic Newcastle disease in a young parrot to VS. A 2-month old yellow-nape Amazon parrot was brought to his clinic showing signs of central nervous system (CNS) dysfunction. A foreign animal disease diagnostician (FADD) investigated the report, confirmed the clinical diagnosis, and sent specimens to the National Veterinary Services Laboratories (NVSL), Ames, Iowa, where the Velogenic Viscerotrophic Newcastle Disease (VVND) virus was isolated and characterized. Based on investigative information, the bird was purchased earlier in March from a vendor near the grounds of a swap meet at Spring Valley, California. All attempts to locate this vendor were unsuccessful.

On April 19, 1991, the California Veterinary Diagnostic Laboratory at San Bernadino, California, isolated a Newcastle disease virus from young yellow-nape Amazon parrots. Subsequently, NVSL at Ames, Iowa, confirmed the isolate as VVND virus. The parrot owners, a couple at Tarzana, California, reported the young birds were purchased as a group of 70 from a person in Venice Beach, California. Approximately 60 of the 70 young parrots died after showing signs of a CNS disorder. A few of the sick birds were examined by several veterinarians before a practitioner in Simi Valley suspected exotic Newcastle disease and reported the findings to State and Federal animal health officials in California. All remaining birds on the
premises were appraised, euthanized, and the premises cleaned and disinfected. Epidemiological investigations were conducted; however, the vendor could not be located and the exact source of the yellow-nape parrots was never found. A national and local press release was issued about the outbreak to inform the public of the outbreak and the hazards of purchasing and dealing in suspicious birds.

In May 1991, another outbreak of VVND occurred in caged pet birds located in Indiana, Illinois, Michigan, and Texas. The point source for these outbreaks was determined to be an aviary in Houston, Texas. The owner of this aviary reportedly purchased 20 immature double yellow-headed Amazon parrots from an unknown source at a swap meet in Pasadena, Texas. He subsequently sold 19 parrots to aviaries in Michigan, Indiana, Maryland, and Texas. The birds in Indiana were distributed to an aviary and private residence in Illinois. Ten of the birds became ill shortly after purchase and died and/or were euthanized for diagnostic purposes. Seven birds were submitted to NVSL where a VVND virus was isolated from five of them. On June 12, 1991, the last case of VVND was confirmed positive. A total of four premises were infected and 29 additional premises were exposed. Birds on the four positive premises which were isolated from the infected birds were swabbed three times. All birds on these 29 premises were swabbed twice and remained negative for Newcastle disease. The point source aviary and one infected aviary in Indiana were depopulated at a cost of approximately $60,000. The total cost of eradicating the disease was $160,000.

All outbreaks of VVND in caged pet birds were quickly eliminated and no domestic poultry was affected.

Dr. J. E. Pearson, APHIS, NVSL presented the report of the Subcommittee on turkey rhinotracheitis and swollen head syndrome, chlamydia, and paramyxovirus.

**Turkey Rhinotracheitis (TRT)/Swollen Head Syndrome (SHS) of Chickens**

The number of samples tested by the National Veterinary Services Laboratories (NVSL) increased substantially over previous years. Between October 1, 1990 and September 30, 1991, 1933 samples were tested (Table 1). The increase in testing was primarily due to reports of a drop in egg production in breeder turkey flocks, and TRT was a suspected cause.

All samples tested in FY 91 were negative for antibodies to TRT/SHS. One submission received at the NVSL on October 4, had 20 positive samples when tested by the enzyme-linked immunosorbent assay (ELISA). The significance of these positive samples is being evaluated.

**Chicken Anemia Agent (CAA)**
TRANSMISSIBLE DISEASES OF POULTRY

In the last year there have been many reports on CAA research. However, from a regulatory standpoint, little has changed. Most flocks, including specific pathogen-free (SPF) flocks, are positive or seroconvert by 30 to 40 weeks of age. There usually is no clinical disease associated with infection.

*Chlamydia psittaci*

Between October 1, 1990 and September 30, 1991, 19 isolations of *C. psittaci* were made from six states and the District of Columbia. All isolates were from psittacine birds. There were no reports of chlamydiosis in turkeys during this time.

Avian Nephritis

An avian nephritis virus (ANV) reference strain was obtained from the Veterinary Research Laboratories, Belfast, Ireland. Antiserum was produced and the capability to perform serology using the indirect fluorescent antibody (IFA) test was developed at the NVSL.

A survey of SPF and commercial flocks was conducted using the IFA procedure. Six of 20 SPF chicken flocks were positive for antibody; one SPF turkey flock tested was negative. Twelve of 25 commercial flocks were positive.

Import Bird Testing

Dr. Hand will provide a summary of import bird activity. There has been a substantial drop in the number of import birds. There continues to be avian influenza (AI) isolates from import birds. There were a total of 105 isolates from seven lots of birds. The number of velogenic viscerotrophic Newcastle disease (VVND) positive lots and percentage of positive lots is the lowest since the import bird testing was started in 1974. There was one pigeon paramyxovirus virus isolate made from a pigeon imported from Germany.

Paramyxovirus (PMV) Subcommittee

There have been no reports of PMV–2 or PMV–3 virus isolates from poultry. However, turkey flocks with positive for antibody to PMV–3 are constantly being identified and the infection has been associated with a drop in egg production. There has been an increase in use of the vaccine. It is an inactivated vaccine that is now licensed in combination with Newcastle disease virus (NDV). There were two PMV–2 isolations made at the NVSL from Michigan psittacine birds and 142 isolations from import birds. All but
12 of the import bird isolates were from finches. There were 54 PMV-3 isolates from import birds and none from domestic birds. Forty-two were from psittacine birds, the remainder were from finches (2), Peking robins (8), and canaries (2).

Eighteen pigeon PMV-1 isolations were made at the NVSL or submitted to the NVSL for identification. The isolates were from nine states: California, Colorado, Delaware, Illinois, Indiana, Maryland, New Jersey, Pennsylvania, and Washington. The PMV-1 isolates are characterized using a panel of monoclonal antibodies (Mab) developed by Dr. David Snyder at the University of Maryland. These Mabs have proven very effective in differentiating between the pigeon PMV, lentogenic NDV, and VNDV. However, any virus with a mean death time (MDT) in embryonating chicken eggs less than 90 hours is tested for pathogenicity by inoculating 6 to 8-week-old susceptible chickens.

**Inactivation of Avian Viruses by Composting**

An experiment was conducted at NVSL to determine if avian influenza virus and the egg drop syndrome –1976 agent (adenovirus 127) could be inactivated by composting infected tissues. Infected tissues were added to dead chickens and composted according to a procedure developed at the University of Maryland. The viruses were inactivated after 20 days.
TABLE 1. TURKEY RHINOTRACHEITIS/SWOLLEN HEAD SYNDROME
SEROLOGY
OCTOBER 1, 1990 – SEPTEMBER 30, 1991
ALL SPECIMENS WERE NEGATIVE

<table>
<thead>
<tr>
<th>Month</th>
<th>State</th>
<th>Samples</th>
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</thead>
<tbody>
<tr>
<td>August</td>
<td>Alabama</td>
<td>16</td>
</tr>
<tr>
<td>December</td>
<td>California</td>
<td>39</td>
</tr>
<tr>
<td>March</td>
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<tr>
<td>March</td>
<td>Canada</td>
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</tr>
<tr>
<td>January</td>
<td>Georgia</td>
<td>12</td>
</tr>
<tr>
<td>April</td>
<td>Georgia</td>
<td>8</td>
</tr>
<tr>
<td>October</td>
<td>Iowa</td>
<td>95</td>
</tr>
<tr>
<td>December</td>
<td>Iowa</td>
<td>40</td>
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<tr>
<td>January</td>
<td>Iowa</td>
<td>160</td>
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</tr>
<tr>
<td>August</td>
<td>Iowa</td>
<td>180</td>
</tr>
<tr>
<td>March</td>
<td>Maryland</td>
<td>30</td>
</tr>
<tr>
<td>August</td>
<td>Minnesota</td>
<td>55</td>
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<tr>
<td>September</td>
<td>Minnesota</td>
<td>20</td>
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<td>July</td>
<td>North Carolina</td>
<td>66</td>
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<td>January</td>
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<td>76</td>
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<tr>
<td>April</td>
<td>West Virginia</td>
<td>60</td>
</tr>
<tr>
<td>August</td>
<td>West Virginia</td>
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1840
TABLE 2. VIRUS ISOLATIONS FROM IMPORTED BIRDS
FY 1990 AND 1991

<table>
<thead>
<tr>
<th>Lots – Private Facilities</th>
<th>FY 1990</th>
<th>FY 1991 thru September 15</th>
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<tbody>
<tr>
<td>Specimens</td>
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<tr>
<td>Private Facilities</td>
<td>20,885</td>
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</tr>
<tr>
<td>USDA Facilities</td>
<td>3,482</td>
<td>2,893</td>
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<td>VVND Positive Lots</td>
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<td>Private Facilities</td>
<td>4</td>
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<td>USDA Facilities</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Confiscated Birds</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>VNDV Positive lots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private Facilities</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Confiscated Birds</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other Virus Isolates</td>
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<td></td>
</tr>
<tr>
<td>Pigeon Paramyxovirus</td>
<td>13</td>
<td>1</td>
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<td>Paramyxovirus–2</td>
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<td>142</td>
</tr>
<tr>
<td>Paramyxovirus–3</td>
<td>215</td>
<td>54</td>
</tr>
<tr>
<td>Lentogenic NDV</td>
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<td>1</td>
</tr>
<tr>
<td>Unidentified Hemagglutinating Virus</td>
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<td>0</td>
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<td>Chlamydia psittaci</td>
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<td>Psittacine Herpesvirus</td>
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<td>2</td>
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<td>Reovirus</td>
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<td>Avian Influenza H4N3</td>
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<td>75</td>
<td>17</td>
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<tr>
<td>Avian Influenza H4N6</td>
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<td>0</td>
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<tr>
<td>Avian Influenza H4N8</td>
<td>55</td>
<td>81</td>
</tr>
<tr>
<td>Avian Influenza H3N8</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

III. AVIAN IMPORT/EXPORT ACTIVITIES

Dr. Irvin Peterson, APHIS–VS, presented a report on avian import activities.

A. Poultry and Hatching Eggs

There were 5,440,976 poultry, including day old chicks, and
TRANSMISSIBLE DISEASES OF POULTRY

11,908,442 hatching eggs imported into the United States from Canada during the first three quarters of FY 1991 through the Canadian land border ports.

Approximately 100,000 turkey hatching eggs were imported from the United Kingdom and quarantined for 8 weeks post hatch with serological testing to determine their status for viral turkey rhinotracheitis prior to release. Quarantines were conducted in West Virginia.

Currently USDA is requiring serological negative certification for adenovirus 127 (egg drop syndrome), Salmonella enteritidis, and the causative agent of viral turkey rhinotracheitis (swollen head syndrome in chickens). Since February 1990, importation of egg type chicks or hatching eggs from Canada must be from flocks that are under a surveillance program like that of the National Poultry Improvement Plan. These requirements are in addition to the required health certification indicated in Part 92.5, of Title 9, Code of Federal Regulation (CFR), for all chickens and turkey hatching eggs.

B. Commercial Birds

The importation of commercial birds continues to decline, both in the number of lots and birds. The decline seen in recent years has been due to less availability of species, combined with a number of countries restricting international trade of birds. This decline was compounded during FY 1991, when a large number of international airlines refused to ship "wild-caught" birds. There were 102 lots, totaling 180,706 birds, released from quarantine during the first three quarters of FY 1991; 3 lots, totaling 963 birds, were refused entry because of VVND.

C. Pet Bird Program

Pet birds are currently being imported and quarantined at New York, New York, Miami, Florida, Los Angeles, California, Honolulu, Hawaii, and Mission, Texas. There were 1,612 birds imported and quarantined during FY 1991. All pet birds were tested for VVND, and no Newcastle disease was isolated.

D. Smuggled Birds

Birds that were illegally entered into the United States and seized were quarantined at USDA facilities. There were 1,184 birds quarantined. Following the successful completion of the quarantine and test procedures, these birds will be sold at public auction as permitted by a Memorandum of Understanding between the Departments of Agriculture, Interior, Justice, and Treasury.
E. Ostrich Importations

On August 21, 1989, the USDA imposed a ban on the importation of all ratites because of the disclosure of exotic ticks on imported adult ostriches. Consequently, ostrich importations were limited to those USDA permits that were outstanding at the time the ban went into effect. Five of these permits were honored during FY 1990. The final permit was honored during FY 1991, and 144 ostrich chicks were imported through the New York Animal Import Center (NYAIC) at Newburgh, New York. Of these, 74 died while in quarantine and 70 were released. These chicks arrived at NYAIC in very poor condition and were not provided adequate heat while they were being transported to the United States.

In order to remove the ban, the Animal and Plant Health Inspection Service promulgated regulations for the importation of ratite birds. A proposed rule was published in the Federal Register on May 30, 1990, and proposed to allow for ratites to be imported through the USDA, NYAIC, Newburgh, New York, and to limit imports of ostriches to chicks less than 36 inches in height and 30 pounds in weight upon arrival. The proposed rule also would allow ratite eggs from domestic flocks to be imported, incubated, and quarantined in commercial bird importation facilities. Over 2,000 comments were received and have been reviewed. A final rule was published on July 12, 1991, to be effective on August 12, 1991. A site inspection of the farm of origin must be completed by a USDA official prior to issuing an import permit to insure that the chicks and/or eggs are domestically produced. These additional restrictions, combined with a limited amount of space available at NYAIC, prevented the importation of additional ostriches during FY 1991.

IV. NATIONAL POULTRY IMPROVEMENT PLAN

The status report on the National Poultry Improvement Plan was presented by Dr. Irvin Peterson, APHIS-VS.

During calendar year 1990, there were 91 outbreaks/isolations of Salmonella pullorum and no isolation of S. gallinarum. More isolations or diagnoses of pullorum were reported; however, many were involved in the same case or same outbreak situation. The total number of isolations or diagnoses reported was 155 from 22 states. The outbreak involving a commercial integrated operation resulted in 52 of these reports. Five states reported 59 outbreaks/isolations. Pullorum was reported in turkeys twice and in a duck once.

In 1991 up to October 1, twelve States reported 92 isolations of pullorum and no fowl typhoid. One State reported 52 isolations or diagnoses of pullorum disease based on epidemiologic information. The commercial broiler/roaster operation was involved in 20 of the reported isolations. Seventy of the reports involved flocks with less than 100 birds. Fifty-five of
the reports are believed to have originated from an outbreak involving a mail-order hatchery.

Montana became the 40th State to be recognized as a "U.S. Pullorum–Typhoid Clean State" under the National Poultry Improvement Plan. Only eight of the 48 contiguous States remain unclassified. All are located in the Western region of the United States.

V. AVIAN INFLUENZA

A. Report of Avian Influenza Subcommittee.

Dr. B. S. Pomeroy, University of Minnesota, stated that this report includes data from questionnaires sent to State Veterinarians and laboratory results reported by NVSL.

Individual States

A questionnaire was sent to 48 State Veterinarians and members of the Committee. Replies were received from 27 states. No corrections were received in the table listing the influenza serotypes isolated from turkeys, chickens and other domestic fowl in the U.S. or based on serology (1964–1990).

Turkeys

California

One flock was identified serologically with H1N1 at NVSL.

Colorado

Serological diagnosis and isolation of H7N3 were reported by NVSL.

Florida

NVSL reported one serological identification of H9N1.

Illinois

Serological diagnosis of H1N1 was made by NVSL on one flock. A serological diagnosis of H1N1 was reported by NVSL on two occasions.

Michigan

Reported one serological identification of H1N1.

Minnesota

Minnesota has an extensive monitoring program of collection of blood samples at processing plants of broilers and turkeys; 269 broiler flocks and 2,004 turkey flocks were sampled in FY91S. No broiler flocks were identified as serologically positive. In FY91F, 28 flocks were identified as positive serologically and/or by virus isolations. Two virus isolations were characterized at NVSL; H13N2
and H6N1. The serotypes involved were H1N1, H4N6, H4N8, H5N2, H6N1, H6N2, H6N8, H7N3, and H13N2. H5N2 involved two flocks on one farm. Several attempts were made to isolate the virus and were unsuccessful. There was no spread to replacement flocks. H7N3 involved two flocks under the same ownership. Attempts to isolate the virus were unsuccessful and no spread occurred. H13N2 was isolated for the first time. It has been reported from shore birds and gulls. H1, H2 and H6 vaccines were used.

Nebraska
NVSL reported the serological identification of H1N1 on a single occasion.

North Carolina
NVSL reported the serological identification of H1N1 on 11 occasions. Sixty-nine flocks were vaccinated with H1N1 vaccine.

Ohio
Twenty-seven flocks were vaccinated with H1 vaccine.

Virginia
NVSL reported the isolation of H10N7 and serological identification of same serotype in three flocks.

Use of Avian Influenza Vaccine FY1991

The use of AI killed vaccine was limited to three states, H1N1 Minnesota, Ohio and North Carolina. In addition, Minnesota used H2 and H6 vaccines. The primary manufacturer of AI vaccine indicated H1N1 was the principal serotype used in 1991.

Chickens

Pennsylvania
Two flocks were identified through AI surveillance program, one dealer and one backyard flock with less than 500 birds in both flocks, isolation of H2N2 serotype was made as well as positive serology.

Ohio
Chickens originated in Ohio, were found serologically positive (H1N2) at live bird market in Pennsylvania. There were no clinical signs in trace backs in Ohio and Pennsylvania and no isolations.

Live Poultry Markets (NVSL)
NVSL reported the results of the live poultry market survey made in June, 1991.
TRANSMISSIBLE DISEASES OF POULTRY

New York
Serotype H6N2 isolate from chickens and H2N2 from an environmental sample.

New Jersey
Serotype H6N8 was isolated from chicken and H2N2 from guinea fowl (2) and from environmental samples H6N8 and four positive isolations of H2N2.

Other Fowl (NVSL)
Quail
Maryland H6N1 or H1N1

Imported Birds (NVSL)

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Month</th>
<th>Type</th>
<th>Number of Isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russia/Hong Kong</td>
<td>Peking Robin</td>
<td>November</td>
<td>H3N8</td>
<td>7</td>
</tr>
<tr>
<td>Belgium</td>
<td>Rusella</td>
<td>January</td>
<td>H4N8</td>
<td></td>
</tr>
<tr>
<td>Argentina/ Malaysia</td>
<td>Mynah Bird and Nanday Conures</td>
<td>January</td>
<td>H4N8</td>
<td>62</td>
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<td>Zimbabwe/Belgium</td>
<td>Cockatiel</td>
<td>January</td>
<td>H4N8</td>
<td>3</td>
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<tr>
<td>Taiwan/People’s Republic of China</td>
<td>Peking Robin, Thrush and Mesia</td>
<td>April</td>
<td>H4N1</td>
<td>21</td>
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<td>Malaysia</td>
<td>Parrot and Parakeet</td>
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<tr>
<td>The Netherlands</td>
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<th>HEMAGGLUTININ ANTAGENGS</th>
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<tr>
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</tr>
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<td>1964</td>
<td>H5, H6, H9; 1991: H1</td>
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<tr>
<td>Massachusetts</td>
<td>1965</td>
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<tr>
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<td>1965</td>
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<td>1971</td>
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<td>Colorado</td>
<td>1972</td>
<td>H1, H5, H9; 1991: H7</td>
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<tr>
<td>Ohio</td>
<td>1975</td>
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<td>1978</td>
<td>H1</td>
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<td>Texas</td>
<td>1979</td>
<td>H5, H7, H9</td>
</tr>
<tr>
<td>Indiana</td>
<td>1980</td>
<td>H1, H2, H10</td>
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<td>Missouri</td>
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<td>Kansas</td>
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<tr>
<td>North Dakota</td>
<td>1981</td>
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<td>North Carolina</td>
<td>1982</td>
<td>H1, H4; 1991: H1</td>
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<tr>
<td>Virginia</td>
<td>1983</td>
<td>H1, H2, H4, H5, H10; 1991: H10</td>
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<tr>
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<td>1983</td>
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<td>Michigan</td>
<td>1985</td>
<td>H1, H9; 1991: H1</td>
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<tr>
<td>Utah</td>
<td>1985</td>
<td>H6, H4, H10</td>
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<tr>
<td>Nebraska</td>
<td>1988</td>
<td>H1; 1991: H1</td>
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<tr>
<td>New York</td>
<td>1988</td>
<td>H9</td>
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<tr>
<td>Illinois</td>
<td>1991</td>
<td>H1</td>
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<tr>
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<td>1991</td>
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<td><strong>Chickens</strong></td>
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<tr>
<td>Alabama</td>
<td>1975</td>
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<td>Minnesota</td>
<td>1978, 88</td>
<td>H6, H9</td>
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TRANSMISSIBLE DISEASES OF POULTRY

Pennsylvania 1983, 86 H1, H5; 1991: H2
Maryland 1983, 84 H5, H9
New Jersey 1983, 86 H5
Virginia 1983 H5, H7
Massachusetts 1986 H5
New York 1986 H5
Ohio 1991 H1

Chickens – Live Market

District of Columbia 1980 H1, H5
Connecticut 1986 H2, H5
Florida 1986 H5
Massachusetts 1986 H5
New Jersey 1986 H2, H5; 1991: H6
New Jersey 1989 Turkey H9
Rhode Island 1986 H5
Delaware 1990 Duck H2
New Jersey 1991 Guinea Fowl H2

Chickens – Dealer

Maryland 1983 H5
Ohio 1986 H5
Georgia 1987 H5

Other Species

Pennsylvania 1969 Ducks NA, H3, H5
Minnesota 1974 Geese NA
1974 Guinea Fowl NA
1980 Pheasants H3, H7, H8
New York 1978 Ducks H3, H4, H5, H6, H11
Pennsylvania 1983 Guinea Fowl, Quail H5
Maryland 1984 Ducks, Guinea Fowl H3
1984 Chukar H5
1985 Ducks H4
Washington 1985 Pheasant H9
Virginia 1985 Ducks, Swans, Geese H7
Oregon 1986 Quail, H5
Pennsylvania 1986 Guinea Fowl H1, H6, H11
1986 Guinea Fowl, Chukar H5
Georgia (Dealer) 1987 Guinea Fowl H5
Maryland 1987 Ducks, Geese H9
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Wisconsin 1988  Pheasant H9
Pennsylvania 1988  Geese H1
North Carolina 1988  Ducks H6
Connecticut 1990  Pheasant H4
New Hampshire 1990  Pheasant H10
California 1990  Quail H4
Maryland 1991  Quail H6 or H1

NA= Not Available

SUMMARY

Avian influenza was identified in the following species and states FY1991. The incidence was low, except for Minnesota, and no highly pathogenic serotypes were identified by USDA-APHIS-NVSL. H13N2 was identified in turkeys for the first time in U.S. Avian influenza was identified in turkeys in two new states, Florida and Illinois. H1N1 was the most common serotype identified.

Turkeys
California  H1N1
Colorado  H7N3
Florida  H9N1
Illinois  H1N1
Iowa  H1N1
Minnesota  H1N1, H4N6, H4N8, H5N2, H6N1, H6N2, H6N8, H7N3, H13N2
Iowa  H1N1
Nebraska  H1N1
North Carolina  H1N1
Virginia  H1N7

Chickens
Pennsylvania  H2N2
Ohio  H1N2

Live Poultry Markets
New York  H6N2, H2N2
New Jersey  H6N8
New Jersey  H2N2 Guinea fowl

Other Fowl
Quail
Maryland  H6N1 or H1N1

Vaccine Use
Avian influenza killed vaccine was used in turkey flocks in three
states, Minnesota, Ohio and North Carolina. H1N1 vaccine was used primarily in breeder flocks in these states. No vaccine was used in chicken flocks.

Import Bird Isolation

NVSL made 105 isolations of AI virus from tissues of birds originating from seven different countries. The serotypes identified were H3N8, H4N1, and H4N8.

B. APHIS-VS/NVSL Activities.

A summary on NVSL avian influenza surveillance activities was presented by Dr. James E. Pearson, APHIS-NVSL.

Avian Influenza (AI) Surveillance


Two live-bird markets in Rhode Island were sampled semi-annually; whereas, four in Massachusetts and five in Connecticut were sampled quarterly. The sampling consisted of cloacal, tracheal, and environmental swabs.

Thirty-eight live-bird markets which includes all of the high risk markets in New York were sampled in May of 1991 by collecting and culturing tracheal and environmental swabs. Avian influenza H2N2 and H6N2 viruses were cultured from two swabs from a guinea fowl and from four environmental swabs.

In New Jersey, sampling consisted of the collection of environmental and cloacal swabs for culture from 25 live-bird markets, two dealer premises, and two auction markets. An H6N8 virus was isolated from a cloacal swab from a chicken and from an environmental swab, and H2N2 viruses were cultured from two swabs from a guinea fowl and from four environmental swabs.

In February of 1991, the State of Pennsylvania sampled the five live-bird markets in Philadelphia. In addition, chickens sold through livestock markets throughout the State were routinely sampled. Monthly, approximately 5000 eggs from layer flocks and 4000 blood samples collected from broilers and turkeys at slaughter were also tested. An H2N2 virus was isolated from a dealer chicken flock which was determined to be non-pathogenic for chickens.

Sentinel birds are still being placed in live-bird markets in Florida. Blood samples are routinely collected from these sentinels and serological
tests are performed for AI antibodies. Cloacal swabs of these chickens are also taken and cultured for AI virus. The last H5N2 AI virus isolated from chickens in the United States was made from swabs collected from sentinel chickens in a live-poultry market in southern Florida in late September of 1989. The virus was identified at NVSL in October of 1989. When the virus was inoculated into susceptible chickens, it did not produce symptoms or lesions, nor did it kill chick embryos. The market was depopulated and disinfected and there have been no further isolations.

C. 3rd International Symposium on Avian Influenza Planning Subcommittee.

Dr. C. W. Beard, USDA-ARS, stated that the Third International Symposium on Avian Influenza will be held May 27–29, 1992 at the Extension Conference Center, University of Wisconsin-Madison, Madison, WI 53706. The theme of the conference will be: AVIAN INFLUENZA: THE CONTINUING THREAT.

No area in the world seems to have experienced major problems with highly pathogenic influenza virus infections of avian species in the recent past. However, it is clear that avian influenza viruses continue to circulate. At the Second Symposium we reflected on the events and the aftermath of the "Pennsylvania, New Jersey, Virginia Experience." It is now timely to review what has happened in the past six years, to share the accumulated new knowledge, and to offer contingency plans for dealing with the localized outbreaks of mild disease and the next outbreak of highly pathogenic avian influenza, whenever and wherever it might occur.

The duration of the Symposium will be two and one-half days with five general topics of one-half day each. Attendance will be limited to one hundred participants. The registration fee is expected to be $150. Housing will be available within easy walking distance from the conference center. The five general topics will be:

- The World Status of Avian Influenza
- Epidemiology – Domestic and Wild Avian and Mammalian Species
- Pathogenicity Mechanisms and Markers
- Diagnostic, Prophylactic and Therapeutic Developments
- Control Philosophies and Recommendations

Co-Chairs: C. W. Beard and B. C. Easterday
Co-Vice Chairs: D. J. Alexander and V. S. Hinshaw
Counselors: R. A. Bankowski and B. S. Pomeroy

Program Committee (with the chairs, vice chairs and counselors):
VI. INFECTIOUS LARYNGOTRACHEITIS SUBCOMMITTEE

The ILT subcommittee report was given by Dr. Daryl C. Johnson, APHIS–VS.

The Subcommittee did not meet during the year since we waited for the USDA response to the 1990 resolution and for further direction from the Transmissible Diseases of Poultry Committee.

The "Guidelines for the Eradication of Infectious Laryngotracheitis Encompassing the Broiler Industry, Table Egg Industry, Exhibition and Backyard Poultry" was presented by subcommittee members at numerous poultry meetings during the year.

A. USDA Response to 1990 Resolution.

The USDA response to the resolution on infectious laryngotracheitis was given by Dr. Irvin Peterson, APHIS–VS.

Dr. Peterson stated that the eradication plan was considered feasible. However, with the current of manpower and funds, it will probably not receive a high priority for immediate action. Some states have expressed interest in an ILT program, and pilot projects with cooperating states will be encouraged.

B. Industry Response and Other Issues.

An industry response and the reaction of the AAAP Committee on Respiratory Diseases was given by Dr. Fred Hoerr, Auburn University. The AAAP Committee on Respiratory Disease has examined the ILT Eradication Guidelines and considers them to be scientifically sound and appropriate for implementation by a state or region if so desired. The opinions of AAAP members representing the commercial egg and broiler industries, and those with information on successful cooperative programs with gamebird raisers and backyard flock owners have been requested. A resolution concerning the ILT Eradication Guidelines is planned for presentation to the AAAP for consideration 1992.

C. Vaccine Issues.

Dr. A. Mutalib, Cornell University presented the results of recent experiments designed to determine if chickens vaccinated with a tissue culture-adapted ILT vaccine would transmit the virus to contact chickens.

Three on-farm trials were carried out in which contact control chickens were housed with commercial layers at different periods after vaccination with tissue-culture-adapted laryngotracheitis virus (LTV) vaccine. Sera were collected from vaccinates and controls before and six weeks after vaccination and were checked for LTV antibody titers by ELISA.

A fourth trial was carried out in the virus isolation unit at Cornell University in which both the vaccinates and controls were specific-pathogen-free (SPF) chickens. Serum LTV antibody titers were measured in both groups as above. At the end of this trial the vaccinates, contacts and a third group of naive SPF chickens were challenged by inoculation of virulent LTV.
into the infraorbital sinus. Birds were observed twice a day for one week after challenge for clinical signs and mortality. All birds were necropsied and pathologic lesions were recorded and compared grossly and histologically.

LTV antibodies were detected in contact controls in all trials and it was concluded that birds vaccinated with tissue–culture–adapted laryngotracheitis vaccine will shed the virus. Contact birds develop immunity comparable to that of vaccinates and it enables them to resist challenge infection.

VII. MYCOPLASMA SUBCMMITTEE

The report of the Mycoplasma Subcommittee was given by Dr. Duncan McMartin, University of California.

A. Sensitivity and Specificity of Plate Antigens and Availability of Standard Test Reagents.

Concerns in these areas led to submission of a resolution from USAHA in 1990, which was positively responded to by USDA. NVSL Labs have distributed 20% more test reagents in 1991 to date than in 1990. The Bacteriology Section, NVSL, is commended for its efforts. However, it is understood that to achieve this, the resources of the Section have been utilized to the absolute maximum. In this connection, therefore, the subcommittee recommends to the TDP Committee that if budget considerations make it impossible for NVSL to address the production of both standard agglutination plate antigens and positive and negative standard reference sera, that priority be given to the letter.

To further enhance the production of improved commercial plate antigens, the subcommittee recommends that the TDP Committee encourages additional communication and interaction between users of serologic reagents, producers of serologic reagents and developers of protocols governing the production of reagents.

B. Proposed Annual Report on Mycoplasma Field Infections in Poultry in the U.S.

Such a report would be valuable for:

- evaluating progress in eradication
- estimating quantities of reagents required for testing
- providing information on risk factors associated with field infection.

The subcommittee recommends that the TDP encourage the publication of an annual report by USDA on confirmed field infections of MG, MS, and MM including numbers of infections, species, breed or strain (if possible), and any special circumstances connected with the infection.

C. Repositories for Mycoplasma Isolates From Field Infections

The subcommittee recommends that the TDP encourage the continued collection and storage of field mycoplasmas or NVSL and other
major mycoplasma research laboratories to facilitate the study of strains which may be atypical.

VIII. EMERGING ISSUES

Dr. R. L. Witter, USDA-ARS, presented the following report on reticuloendotheliosis virus.

Reticuloendotheliosis virus (REV) is an avian retrovirus, distinct from avian leukemia virus, that causes immunodepression, B-lymphomas, and T-lymphomas in chickens, and undefined lymphomas in many avian species. Infection as measured by serology is moderately widespread in the United States and other countries. However, clinical disease is rare. The virus is transmitted congenitally and by contact with undetermined sources of infection in the environment. The virus is well recognized as a potential contaminant of biologic products. Severe losses due to stunting and immunodepression were noted in the 1970's in Australia and Japan, and more recently in Brazil, due to the administration of Marek's disease vaccines contaminated with REV. In the United States, spot checks of poultry vaccine master seed stocks have revealed no evidence of REV contamination. Therefore, no regular testing of production serials is required or done.

Although REV infection in poultry has been assumed by disease control authorities to be of little sequence, several recent developments which may impact this assessment should be considered.

Studies over the past several years have shown that lymphomas and nerve lesions induced by REV resemble those of Marek's disease or lymphoid leukemia so closely that misdiagnoses could easily occur. Even if a diagnostician wished to include RE in the differential diagnosis, simple and reliable procedures for distinguishing RE lesions from other viral neoplasia are lacking. This situation is not well appreciated by the diagnostic community and accurate assessments of tumor frequencies may be difficult to obtain at the present time.

Secondly, recent experiences support the potential for REV infection to emerge as a significant economic disease in poultry. Infection is clearly widespread in both turkeys and chickens. A turkey breeder complex in Pennsylvania has been made unprofitable by losses due to endemic REV infection. Although reports of chickens that have developed disease from natural exposure are rare, much concern over this possibility currently exists in the Middle East where REV-associated lymphomas appear to be associated with significant death loss in certain chicken flocks. If REV was to emerge as an economic problem in chickens or turkeys, no proven method to control or eradicate the infection exists.

Finally, REV infection is presently being considered as a barrier to importation of breeder stock by one Middle Eastern country. This policy is prompted by the increased prevalence of what may be REV-induced
REPORT OF THE COMMITTEE

lymphomas in chickens and the suspicion that this problem has been introduced by importation. As a result, breeder companies in the United States are being required to certify that their flocks are free of REV infection prior to importation. The ELISA antibody test is usually employed, but some problems in interpretation of false positive reactions have been identified. Methods to insure the freedom of breeder flocks from REV infection may be increasingly needed, but no uniform tests or policy have yet been developed.

Based on its newly recognized potential to confuse differential diagnoses, cause economic loss and impede exports, REV infection may warrant increased concern. Surely, it would seem prudent to develop better diagnostic and disease control procedures. Indeed, until better diagnostic tests are in use, it may be impossible even to assess the incidence of REV-associated disease. Regulatory officials should be knowledgeable about the potential of REV to negatively impact the poultry industry of the United States and may want to consider closer surveillance of the situation.

IX. 1990 MIGRATORY BIRD DISEASE SURVEILLANCE

Dr. Milton Friend, US Fish and Wildlife Service gave the following report on wild/feral bird die-offs.

The U.S. Fish and Wildlife Service's National Wildlife Health Research Center recorded 215 bird die-offs during the period of July 1, 1990 through June 30, 1991. This is slightly higher than the 206 average for the previous three years and 19% greater than the 180 average for the previous six year period. The diagnosis of velogenic Newcastle Disease as the cause of death of an estimated 6,500 cormorants and 300 white pelicans and gulls in Canada was the most noteworthy wild bird mortality event. The largest loss of bird life occurred on marshes in the vicinity of the Great Salt Lake, Utah, where 50,000 aquatic birds died from avian botulism (type C).

Botulism was once again the most frequently diagnosed cause of bird die-offs (62 events), followed by 34 events of avian cholera. The first confirmed occurrence of avian botulism in Hawaii and in wild birds in Colorado were among these events. Also, nearly 60 endangered Aleutian Canada geese died during one of the avian cholera events. Salmonellosis was diagnosed as the primary cause of songbird mortality in seven states -- Georgia, Idaho, Illinois, Missouri, Oregon, Washington, and Wisconsin. Salmonellosis was also a secondary finding in a gull die-off on Lake Champlain in Vermont. Duck plague (duck virus enteritis) was diagnosed for the first time in Indiana. A confirmed outbreak occurred in muscovy ducks in Vanderburgh County and a suspect outbreak (no virus isolation) occurred in Posey County. Other outbreaks occurred in Maryland (2 confirmed, 3 suspect), Pennsylvania (1 confirmed), and Virginia (2 confirmed, 1 suspect). Muscovy ducks were the principal species involved in all of these events. Captive-propagated mallards were also a principal species in two of the
Maryland events. Concern continues to mount regarding the potential establishment of this disease in free-ranging waterfowl.

A total of 42 states had one or more bird die-offs during the reporting period. California led all states with 28, followed by Illinois with 19, Wisconsin with 16, and Texas and Maryland with 12 each. Ohio and Florida with 10 each were the only states with 10 or more reported events.

X. OLD AND NEW BUSINESS

Statement of Purpose.

Following a short discussion, the following Statement of Purpose was unanimously approved by the Committee.

Statement of Purpose

Committee on Transmissible Diseases of Poultry and Other Avian Species
United States Animal Health Association

The purpose of the Committee on Transmissible Diseases of Poultry and Other Avian Species shall be to provide information and advice to the United States Animal Health Association on issues pertaining to the health of animals of the avian species and the hygiene of raw foods of avian origin.

Specifically, the Committee will serve USAHA as a representative forum for interested private and public sector individuals and organizations to:

A. Study the science of the health of animals of the avian species and the hygiene of raw foods of avian origin and convey, through comprehensive Committee reports, pertinent information regarding current issues in these areas.

B. Advise, through recommendations and proposed resolutions, means to unify, so far as possible, laws, regulations, policies and methods pertaining to the: prevention, control and eradication of transmissible diseases of the avian species; and hygiene of raw foods of avian origin.

The principal agencies to which approved Committee recommendations and adopted resolutions are directed to are as follows:

1. Federal Agencies: USDA, including APHIS, FSIS, CSRS, ARS; US Health and Human Services, including FDA Center for Veterinary Medicine, Centers for Disease Control, and Center for Food Safety and Applied Nutrition; US Environmental Protection Agency;
REPORT OF THE COMMITTEE

Department of the Interior, including the National Wildlife Health Research Center.


3. Academic Institutions: State universities including schools of veterinary medicine, departments of veterinary science, agricultural experiment station units, cooperative extension units and veterinary diagnostic laboratories.

4. Avian industries and owners: The broiler, table egg and turkey, game bird, caged bird industries, owners and associated health professionals.

5. Allied industries: The pharmaceutical and biologics industries; private health professional practitioners; the feed industry.

USAHA Allied Industry Organization Membership Requirements for the Turkey, Broiler, and Table Egg Industries.

Concern was expressed that the major allied industry broiler, turkey, and table egg organization are not represented on the USAHA Executive Committee. One of the requirements for such representation is that such organizations must have at least 50 USAHA members. The National Broiler Council, for example, is represented by fewer than 50 companies, making it difficult for them to meet the USAHA membership requirements. It was suggested that the Committee recommend that USAHA consider waiving the Constitution and By-Laws membership requirements so that poultry industry trade organizations could be considered for election to allied industry organization membership and representation on the Executive Committee.

Several Committee members expressed the view that even though such organizations were represented by only a small number of companies, each company could have several members of USAHA. There was also concern that USAHA would not be willing to waive its membership requirements. A motion to table this issue until next year was passed by a unanimous vote.

Third International Symposium on Avian Influenza.

A motion was made that the Committee on Transmissible Disease of Poultry authorize the 3rd International Symposium on Avian Influenza Subcommittee to apply leftover funds from the 2nd Symposium to help to defray the costs of the 3rd Symposium. The motion carried unanimously.
XI. THE FOLLOWING SUBCOMMITTEES WERE CONTINUED.

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; S. H. Kleven; E. T. Mallinson; H. O. Optiz; 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, Chairperson.

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; B. Baisley; E. Odor; T. Holder; H. M. Gholi; and D. C. Johnson, Chairperson.

Third International Symposium on AI: R. Bankowski; B. Pomeroy; B. Easterday, Co-chairperson; and C. Beard, Chairperson.
Parameters of progress in the US pseudorabies eradication program have been calculated from the quarterly reports submitted by participating states. Except for data reporting numbers of known infected herds by quarters which are shown in Figure 1, data are shown for calendars years 1989 and 1990 (CY89 & CY90). During the first reporting quarter (4thQ CY88), 5813 infected herds were reported in the US. Case finding has progressed more rapidly than herd clean-up, with the number of infected herds reported in 2ndQ CY91 being 16.7% higher than that initial report.

The numbers of infected herds reported at the beginning of CY90, identified and cleaned up during the year and status at the end of the year are shown in Figure 2. The total numbers of known infected herds was increased by 44.4% during the year, but of the total, 16.4% were cleaned up during the year. At the end of CY90, 42.3% of the known infected herds were under clean-up plans.

The prevalence of identified infected herds in states reporting in CY90 is shown in Figure 3, calculated as the number of recorded infected herds divided by the number of herds in each state. Following completion of state infected herd case finding, progress toward eradication is measured by a decreasing prevalence. Eleven states, AR., AZ., CA., GA., KS., MA., OH., OK., SC., TN., and WI. reported a prevalence decrease ≥ 10% from CY89–90 while 13 states, CO., FL., IA., KY., MI., MN., MO., NE., NC., PA., SD., TX., and VA. reported an increase.

The prevalence of infection recorded in breeding swine in states reporting in CY90 is shown in Figure 4, calculated as the number of infected breeding swine detected divided by the state breeding swine population. Progress toward eradication is measured by a decreasing prevalence. Eleven states, AR., AZ., CA., MS., NC., ND., OK., PA., SD., VA., and WI. reported a prevalence decrease ≥ 10% from CY89–90 while fourteen states, AL., CO., DE., GA., IL., IA., KS., KY., MA., MI, MO., NJ., OH., and SC. reported an increase.

The surveillance indices of states reporting in CY90 are shown in Figure 5. The surveillance index is calculated by the percentage of sows and boars tested in the state during the year multiplied by the percentage of positive swine traced to herds of origin. By Stage III in the eradication program, states must reach an annual level of testing ≥ 10% of their breeding stock with ≥ 80% successful traceback of positive sera, achieving surveillance indices ≥ 0.08. In CY90, the overall US achievement was 5.7%
of the breeding swine tested with 78.6% of positive sera successfully traced back, giving a national surveillance index of 0.045. A rising state surveillance index is a measure of progress. Between CY89 90, 20 states, CT., DE., FL., HI, ID., IN., ME., MA., MN., MS., NH., NM., NC., ND., PA., RI., SC., TN., TX., & VA. achieved higher surveillance indices. Fourteen states, AL., AK., AR., AZ., CA., CO., GA., IL., KS., MI., NJ., OH., SD., and WI. experienced lower records.

The ratios of herds under monitoring programs, ie, Feeder Pig Monitored herds, Qualified Negative herds and Qualified Negative Vaccinated herds, to total herds in each state are shown in Figure 6. Between CY89 and CY90, 17 states AL., CA., CO., DE., GA., IL., IA., KS., MI., MO., MN., NE., OK., SC., TN., TX., and WI. achieved ≥ 10% increase in herd monitoring while 8 states, FL., NH., NJ., NM., NC., ND., SD., and WV. decreased an equivalent percentage.

Records of epidemiological identification of sources of new herd infections are shown in Figure 7. While during CY90, only 57% of the 1606 newly recorded herd infections were reported, of these only 60% were identified as to source of herd exposures. Among the 955 identified sources, 51% were traced to various categories of contact with infected swine, including feral swine, and 48% to area spread of the virus. Eleven states, AL., AR., CA., FL., GA., IL., MI., NE., NC., OH., and SC. increased their epidemiological investigation success rate by ≥ 10% while 9 states, IN., IA., KS., KY., MO., NJ., OK., SD., and TN. fell behind in identifying sources of new infections.

The number of swine movement tests recorded in each state divided by the number of herds in the state is shown for CY90 in Figure 8. This figure should rise until all movements of swine from non-monitored herds are of tested negative swine. Between CY89 and CY90, 32 states, AL., AR., AZ., DE., FL., GA., HI., ID., IL., IN., IA., KS., KY., ME., MA., MI., MN., MO., MS., NE., NH., NJ., NC., ND., OH., OK., PA., SD., TN., TX., VA., and WI. increased their swine movement testing by ≥ 10% while three states, NM., RI., and SC. fell behind in their numbers of swine movement tests.

The percentage of known infected herds under cleanup plans in each state is shown in Figure 9. Twelve states, CA., GA., IA., KS., MA., MI., MN., NE., NC., OK., PA., and SD. were able to achieve ≥ 10% increase in CY90 over CY89 while three states, FL., MO., and NJ. fell behind. The national average is 42.3% of identified infected herds under clean up plans.

Appropriate goals for all states in the coming year must be to achieve a surveillance index of 0.08, to bring all feeder pig producers into the monitoring program, to carry out epidemiological investigations to determine sources of new herd infections and to carry out traceback and circle testing on herds related to these infections, to test all swine movements except to slaughter from non-monitored herds, and to place all newly recognized infected herds under cleanup plans.
Fig. 1
TRENDS IN KNOWN INFECTED HERD STATUS

Fig. 2
INFECTED HERD STATUS
United States, 1990
Fig. 3
PERCENTAGE OF KNOWN INFECTED HERDS
1990

Fig. 4
SEROPREVALENCE OF BREEDING SWINE
1990
Fig. 5
SURVEILLANCE INDICES 1990

Fig. 6
PERCENTAGE OF HERDS UNDER MONITORING PROGRAM, 1990
Fig. 7
PERCENTAGE OF INFECTION SOURCES IDENTIFIED, 1990

Fig. 8
SWINE MOVEMENT CONTROL 1990
Fig. 9
PERCENTAGE OF KNOWN INFECTED HERD UNDER CLEANUP PLANS, 1990

BERAN,YANG

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The State–Federal–Industry Cooperative Pseudorabies Eradication Program has been a model of producer and Government teamwork. The guidelines for this eradication campaign, which began January 1, 1989, are called the Pseudorabies Program Standards. These Program Standards were developed jointly by APHIS, State and industry leaders. Participation in the Program requires the formation of a State Pseudorabies Committee consisting of swine producers, animal scientists, veterinarians, State and Federal regulatory officials as well as other representatives of the swine industry. Additionally, incorporating the program standards into the State’s regulations is required for full participation in the Program.

Table I. State Pseudorabies Program status as of September 1991.

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* Pending passage of regulations
** NC has split status II/III.
All 50 States, plus the Virgin Islands and Puerto Rico, are participating in and receiving Federal funds for this industry initiated program. Progress in the Program is measured by advancement through each of five stages (Table I). Entry into Stage V requires having no infected herds and maintaining adequate surveillance, testing 10 percent of the breeding swine in the State annually, for the previous 2 years. Once Stage V is achieved the surveillance can be reduced to 5 percent of the breeding swine in the State, thereby reducing the cost of the Program. No States have yet attained Stage V status. It is quite an accomplishment, however, for four States to have already achieved Stage IV status in only the third year of this 10-year program. Mandatory cleanup of infected herds is the requirement of the 14 States in Stage III. Stage II is the control phase of the Program and 20 States are at this level. The preparation stage, Stage I, is the entry level of the Program. Stage I only requires that the State Pseudorabies Committee formulate plans for a reliable determination of prevalence and seek the regulatory/legislative authority to conduct an effective control/eradication program. Unfortunately, at this point in the Program there are still 11 States and Puerto Rico in this preliminary category.

![Figure 7. States reporting infected herds as of June 30, 1991.](image)

* Iowa reported 2896 infected herds
Tremendous progress is being made in many States. Arizona, Hawaii, and North Dakota have already eradicated pseudorabies from their swine herds since the start of the Pseudorabies Eradication Program. Twenty-three States do not report having any pseudorabies in their herds. Currently, only 27 States have pseudorabies infected herds and 16 of these States have less than 15 herds with pseudorabies (Figure 1). It is expected that States with less than 15 infected herds will move rapidly toward eliminating virus from these herds and be eligible for advanced status shortly.

APHIS is continuing to provide funding and technical support to contribute innovative solutions to problem areas such as large herd cleanup, feral swine and area based control strategies. The Large Herd Cleanup project, originally begun in seven States (IL, IN, IA, MN, NE, NC, OH) is completing its second year and has been expanded to include 4 additional States (GA, KY, MI, TN) plus 22 additional herds in North Carolina. Preliminary results from this equal cost sharing (State, Federal, producer) program indicate that elimination of pseudorabies from herds of greater than 400 sows is economically feasible and achievable. The Mercer County, Ohio, project is being coordinated by Ohio State University in association with the Ohio Department of Agriculture and APHIS. The object of the Mercer County area control program is to develop efficient, economical methods to eliminate pseudorabies from dense swine-raising areas. A Feral Swine Technical group has been established to recommend procedures to reduce the risk of reinfection of domestic swine from feral swine reservoirs. The Feral Swine Technical Group along with a Pseudorabies Technical Group provides APHIS with guidance to continually improve the Program so it is cost effective, practical, and producer friendly. In addition to Federal allocations, funding for the program is provided through State funds and producer contributions. An effective disease eradication program depends on finding infected herds and cleaning them up as soon as possible. APHIS provides Federal funds to support herd cleanup activities based on the number of infected herds in a State. Finding these infected herds depends on an adequate surveillance system. The Program provides flexibility for States to determine what method of surveillance best suits the need of that State. Whatever method of surveillance is chosen, a State must test at least 10 percent of its breeding swine to advance in the program. APHIS provides funding for this aspect of the Program based on the number of breeding swine in the State.

Short-range Pseudorabies Program goals call for all States to be in Stage II or higher and 22 States in at least Stage IV by 1992. Program performance monitors indicate that the 1992 goals can be met or even exceeded with continued State, Federal, and industry support and cooperation. The definitive Program goal is to have all domestic swine herds in the United States free of pseudorabies by the year 2000.
REPORT OF THE COMMITTEE ON PSEUDORABIES

Chairman: Mr. Don D. Gingerich, Pamelia, IA
Vice Chairman: Dr. George W. Beran, Ames, IA

W.L. Adams, GA; J. Alumbaugh, IA; N. Black, MN; P.E. Bradshaw, IL; D.R. Bridgewater, CO; R.G. Brown, NC; D.J. Carr, WI; R. Dykhuis, MI; G.C. Edwards, NC; C.Y. Erbel, NC; W.D. Felker, IA; T.W. Freas, IN; D. Galbreath, MD; A.M. Gallina, WA; L. Granger, MI; T.J. Hagerty, MN; M. Hammer, NC; J.A. Hennessey, MO; H.T. Hill, IA; D. Hoogestraat, SD; I.H. Huff, ND; R.D. Hull, IL; J.P. Huntley, NY; D.D. Hupe, KS; C.L. Kanitz, IN; J.P. Kluge, IA; W. Korsmeyer, IL; M.H. Lang, WI; W.J. Mackey, MN; B.D. Marsh, IN; C. Massengill, MO; T.J. McGinn, NC; W.L. Mengeling, IA; H.F. Moberly, IL; A.D. Moles, MO; R.B. Morrison, MN; F.J. Mulhern, MD; R.R. Ormiston, MD; D.H. Person, MN; N. Pfeiffer, NE; R. Polen, NJ; J.R. Ragan, TN; H.B. Schroeder, WI; R.L. Sharpee, NE; G.P. Shibley, KS, 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; W.L. Thomas, OH; D.L. Thompson, CA; E. Thurber, NE; J.W. Van Buren, MI; J.A. Vansickle, MN; W.H. Waldo, NE; D.L. Weiss, MN; L. Williams, NE.

Thirty seven committee members were in attendance. There were more than 100 guests.

Willard Korsmeyer presented the National Pork Producers Council Oversight committee report. The Oversight Committee reviews the PRV eradication program from a producers perspective. He reported the federal appropriation for FY92 is $7.554 million compared to $6.7 million in FY91. The Oversight Committee also recognizes significant contributions to the program by producers. Producers contribute to the program through vaccine purchases, changes in production practices and in some states, direct payments for blood testing.

Dr. Joe Annelli gave the USDA report of program progress and future objectives. He reported the goal of the program should be to increase the number and percentage of herds on cleanup plans. Twelve applications for program stage up grades have been approved by the PRV Control Board this year. USDA/APHIS conducted 5 program reviews in 1991. The states were IA, GA, CA, IN and PA. The 1991 program in Iowa is a success story, with almost triple the activity in 1991.

The control board report was presented by its chairman Phil Bradshaw. The control Board reviews applications for state status. He reported that one state, Maine, has been granted Stage 5, free of PRV.

Dr. George Beran, Committee Vice Chairman reported on program costs and the federal 1990 report. He reported producers are contributing 59.8% of program costs. Government expenditures represent 40.2% of total program costs. Government expenditures include federal and state funds. He suggested another cost: benefit analysis be done on the PRV eradication program.
Dr. Peter Cowan gave the Technical Group report. He stated the PRV eradication program can be an example to all future animal disease eradication programs. He discussed the development of a nationally consistent database. Dr. Cowan reported feral swine will be a problem in the later stages of the program.

Dr. Frank Mulhern reported program obstacles and how to overcome them. He stated that continual cost-benefit analysis is needed. The year 1992 could be a critical year as to how a producer oriented eradication program can be advanced to reach its ten year goal.

Dr. Dennis Thompson discussed low incidence states problems. Lack of disease means a lack of concern by producers. The use of vaccine is highly debated and must be controlled. These states won't be safe until major states decrease or eradicate PRV.

Dr. Lowell Anderson updated the committee on Iowa's progress. Iowa's program for clean-up, and controlling the spread of PRV are accomplished on an area basis. He explained how the program tries to keep producers in business during PRV eradication. The state has tested 4,488 new herds in the past 10 months. 9,557 herds have a known herd status. 3,311 herds are infected statewide with 66% of the herds on clean-up plans. In program counties, 80% of the infected herds are on clean-up plans. During 1991, 433 veterinarians have been trained and certified to set-up herd clean-up plans.

Dr. Tom McGinn reported on vaccine use and herd clean-up in North Carolina. They do geographic disease mapping to help identify herds and assist with herd clean-up. They also carefully monitor movement techniques including traffic control.

Dr. John Katz reported new strategies in PRV diagnosis. Techniques have been developed to determine latent infection, latent vaccination or a combination of latent infection and vaccination. The technique should be used on a limited basis because of practicalities.

Dr. William Mengeling reviewed routes of delivery for vaccines and the latency problem with vaccine antibodies compared to latent infection.

Dr. Donald Bridgewater outlined swine identification for sows and boars for slaughter surveillance. He discussed the identification field trial in Montana. The trial uses a two piece plastic bangle eartag. Montana officials have faith that the tag will serve its purpose if everyone involved supports it. This tag will be applied by the producer of the animals before going to market.

Dr. Walter Mackey reported the implications of wild hogs on Northern States. In Minnesota, wild hogs are being imported and raised for hobbies, exhibition, hunting and sale as meat. A survey of states was undertaken to study wild hog impact on livestock, wildlife, crops and the environment. Wild hogs could pose a serious threat to swine disease control, especially PRV and Brucellosis.
PSEUDORABIES

Dr. Robert Morrison reported on the International PRV Eradication Symposium. There was general consensus at the symposium that PRV can be reliably eliminated from most swine herds. Several symposium speakers discussed PRV transmission between herds including airborne transmission. A resounding point expressed at the international symposium was that eradication programs must be conducted on an area basis and vaccination is a very important tool in any eradication program.

Dr. William Buisch reported on state compliance with stage status requirements. The major problem states face in achieving and maintaining status is meeting surveillance index requirements. Regulatory officials and the swine industry must work together to improve the effectiveness of slaughter surveillance.

Dr. Joe Annelli presented Dr. Beth Lautner's report on the transmission of pseudorabies virus between and within herds. Although vaccine can limit virus circulation, management changes can also greatly reduce virus transmission.

Dr. David Kinker reported on more rapid diagnostics using PCFIA. These tests could greatly increase laboratory testing capacity and timeliness, provided they are accepted for program use.

National surveillance procedures were reported by Dr. Mark Schoenbaum. Computer pascal language was used to write a program to simulate various surveillance systems. Further studies of computer simulations of surveillance systems may be helpful in decision making and program planning.

Dr. Robert Morrison presented Dr. Ned Hahn's report on the immunosuppression of PRV by reviewing PRV-associated diseases. Several swine diseases intensify within a swine herd if the herd is PRV infected.

Dr. Brad Thacker reported on comparative efficacy studies for vaccines.

Susan Kittelson explained the state pseudorabies regulations survey. The Livestock Conservation Institute (LCI) conducted the survey and can be used as a reference for anyone desiring this kind of information. Updates will be done on an annual basis and will be available through LCI.

Three vaccine companies and one diagnostic test kit company gave reports of gene-deleted vaccines. All reported working on increased accuracy of diagnostic tests. Syntrovet announced that a license was approved for "PRV Gold", a GI and GX deleted vaccine.

Dr. Thomas Hagerty gave the program standards subcommittee report. Dr. Hill moved that the USAHA Committee on Pseudorabies approve the program standard subcommittee report, amended as follows:

With respect to a request for a change in Stage IV requirements on importation of feeder pigs from other than IV or V states, the committee approved the following proposed change:

Feeder pigs be allowed entry to Stage IV states from Stage III
states/area or from feeder pig monitored herds in Stage II states on the following conditions: (1) that they enter on permit directly to a designated feedlot and not through an all-class market; (2) that they originate from an approved feeder pig market or direct from a Qualified Negative herd (QN), Qualified Negative Vaccinated herd (QNV), Feeder Pig Monitored herd (FPM), or Feeder Pig Monitored Vaccinated herd (FPMV); (3) that they be quarantined to slaughter only; (4) that the designated feedlot have no breeding swine on the premises and no breeding herds within 1.5 miles; (5) that the feeding herd must be part of the feeder pig surveillance system required for Stage IV with testing of a sample of pigs from the feedlot, using the official random sample test (95/10) as defined in the Program Standards as revised in 1991, and that tests be conducted in each such feedlot at least every 6 months.

With respect to the use of gene-deleted vaccines in Stage IV and Stage V states in herds around an outbreak or in other high-risk situations, which is now prohibited, the committee approved the following change:

That the use of gene-deleted differential vaccines in high-risk herds in Stage IV and V states be allowed on permit from the state veterinarian. While an outbreak in a Stage V state prompts immediate reversion to Stage IV, it was pointed out that there might be other situations, such as an outbreak in a neighboring state along the border with a Stage V state, which might make such vaccine use appropriate.

With respect to interstate movements of such animals the committee feels that: (1) such movements are not consistent with past practices on movement from infected herds; (2) states would not accept such animals for importation; (3) such a plan probably would not be approved by USAHA, and (4) it would be in conflict with long-standing interstate movement regulations and quarantine release provisions in the CFR.

But if a state wishes to approve such movement within its borders these guidelines are suggested: (1) The breeding herds must be vaccinated at least twice a year with a differentiable vaccine; (2) Movement of the progeny either from one site to another or from the final site, shall be in-state only; (3) The plan shall be part of a state-approved cleanup plan for the breeding herd which will provide a maximum of 18 months for elimination of the virus from the infected breeding herd; (4) Progeny shall be tested monthly; (5) Before movement of progeny as breeding stock, 100% of the progeny must be tested.

The committee endorsed the present system of including only slaughter testing, first-point testing or on-farm area testing in computing the surveillance index. The committee did approve the following recommendation:

Since circle testing has been found to be the most effective method of finding infected herds in the two years of the program and since Stage II of the program is the stage for finding and quarantining infected herds, this
effective method of case finding should be used in Stage II; therefore, the
subcommittee recommends that circle testing for 1.5 miles around all infected
herds be required in Stage II of the program. It is recommended that this be
required for all states entering Stage II after the effective date of the change
in program standards, and one year later for all states already in Stage II.

The committee approved the following proposed changes in the
program standards with regard to feral pigs:

Add to Qualified Negative and Monitored Feeder Pig herd require-
ments the following: "Any such herds must be entirely confined or separated
by physical barriers so as to preclude any contact with free-roaming swine."

Add to quarantine rules: "(a) Any swine herds in contact with
free-roaming swine of known positive or unknown pseudorabies status which
do not maintain physical separation shall be quarantined; (b) Herds
quarantined for pseudorabies for more than 3 months must develop physical
separation from free-roaming swine; (c) Quarantined feedlots must have
physical separation from freeroaming swine."

Add to rules for game farms/hunting preserves: "(a) Such facilities
must be approved as quarantined feedlots; (b) Any swine which escape from
such facilities may be returned only if negative by two tests over 60 days."

With regard to release of quarantine by statistical sampling, the
committee approved the following change:

Release of quarantine by a statistical sample test, rather than a
whole herd test, be permitted in Stage III, the quarantine to be released
following two negative tests by official random sample test (95/5) as defined
in the program standards as revised in 1991, such tests to be 90 days apart,
with a similar official random sample test in the herd a year later
recommended.

A question was raised about the legality of moving test negative,
unvaccinated adult progeny from Qualified Negative Vaccinated herds across
state lines. The committee concurred that there are presently no prohibitions
either in the program standards or the CFR on such movements. The
motion carried.

Black moved that the PRV Committee of USAHA supports the
recommendations of the 1990 state program reviews, which have been
examined and endorsed by the NPPC PRV oversight committee, and further
that the committee urges that in all cases of program reviews in the future
the state being reviewed be required to respond to the review and both the
results of the review and the state response be made available to anyone
interested. The motion carried.

Mulhern moved that the chairman of the PRV committee appoint two
subcommittees of technical experts, one on PRV vaccines and a second on
PRV diagnostic tests. Each subgroup would report to the PRV
committee each year on its evaluation of products available and
recommendations for use. The motion carried.
REPORT OF THE COMMITTEE

Other resolutions brought to the floor passed were:

1. The Feral Swine Subcommittee recommends to APHIS, NPPC and the Southeast Wildlife Disease Research Center that pilot studies be undertaken in states of high feral swine populations with the objectives of developing effective, practical methods for prevention of transmission of pseudorabies and swine brucellosis between feral and domestic swine and for control/elmination of infection from feral swine. Suggested for studies are Florida, Georgia, Texas and California.

2. The United States Animal Health Association, the National Pork Producers Council, and the United States Department of Agriculture encourage the manufacturers of PRV vaccine to work toward a common gene deletion, especially for diagnostic purposes.

3. The United States Animal Health Association endorses the concept that states that do not have free-roaming feral pigs be encouraged to prohibit their entry.
Sir William Osler said "Know one disease completely and you know all of medicine". My contention is "understand the technical basis for a successful disease eradication campaign and you know all of epidemiology". A quick scan of the agenda items for the upcoming Technical Group meeting or the objectives of the Agricultural Experimental Station's cooperative research project on the disease reveals the breadth of items covered from sensitivity and specificity of tests to economic impacts to strategies for the surveillance of Aujesky's Disease (see appendix A). This disease control program has its roots and owes its current success to technical innovations but I believe we have just begun to scratch the surface. This program can incorporate an amazingly wide spectrum of technical innovations, many of which can result in new tactics for disease control programs in general. Before anyone concludes that I have overstated the case, let us carefully examine the technical innovations that have already been incorporated to date – at least in some states.

First of all, recognize that the Aujesky's Disease (AD) eradication campaign is the first disease control program in either human or veterinary medicine which relies so heavily on the use of a genetically altered vaccine and its companion diagnostic tests. This, of course, represents the fruits of the well known buzzword "biotechnology". It provides us with a tremendous advance in that we can now theoretically differentiate a field strain from vaccine strains of virus. Traditionally, we had a mutually exclusive choice among disease control tactics. One choice was to vaccinate and forget about detecting new case, never really having a very good idea of disease prevalence and more importantly never really making any progress towards eradicating the disease. Hence, with vaccination alone, we always bear the persistent cost of control and never move towards eradication. A second choice was to test for the disease in unvaccinated populations and slaughter the reactors. The testing allows the identification and characterization of the disease in our animal populations but also leaves the population naked of protection. The technical change which created differentiable vaccines and diagnostic tests allow us to move beyond the old paradigms outlined above. Let me suggest that other technical innovations may also allow us to move beyond some of our current options when making difficult decisions.

Of course, no change for the better occurs without some downsides. The development of vaccines and differential tests is not without its problems, particularly concerning test interpretation. Others will address this very significant problem later in this session. Let me just say that many of the
new ELISA tests are probabilistic in nature in that the optical density readouts are tied to an underlying probability distribution. A certain ELISA value can only be interpreted with a probability that a particular animal is infected. Combine this notion with a statistically valid sample providing the basis for most herd tests and the conclusion that we must deal with probabilities of infection rather than certainties of infected swine or absence of infection becomes crystal clear.

A second major technical innovation that can substantially influence the way we manage the Aujesky's Disease control program is the development of Geographic Information Systems (GIS). The experience that the North Carolina State Veterinarian's office has had to date, demonstrates the utility of using geographically based data for making disease control decisions. Geographic information systems are a major technical innovation which developed by leaps and bounds during the 1980's. It allows the combination of different layers of spatially oriented data to be collated and analyzed as a single dataset. Most GIS digitize information on water source, roads, soils, vegetation and population density as well as other specific information of interest. In the work done in North Carolina, information on farm location, swine farm density, herdsize, type and ownership has been combined to make day to day decision that enable better disease management decision concerning the control of disease.

Last year at USAHA, Dr. Tom McGinn detailed how he uses GIS systems in the North Carolina program. The following aspects can be addressed using GIS technology: 1) epidemiology/disease investigations which allows one to: notify adjacent owners, determine possible sources of infection, arrange circle testing, and evaluate disease status of an area. 2) personnel/program management which must be increasingly efficient. A shrinking field work force means larger areas for each VMO or AHT. Directions to a premises can be simplified and unambiguous. Assignments for field personnel can be better planned using GIS data. The value of a geographically based unique identification number is also evident when dealing with allied programs i. e. locating low hazard waste plants, dead animals and nuisance complaints. 3) Disease mapping using GIS is also a method of monitoring and controlling disease expansion. Mapping can be used to find best location to place feeder pigs out of 1000 sow units which could finish out on several alternative sites. Optimum location of expansion farms of negative status in conjunction with design of local facilities can help minimize risk PRV (AD) infection to new farms. 4) Research into the evaluation of nearest neighbor PRV (AD) status as a risk factor for disease. Such studies have showed that while the PRV (AD) status of the nearest neighbor to a swine farms is a risk factor, other risk factors such as type of operation, herdsize or commercial affiliation are more likely to be truly causal. 

The incorporation of GIS technology can be a major addition to the
national Psuedorabies control program. The ability to map and visualize the occurrence of the disease should not be underrated. Unfortunately, the development of data on farm location is not always straight forward, although the new hand held compasses should simplify this task and even skirt the data entry bottleneck. The second obstacle to utilizing GIS technology is the necessity to keep the database updated in terms of new swine farms and ones that have gone out of business. Thus, mapping of swine farms takes considerable effort but the benefits for not only pseudorabies (AD), but also foreign animal disease preparedness, waste management concerns and other nuisance complaints.

Technological advances don't always have to arise from the laboratory and they don't always have to originate in some form of electronic wizardry. A third major technical innovation which will impact the national PRV (AD) program is the development of 3 site production systems. There are fairly strong indications that we may be able to consistently produce negative finishing swine from positive sow farms using 3 site technology. There is also an interest in using 3 site for the production of breeding swine. The 3 site production technology may benefit swine husbandry by enhanced disease control for many diseases. In terms of PRV (AD) control tactics, 3 site production really amounts to an extremely physically separated form of offspring segregation. The PRV (AD) control program may be able to incorporate this new production technology as one of it's more economical herd clean up technics.

Remembering that we are indeed relatively early in the PRV (AD) eradication program and that the rate of technical advancements has rocketed in the last couple of decades, it seems highly probable that different technical innovations may be helpful in solving some the unsolved questions impacting the PRV (AD) program. One problem that has been discussed both in the PRV (AD) Technical Group and AES NC - 197 PRV research conference is how to develop a national database, so that we can gather better information on what puts farms at risk of PRV (AD) infection. The development of a nationally consistent database has two major requisites: a commonality in the way that various state program record and analyze their data and secondly an agreed upon outbreak investigation protocol.

Recent advances in management information systems hardware and software maybe helpful at both the data collection and analysis levels. Development of an integrated, modular information systems approach to collecting program data on both new outbreaks and prevalence is technically possible now. Such systems could be allied with other management information system tools such as decision support systems. It would take a great deal of effort to develop such computer based information systems but many businesses have found that such a transition provides profound and unexpected benefits. The development of a better understanding of the economics of PRV (AD) might be one example.
Technical advancements may have impacts in other parts of the program. As the PRV (AD) control program progresses toward eradication, the problem of feral swine as a reservoir of disease grows in importance. Certainly the eradication of the feral swine population in the United States would provide substantial technical and socio-economic obstacles. This problem provides another challenge to which novel technology concerning vectored vaccines for wildlife populations can be brought to bear. Studies exploring the potential for the construction of a recombinant swinepox virus expressing the PRV (AD) genes that code for the immunogenic proteins. Further work by the same group has indicated that a swinepox vectored PRV (AD) vaccine will elicit a strong immune response similar to natural infection. Such a vaccine would have to be thermostable and administered orally as a bait. While certainly many problems remain in the development of such a system for vaccination of feral swine against PRV (AD), a successful analogy can be found in the control of rabies in wildlife populations.

Another area that may benefit by our increased technical understanding of the molecular biology of the disease is the problem of singleton reactors and in fact, the whole question of latency. PRV is complicated by the fact that the virus can be latent and reactivated by stress of one sort or another. If polymerase chain reaction techniques can be used to understand the mechanism of and causes of reactivation of PRV (ADV), then new and advanced methods may be developed for elimination of the disease from swine herds.

One of our biggest challenges now is the identification of the source of new herd infections and the development of practical preventive strategies. A technical advance which would allow us the ability to identify when herds that don't have a clinical outbreak become infected would at first glance seem to be a tremendous tool. However, some caution on the potential of the development of such a tool must be given by data which indicate that the source of infection is no more easily identified in clinical versus non-clinical herd infections.

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COWEN, MILLER


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APPENDIX A

TECHNICAL ADVISORY GROUP

DISCUSSION ITEMS

1) Evaluation and guidelines for case finding through slaughter sampling. 2) Use of differential tests in slaughter sampling. 3) Review of cost estimates for case finding by slaughter/area testing. 4) Surveillance plans for states using area testing in case finding. 5) Review of clean up of infected herds. 6) Review of status and needs of large herd clean up studies. 7) Review of three site plans for production of breeding swine. 8) Review of three site plans for production of finishing swine. 9) Review of quarterly reports, evaluations and summaries. 10) Objectives for study to validate area testing. 11) Recommendations for program standards for feral swine. 12) Objectives for economic studies on pseudorabies. 13) Testing in herds with single identified reactor swine. 14) Use of polymerase chain reaction tests for GP1 antigens.

AES NC 197

RESEARCH GROUP OBJECTIVES

1) Establish a uniform computerized database for epidemiologic research. 2) Determine effectiveness and cost of surveillance and eradication techniques. 3) Determine factors that influence viral spread within and between herds. 4) Determine effectiveness of vaccination strategies. 5) Determine the interaction of PRV(AD) with the immune system and its impact on persistence and protection. 6) Develop and evaluate diagnostic procedures.
Program Obstacles and How We Overcome Them

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Consultant, Government and Scientific Affairs
National Pork Producers Council
Annual Meeting – USAHA – San Diego, California
October 27-31, 1991

Here I am again acting like one's conscience talking about Aujeskey's Disease eradication in the United States as I have done each year since the eradication program began. There is good news and bad news and I will dwell on the good news first.

All the states are actively involved in one of the stages of the eradication program. Some states have been aggressive in implementing their programs. More hogs are being vaccinated that provides more protection to our national herd. The goal of getting 22 states in stage IV during 1992 appears feasible. Congress, even though providing less funds than we requested each year, has increased our appropriations by at least one to two million dollars. During these times of deficit budgeting it indicates their support. This also means that producers are showing their commitment to the eradication goal by emphasizing its importance to their congressman. There seems to be a general agreement to move to a gene I deleted vaccine that should eliminate some of the confusion in diagnosis that has occurred in recent years.

Training programs for state and federal personnel and veterinary practitioners are being well received. Reactions are that attendees are being well informed on the epidemiology of the disease and how the program is designed to control and eradicate the virus.

I think we all need to recognize and appreciate what Iowa is trying to do to eradicate the disease through the development of a "Producer friendly program". We all must admit that their claim of the density of the hog population in that state and the high rate of infection and indifference to the program by many of their producers makes their problem of eradication unique. There are potential flaws that need to be addressed but I look upon what they have accomplished in the past year as good news.

Finally I'm pleased that some action has been taken on wild swine as NPPC has funded studies on the epidemiology of PRV in such swine. Georgia pork producers and APHIS/VS may participate in similar studies. Studies are being proposed and hopefully funded to use oral vaccines to control diseases in feral swine that would provide a real breakthrough. Finger printing of the virus in feral swine and the information pamphlets communicating the problem of feral swine to our domestic swine shows that these meetings are important and that concerned people and organizations respond to them.
PROGRAM OBSTACLES

NPPC leadership and commitment to the eradication of PRV by providing Don Gingerich, David Meeker and Beth Lautner and their PRV oversee committee to direct and monitor the program from an industry standpoint is developing a model for any future producer control and eradication program. The Washington office continually strives to get increased funding for the program.

Eradicating a Disease in a Chronic Stage

Now let us tell "the other side of the story". I think the number one obstacle is that we are trying to build up support and enthusiasm to eradicate a disease that is currently in a chronic or endemic stage. Between natural transmission and the use of vaccines, clinical manifestations are suppressed by increasing antibodies in the population where a lot of the infection exists. However, there are periodic outbreaks and some herds have been reinfected several times to let producers know that the virus is still there. Also, read the recent Iowa Producers PRV newsletter. They stress the real economic losses are more often due during the chronic stage of the disease to the PRV virus predisposing their swine to losses from other disease agents in the herd. Once PRV is eradicated in the herd the losses from other disease agents disappears.

Need Continual Cost/Benefit Studies

It is very important that cost/benefit analysis studies be conducted during the program as a motivating factor to keep producer’s support. This is especially important when the national incidence is in this current stage of control and eradication. I would like to see Glenn Grimes be linked in some way to these studies so he could present his annual estimates as to the effect of this disease and the eradication program on the cost of raising hogs in the United States.

Industry Support

Following a similar format that I have given the past two years on obstacles, active industry support is so important that it must always be listed close to the top. I mentioned earlier that the leadership has done their part at the national and state level but we are going to need more direct action by a greater percentage of producers if there is any chance to reach the 10 year goal of eradication.

For example, the national incidence of this disease based on current data available is between 2 and 3 percent. I don’t know how valid that statistic is because we don’t have a national surveillance system in place that
will indicate how much infection exists in many states. However, even if it is double, it would mean that 94% of the producer's herds are not infected and they are the one to receive major benefit from the eradication program. I call them the "silent majority", otherwise there would be more of a demand that we get this job done as soon as possible. in evaluating the program at this stage, I consider this a major obstacle.

To repeat, we must arouse more producer interest and active support to get this job done as soon as it is reasonably possible. After all, besides producers paying for vaccine, state and federal tax dollars are being spent on the eradication program and the longer it goes, more of the tax dollars will be required to complete the job.

Some producers are indifferent towards eradication because vaccines are available. They need to be reminded that the annual sale of hog cholera vaccines before it was eradicated was $25 million in 1970's dollars. In addition, there were losses from the disease and marketing restrictions. The costs of living with such diseases becomes an annual operating expense if they are not eradicated. When hog prices are high, it may not seem like much of a problem, but when they are low it can be a real economic burden.

Last year I mentioned that eradication programs pass through three stages, namely, panic, cooperation, and apathy. In several states we are in the cooperation stage but in others, unfortunately, it is in the apathy stage or they have not accelerated their program and that is our obstacle.

**Raise the Priority of PRV Eradication**

If we are really serious about a ten year eradication program, producers and regulatory officials must recognize the need. There must be more evidence by many more states that this program is on the front burner and we are united to get this job done in that time frame.

We have spent four or five years preparing for the program conducting pilot studies and have just completed three years of the ten year program so "time is marching on". Another way to put it is that we have only seven years left!

**Adequate Funding**

Since the beginning of the program, we have had five proposed budgets. The original one was in 1987. We revised it to begin the program in 1989. Because the third budget came late during the Federal budget cycle, we stuck with the #2 revised budget, Then efforts were made to include APHIS headquarter costs in the fourth budget. Shortly after that, the fifth budget was developed to also include APHIS regional
PROGRAM OBSTACLES

and state salary costs.

Needless to say, in this era of deficit budgets, no matter what efforts we have made to develop an objective budget, our proposals are far above what is realistic available from Congress. Let us look at this visual that shows what APHTS–USDA NPPC proposed for annual funding of the PRV eradication program and what Congress provided. Note the difference in the last two years – in 1991, it was $6.15 million short and in the current year of 1992 it was $5 million short – so in the last 2 years we have not received $11.5 million than we requested and that is bound to have a negative effect on the progress of the program. In fact, Iowa and several other states have reported that litany more producers would be actively involved in their programs if funds were available. They claim this is a major obstacle that prevents their program from moving into more advanced stages of eradication.

My concern is that we need a revised budget because the gap between what we requested and what we received must have an effect on the length of the program unless the current and past proposals were not realistic. We shouldn’t wait for Congress to question this – we need a revised budget as soon as possible. This budget should be related to the proposed cost/benefit studies.

Epidemiological Data – Surveillance

Unfortunately, I must repeat what I said last year, our lack of so-called ground intelligence or effective surveillance continues to be a major flaw in our program. Despite improvements in some states we must show more this year.

When I asked Dr. Annelli how good is our surveillance data, he pointed out to me if you look at the chart on sources of outbreaks and add the unknown and area spread categories together it is over seventy percent. I would say that is nothing to brag about. I am concerned that despite some progress, this continues to be an area that demands a higher program priority. A national effective surveillance system is essential to determine if we are making progress in eradicating the disease, requesting funds from Congress or determining the length of time to eradicate the disease.

If our data is lacking on the number of infected herds in some of the infected states how is the disease being prevented from spreading intrastate and interstate front unknown infected herds? If some states don’t have a reliable monitoring system, that lets them know how much infection is in their state then how do they know whether their program to eradicate pseudorabies is making progress?
Swine Identification

In my inquiry as to our ability to find sources of infection from hogs being marketed and slaughtered, the lack of an effective identification method still is a great problem in many states. Unless a better retention and easy applied tag or a bangle tag acceptable to marketing interests is found the only way this will substantially improve is to have it done by producers with all the problems associated with it.

Diagnosis and Vaccines

There is still too many complaints about results or interpretations of diagnostic tests. There is also a need for producers to have information as to the efficacy of the various vaccines that are available if we refer to similar problems in the brucellosis eradication program during the late 70's they set up subcommittees to report on both of those areas each year at the annual meetings of USAHA. The technical committee reviews these areas but I am proposing that there be more inputs by other members of AAVLD and those involved with veterinary biologics. These two subgroups could report to the technical committee or the USAHA pseudorabies eradication committee.

Feral Swine

As stated earlier, we are recognizing the importance of this problem and certain actions are being taken. however, I think we need some pilot studies in states that have the problem to try different methods to prevent spread of diseases like brucellosis and pseudorabies from feral swine to domestic swine. I mentioned in previous reports that this could be an embarrassing development if we spend more years on a national eradication program and eventually find that we can't identify a practical method to prevent exposure of pseudorabies from feral swine to domestic swine.

Program Trends

It has been interesting to observe the development of this so called producer oriented program. I think it is producing some promising results. However, I believe during 1992, the fourth year of the program will be a real challenge.

In past programs, the program momentum continued because as states invested a lot of manpower and funds and became free, they didn't have much tolerance for states that were not complying with the agreed upon methods and rules. So they either insisted that the Federal government restrict movements from those states or they would or in some cases, both would.
PROGRAM OBSTACLES

I noticed that the gap is beginning to widen between infected and free states. This is going to be a difficult period for a producer-directed program. It is going to be difficult for producers in one state being responsible for advocating restrictions on the marketing of hogs by producers in other states.

Pushing the advancement of the program until producers are asking for it was a concern raised by some producers who endorsed the eradication program. I believe Iowa intends to accomplish this through their information/education approach and county referendums. However it is resolved, I think it will be a challenge that will come into focus during 1992.

Information, Education, Communications

As the program progresses, the need for emphasis on information education becomes more important as effective communication between various groups becomes even more essential. Fortunately, the importance of this is being recognized. The Iowa report on its program and its newsletter is a good example of what state producer organizations are doing. The more exchange of information between states, the better for all. Also, the agricultural media of press and radio that follows these programs have always played an effective role. NPPC and LCI recognize the importance of this component as they monitor and report developments during the year. I don't believe this part of the eradication program can be overdone.

Conclusions

1. In our eagerness to see the challenges of the future, we must be pleased with the accomplishments of the past 3 years,
2. Many of today's eradication problems are because we began an eradication program during its chronic stages rather than when the disease was generally acute nationally,
3. Update and continual cost/benefit analysis is needed.
4. Among the many obstacles ahead, more producer active support and commitment is essential for program advancement and congressional funding.
5. Time is passing quickly we are beginning the 4th year of a 10 year program.
6. Increased annual funding is necessary or a ten year eradication goal is not feasible – Also we need a new 7–year budget.
7. Our epidemiological data relative to surveillance, identification, controls over movements from infected herds needs a much higher priority of importance.
8. We need subcommittees on diagnosis and vaccines so that each year there is a report on its status.
9. There should be field trials on how diseases like pseudorabies and brucellosis can be prevented from feral swine to domestic swine.

10. 1992 could be a critical year as to how a producer oriented eradication program can be advanced to reach its ten year goal.
Although California is a major producer of many agricultural commodities, it has a small swine industry. There are about 160,000 breeding swine in California. During the last four years, there have been as many as 20 infected herds at one time and now there are two known infected herds, our lowest number yet.

With our small population and low prevalence we obviously don't have the problems that major swine producing states have. However we do have problems. As requested I'll describe some and emphasize those which some other states might have in common.

First, the lack of disease means there's often a lack of concern by producers. Many don't feel personally threatened by pseudorabies. They haven't experienced losses from it and haven't come in contact with anyone else who has. Lack of concern leads some producers to a lack of precautions about protecting their herd. A lack of concern also makes the task of informing producers about pseudorabies more difficult. They don't read articles on the subject and they don't attend meetings either.

It's difficult to consistently get good attendance at meetings regarding pseudorabies. The few regular attendees get tired of doing all the work. This is especially true in the western states where a 3-6 hour drive might be necessary to attend a meeting.

Low prevalence results in fewer people being well informed and experienced with pseudorabies. This creates an environment where incorrect information can spread quickly.

A frenzy of fear and rumors spread throughout much of California during an epidemic in 1987, and to a lesser extent in 1989. Airborne transmission across great distances was widely discussed. Many became convinced that pseudorabies was in the air everywhere and spreading up and down the state. Much disruption occurred. Fairs required testing of all swine which added considerable expense to the 4H efforts. Sheep producers refused to bring their sheep to fairs because of fears that exposure to "infected" swine would kill their sheep.

Letters to State legislators demanded to know why the government let in pseudorabies and naturally we had to respond to legislative inquiries.

As you know pseudorabies dramatically kills infected sheep, goats, cattle, dogs, cats and many other species. This led some 4H leaders and
others to fear that the entire livestock population of California was threatened and maybe even people too. The fact that pseudorabies has rabies in its name didn't help matters.

During this frenzied time, a major slaughter establishment refused to accept swine from a large infected herd if they were accompanied by any document which stated that the animals were exposed to pseudorabies. This company became acutely aware of the potential damage to their retail name. They feared the media might exploit the fact that the company sold meat from an infected herd. Of course this establishment also received regular shipments of untested swine from Iowa and other midwestern states, but the company did not consider those imported swine to be a threat because they were not officially designated as being from a known infected herd.

Los Angeles area newspapers began calling about pseudorabies and many feared that the misinformation was really going to seriously hurt pork consumption i.e. if all of Los Angeles suddenly stopped eating pork after reading the morning newspaper. Maybe we were just lucky and never had one of those “slow news days” but the subject was downplayed and only appeared in the back pages of those publications. Eventually educational meetings all around the state, and 25,000 mostly negative surveillance samples convinced most that the epidemic was small and pseudorabies could be controlled and eradicated.

Misinformation still continues though. Even today there is a great fear of swine vaccinated for pseudorabies. Three uninfected herds have been allowed to use a gene deleted vaccine. These three herds have been infected with pseudorabies twice in 3-1/2 years. The State Fair and other fairs refuse to allow animals from these herds into their fair even though all three herds continue to be Qualified Negative Vaccinated Herds and are therefore monitored closely. The fairs have barred them because so many other swine producers called and said they would not participate in the fair if any of the vaccinated swine were allowed to come. So, we still have some work to do.

Having a small swine industry means having an unconcerned public. Not many jobs depend upon swine production so it’s not important to the average person on the street. Other issues such as animal rights have a higher priority. One result of this is how impractical it is to test swine at saleyards. Saleyards are public and animal rightists would soon be protesting where blood and squealing pigs could be found. Such protests are listened to more when the public perceives little at stake which is important to them. So, testing at public saleyards is not very viable in California.

Limiting the use of vaccines is also a problem in states with low prevalence and a small swine population. Many uninformed or especially cautious owners want to vaccinate their animals for pseudorabies even though the threat of infection is very low. We even get requests to vaccinate pot bellied pigs despite the fact that the animals will live indoors in a city.
We need to limit vaccine use for several reasons. First, it is too expensive for the laboratory to stock test kits for all brands of differentiable vaccines. Also, unnecessary vaccinates interfere with surveillance efforts. In our case and other states with low prevalence, surveillance can only be efficiently done at slaughter. Use of several vaccines in the state would mean having to spend up to $100.00 for each positive sample. The positive samples would be so costly because they are infrequent and that means testing only one or two positive samples at a time. Testing one or two samples means using up a whole row of a microtiter tray or the whole tray, so economics of scale are reversed. If many vaccines were allowed, it might mean having to test a sample with several differential tests after the standard tests were completed. This would be necessary when the vaccine status or the brand of vaccine was unknown.

By far the biggest problem in California is the potential for infection to be imported into California. We are an importing state and nothing we do internally can have as big an impact as activities in major swine producing states. Our industry won’t be safe until the major states are successful at decreasing or eradicating pseudorabies. This is true of most or all of the states with a small swine population. It is especially true for California where our huge human population creates a tremendous demand for imported swine. Our large ethnic populations not only prefer pork but prefer buying and preparing it in non-traditional ways.

The demand for lightweight swine between 30 and 110 lbs. is large and growing. As a result of this increasing, relatively new demand, non-traditional marketing channels have developed. These channels involve much smaller operations than the traditional pattern where large plants receive fat hogs and sell wholesale to grocery store outlets. The new marketing involves numerous small operations, dealers and purchasers of 1 or 2 pigs. But, there are thousands of people buying 1 or 2 pigs. Since the new demand for lightweight swine involves many more small operations and small informal transactions it also makes surveillance more difficult and it makes locating the source of test positive animals more difficult.

I’m going to stop here and let Victor Velez describe some of his experience with the diverse ethnic market place in southern California.

In the past 5 years, Southern California has been affected by two minor pseudorabies outbreaks. Although not known for large-scale swine production, this area has numerous pockets of backyard farming and some small producers scattered throughout. In an ever changing Southern California, it is not unusual to find recent immigrants and first generation Americans involved with swine production. In many cases these producers are unable to communicate effectively in English. Yet, in order to control pseudorabies under these conditions, it is paramount to understand the needs of the different cultures and be able to communicate in their respective native language, if necessary.
PROBLEMS WITH LOW PREVALENCE IN CALIFORNIA

Both outbreaks occurred in an area, where Portuguese, Mexicans, Cubans, Nicaraguans, Samoans, and other ethnic groups have settled and become involved in the swine business. Additionally, three state-licensed custom slaughter houses and many illegal backyard slaughter facilities do business in the area. These hog producers and slaughter establishments are catering to the needs of the different ethnic groups that want to continue their traditional methods of food preparation and consumption.

The variation of customs from culture to culture is tremendous. While the Samoan may prefer a 600 pound sow to be consumed at a burial ceremony, an Argentinean may want the 20–30 pound weaner for a wedding. But one commonality is shared by all these cultures, the desire to see the animal alive prior to slaughter. For this reason custom slaughtering appeals to people wanting to select the animal alive and who desire those animal parts that are not normally sold at the store. Whereas to the Vietnamese and Chinese, the uterus and fallopian tubes are considered a delicacy, to the Mexican, the stomach and intestine are an essential ingredient for a breakfast soup (Menudo).

In order to satisfy the growing demand for different classes of pigs, custom slaughter houses, producers and individuals have resorted to the importation of pigs from other states. It is not unusual to find a shipment of 100 feeders bound for a custom slaughter house, to supply an ethnic group with the animals needed for a special holiday.

Unfortunately, this emerging market has also seen the presence of unscrupulous individuals that illegally bring animals of unknown pseudorabies status to the area. To complicate matters, other illegal activities such as livestock theft and drug dealing may be simultaneously occurring. Therefore, traditional approaches to disease control may not prove effective under these circumstances.

As Southern California's cultural make-up continues to evolve, it is essential for individuals or agencies to be prepared to deal with cultural and language hurdles. Any efforts made to educate producers and the public in disease management and control will ultimately have a twofold effect. First, it will provide the consumer and producer with a healthier animal. Second, it will facilitate the government's role in effective disease management and control.
An active advisory committee, dedicated allied industry officials, and enthusiastic producers have worked together to make measurable progress in Iowa during the past year. Herd plans restrict movement of infected swine, but positively encourage the herd owner to clean up the herd. Allowing offspring segregated feeder pigs to be sold within Iowa's borders positively encourages voluntary testing of herds to determine status. Iowa's Quarterly Report will report all testing statewide as of September, 1991. Doses of PRV vaccine distributed to breeding swine from infected herds are entered only from Iowa's program areas. All other vaccine use is not recorded. The program in Iowa has tested over 5,900 new herds since January, 1989 (4488 new herds in the past 10 months). More than 4,800 herds have been statistically sampled and found to be noninfected. Cleanup plans are written for 80% of the infected herds found in the program areas. Four Hundred thirty-three accredited veterinarians have been certified to assist in the initiation and development of cleanup plans in the remaining herds. One thousand four hundred seventy-nine herds have been released from infected status according to guidelines set forth in Program Standards. The program in Iowa is progressing as fast as producer willingness and funding will permit.

The Control and Eradication Program in Iowa is spearheaded by a 7 member advisory committee. Four of these members are required by Iowa law to be pork producers. Currently, five members are pork producers. A practicing veterinarian and a livestock auction market owner/operator round out the committee members. Ex-officio members include the State Aujeszky's Disease Coordinator; Veterinary Services AVIC; Iowa Department of Agriculture State Veterinarian; Extension Swine Veterinarian; Veterinary Services Area Epidemiologist; State Senator; Chairman, Iowa Veterinary Medical Association Swine Committee; Executive Secretary, Livestock Marketing Association; Head of Microbiology, Iowa Veterinary Diagnostic Laboratory.

The Committee is small enough that decisions and program plans are debated and voted upon efficiently. The make-up of the committee
assures that in Iowa the control an eradication program maintains the pork producer's point of view.

The Iowa program goals are:

1. To eradicate Aujeszky's Disease from the State of Iowa by the year 2000.
2. To eradicate Aujeszky's Disease without eradicating the pork producer (making sure Aujeszky's Disease regulations are not the cause of pork producers going out of business).
3. To educate and inform the pork producer that producing hogs without being under the influence of Aujeszky's Disease virus can be more profitable.
4. To evaluate technical information as it becomes available and to implement it into the program if it will prove to assist in the common goal.
5. To test all swine herds in the state and to establish a status as quickly as program funding allows.
6. To assist owners having infected herds by:
   a. Encouraging the herd owner to establish a plan to eliminate Aujeszky's Disease.
   b. Encouraging the recommended "hyperimmunization" of breeding swine by providing a monetary incentive from program funds to offset this vaccine cost. This monetary incentive assures that the recommended schedule of vaccination is continued, during years having down trends in markets.
   c. Encourage 100% participation of all swine producers through education, personal contacts by participating producers and establishing a program that allows the continual marketing of swine.

The program in Iowa may differ slightly when comparing it or what is perceived to the program in other states. The unique program in Iowa is due to Iowa's unique problems. The program in Iowa is making measurable progress.

The program in Iowa does restrict the movement of swine from herds found to be infected. The following statement is contained on each herd plan the owner is required to sign:

"I agree to restrict movement of all swine from my herd to an approved premise or to slaughter. However, if a signed Feeder Pig Cooperator Plan Agreement is attached and the requirements of this plan are followed, then feeder pigs from my herd may be sold within
the State of Iowa as pigs of unknown pseudorabies status. No swine may be represented as BREEDING SWINE when originating from infected herds. I further agree to allow testing at intervals determined by the department and request no indemnity of animals sent to slaughter, and submit no claims against the department, veterinarian, or vaccine producer for losses or adverse effects of any vaccine or adverse effects from any restraint and blood collection procedure associated with this plan. I further agree that program funding for vaccine usage as outlined above, will not be extended past ______________ (date) ____________ months from commencement of this herd plan agreement. In the event that State or Federal funds are not available, I may continue the herd plan at my own expense. I further agree that if this plan is not followed, is discontinued, or is not progressing in a satisfactory manner, as determined by the department, this herd is a quarantined herd."

This statement, when signed by the herd owner, serves to establish at least four main goals:

1. Agreement to develop and implement a plan to clean up the infected herd.
2. Agreement to restrict the movement of breeding swine and feeder pigs.
3. Agreement to limit the amount of program funded vaccine to two years.
4. Requires the herd owner, practicing veterinarian, and regulatory official to sign this plan, assuring all parties understand and agree to the contents of this plan.

Failure of the herd owner to sign this herd plan within thirty days of determining the herd to be infected will impose a State Quarantine. Release of the restrictions contained within the herd plan and a quarantine are consistent with Program Standards.

The feeder pig producer has projected a concern of being put out of business if the herd tests are interpreted infected. Iowa does not have enough approved premises (Quarantined Feedlots) to receive the estimated feeder pig supply originating from infected herds. It is felt that it is better to identify the infected herds and control them, than to have feeder pig producers not participate at all. The method of control imposed on the feeder pigs, agreed to by the owner, is a "carbon copy" of offspring segregation as outlined in the LCI published "A.D. Herd Plan Manual." Most program officials agree that offspring segregation is an accepted method of producing Aujeszky's Disease free pigs from infected breeding animals. The Feeder Pig Cooperator Agreement in Iowa applies this concept to all progeny, not only replacement gilt pigs.

Iowa's regulations do not encourage the approval of quarantined
feedlots. In fact this graph (A-1) depicts a downward trend has occurred since tighter restrictions have been imposed due to changes in the regulations. For example, regulations require vaccination upon arrival to the approved premise.

Iowa's feeder pig monitored program is designed to credit a herd (feeder pig producer or farrow to finish) with noninfected status if the herd has been statistically sampled and exhibits negative test results. Producers are sent a monitored identification card containing a twelve-month expiration date. Continued representation of monitored status after this date without annual retesting is prohibited. Noninfected herds can only be one of the following: monitored, qualified negative, or qualified negative vaccinated.

Iowa's regulations do not revert statistically sampled negative herds back to unknown status after only a twelve-month period. Lack of program funding does not allow a sampling frequency of these herds each year. Producers exporting feeder pigs out of state do test annually to qualify for other states', monitored herd of origin, import regulations.

The Quarterly Report (VS 7-1) is computer generated. Iowa officials feel that reporting the numbers as generated from the computer is accurate and meaningful. Satisfactory explanations are indicated on each report if numbers generated appear to be projecting erroneous information. I would encourage those individuals summarizing the Iowa data to read the explanations carefully so that false conclusions are not made.

The Quarterly Report potentially may give misleading conclusions about the program in Iowa.

For example:

Section A, column C and E reports qualified negative and qualified negative vaccinated herd totals as well as additions and removals affecting these totals during the quarter. Do not assume that every removal means a qualified herd has been found to be infected with Aujeszky's Disease. Loss of status due to not completing quarterly testing on time or going out of business may be additional reasons for removal from this status. Many qualified herds not selling seedstock outside of Iowa have initiated pseudorabies vaccination in the breeding herd, thus achieving qualified negative gene-altered vaccinated status. A qualified negative status is "removed" and a qualified negative vaccinated status "added" in this case.

Section D is a summary of pseudorabies vaccination. The figure is low since Iowa data entry operators are directed to enter only doses of vaccine supplied at program expense. Doses of vaccine administered to swine originating in non-program areas are not reported. Vaccine administered to grower swine statewide is not reported.

Section E reports "source of new herd infections". The groupings, "Area Spread" and "Unknown" have been identified by most herd owners as the most probable source of infection. Many times herd owners do not realize Aujeszky's Disease is present in their herds at the time of initial
statistical sampling. Therefore, the exact virus entry time is difficult to pinpoint. The reporting is accurate in that spread may have come to their herd due to being located in an endemic area or they simply do not know.

Iowa has begun entering all Pseudorabies testing data into the DEC/VAX computer from all 99 counties as of September 1, 1991. The addition of two clerical staff has made this possible. This should improve the completeness of the Quarterly Report being generated for the 4th quarter of 1991.

Previous to this time many of the test reasons reported on the Pseudorabies Quarterly Report, VS 7-1, had been from data entered only from Iowa program areas and not the entire state.

Section F, Column C incorrectly summarizes herds disclosed as infected in which one or more positive PRV tests were found. Many of the samples tested in Iowa are collected from swine vaccinated with a pseudorabies vaccine. Tests that are reported by the State Diagnostic Laboratory as "non" negative are entered as positive. Many times on retest of the same animal the interpretation of the test has changed to negative. Unfortunately, the VS 7-1 report translates this transaction as a herd where infection was found. Changing the results will increase clerical work and will not properly credit fee basis expenses. Therefore, I would suggest the column heading be retitled "Herds tested having positive test results."

Here are some of the facts showing the progress the program in Iowa has made over the past three years:

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<thead>
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<th></th>
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<tr>
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<td>3633</td>
<td>4190</td>
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<td></td>
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<td></td>
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The program in Iowa is designed to allow the pork producer to volunteer their county into area participation and to continue to raise hogs
despite a 32% chance of testing infected. If infected, the herd owner will have available 433 swine practitioners specifically trained in PRV herd plan initiation and development to assist in formulating an individualized cleanup plan and to review the integrity of this plan one year later.

A mandatory program is initiated in each area at the time 50% of the swine herds within the areas have tested.

Swine movement is restricted to only noninfected swine (herds monitored each 12 months, qualified negative, qualified negative vaccinated, or individually tested negative) when the prevalence in the area reduces to 10% infection. This is when the program in Iowa compares similarly to programs in other states.

Strict adherence to all guidelines in Program Standards is the goal in Iowa. The Iowa producers are participating in this program with greater interest. The program in Iowa will continue to require patience and understanding from neighboring states, financial assistance from State and Federal Program dollars, producer willingness to participate, and an understanding of the area–by–area approach as it becomes fully implemented in all 99 counties (statewide).

PROGRAM AREA STATISTICS: October 1, 1991 – IOWA

<table>
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<th>COUNTIES ENROLLED 1987–1988</th>
<th>HERDS IN COUNTY</th>
<th>% HERDS TESTED</th>
<th>% HERDS INFECTED</th>
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1989
Cherokee

<table>
<thead>
<tr>
<th></th>
<th>HERDS IN COUNTY</th>
<th>% HERDS TESTED</th>
<th>% HERDS INFECTED</th>
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<tr>
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<td>88</td>
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<td>Black Hawk</td>
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<td>Buchanan</td>
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<td>Clay</td>
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<td>Clayton</td>
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378
### ANDERSON

<table>
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<tr>
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<th>PRV Sows</th>
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<tr>
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<td>Henry</td>
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<td>Jasper</td>
<td>501</td>
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<td>Jefferson</td>
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<td>Keokuk</td>
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<tr>
<td>Lee</td>
<td>229</td>
<td>73</td>
<td>23</td>
</tr>
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<td>Louisa</td>
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<td>Lyon</td>
<td>360</td>
<td>65</td>
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<td>Mahaska</td>
<td>473</td>
<td>66</td>
<td>42</td>
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<td>Osceola</td>
<td>150</td>
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<td>Shelby</td>
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<td>41</td>
<td>43</td>
</tr>
<tr>
<td>Sioux</td>
<td>850</td>
<td>60</td>
<td>50</td>
</tr>
</tbody>
</table>

Carroll County PRV Project (CCPP) 1990
Carroll*** 550 81 58

* Marshall was an original PRV Pilot Project, 1983
*** Carroll is a PRV Project County

There are five herds participating in the Large Herd Cleanup Project in Iowa. Four of the herds have 500 sows each and the fifth herd has 750 sows. All herds have shown a decline in the percentage of positive tests as the graphic shows (A–7). Two major upsets have occurred in the course of the cleanup procedures. A PRV outbreak occurred within one of the herds concurrently with a TGE outbreak. In another herd the finisher converted to positive status when it was discovered that culled breeding stock were transferred to finisher pens prior to sale for slaughter. They apparently exposed the negative finishers to the virus causing an outbreak of positive tests within the finishing herd.

Plans are underway to begin a surveillance study project. The project will involve two program areas. The study will be designed to develop the protocol for establishing surveillance methodology for herds that have previously tested and have been determined to be noninfected. The methodology will be designed specifically for states or areas having dense swine populations and a high prevalence of infected herds. The chairman of the PRV Technical Advisory Committee has agreed to discuss the development of guidelines to conduct this study at their upcoming meeting in November.
AQESZKY'S DISEASE CONTROL IN IOWA

IOWA APPROVED PREMISES

SWINE HERDS TESTED...IOWA
AUJESZKY'S DISEASE CONTROL IN IOWA

MONITORED HERDS...IOWA

NUMBER OF HERDS

5000
4000
3000
2000
1000
0


INFECTED VS RELEASED HERDS.....IOWA

NUMBER OF HERDS

4000
3000
2000
1000
0


- INFECTED
- RELEASED

382
IOWA-LARGE HERD CLEANUP PROJECT
FIVE HERDS

% TESTS POS.

YEAR

6/90 4/91 7/91 9/91

RH  DE  JS

MP  MB
Overview of North Carolina swine industry and quarantines for PRV

A. North Carolina sow population as of July 1991 was 417,000 sows. By October, it had grown to 441,000 sows. North Carolina is the most rapidly expanding swine producing state in the nation. (See Figure 1)

B. The number of state swine herds is decreasing, but the size of herds is dramatically increasing. (See Figure 2)

C. Although the 426 quarantined premises represent only 5% of North Carolina's premises, yet the 108,000 quarantined sows reflect 25% of North Carolina's total and the 769,000 finishing spaces are 29% of our state's production. (See Figure 3) Obviously, most of the quarantined premises are large herds. Sampson County, the nation's number one swine producing county, and Duplin County, the nation's 12th largest swine producing county, also have the greatest number of quarantined herds, respectively. Swine production is concentrated in the eastern third of the state.

The North Carolina Department of Agriculture (NCDA) has 3 types of production units under quarantine: 1) Sow/Finishing, 2) Sows only (no finishing), 3) Finishing only.

D. There are 92 premises with 47,000 sows (44.9% of all quarantined sows) and 183,000 finishing spaces (24% of all quarantined finishing) in North Carolina. These farms are mostly large conventional units with an average size of 510 sows. (See Figure 4)

E. Of the 426 herds under quarantine, 91 have no finishing. These herds represent 60,000 sows, the average size being 660 sows or 55% of the quarantined sows. The future of swine growth in North Carolina is largely off-site facilities. Currently, approximately 1.2 million pigs are moved to finish at another quarantined location. We must continue to grow in our understanding of how to control disease in multiple site production units as the industry expands in this direction. (See Figure 5)

F. These 243 finishing only premises are over half of all our quarantined farms and swine. Their average capacity
is 2400 finishers. In terms of area cleanup, North Carolina has been waging a significant battle to stop virus circulation on these premises. A more detailed review of these 243 premises is presented in Figure 6.

How we determine our success in managing PRV

By estimating the prevalence of PRV in the overall swine population, in sow farms, and in off-site finishing, we can identify management opportunities. North Carolina has been vaccinating infected herds for years without making progress. It was not until we concentrated on managing the tools available to us, not just vaccines, that we began to decrease virus circulation.

Looking at herds as a means of measuring progress, we compared newly infected herds to herds released from quarantine, Figure 7. Progress can only be measured if surveillance is adequate and animal movements from quarantined herds are restricted. As the graph indicates, we did not see a decrease in infected herds in the Spring of 1989 or 1990. In the Summer of 1990, with herd plans taking shape and diseased animal movement under better control, these two lines crossed and have remained beneficial since. However, in some quarters of the year, infected herds decreased while infected swine increased. Because of this, just following herds quarantined or released can be misleading. We have developed a measurement of progress which addresses large herd management challenges because whether or not a large herd is released from quarantine as indicated on this chart depends on progress over several years. As progress is monitored, management practices are changed based on the findings.

Testing to establish PRV prevalence within sow farms enables us to identify the steps management needs to take to clean up large herds. We conduct parity specific testing in these large herds by taking 15 samples from gilts, first parity, second parity, third to fourth parity, and greater than fourth parity. We then analyze these test results using the percent of animals within each parity in the herd. We try to test every 6–12 months in large herds to monitor progress. This is a great management tool. Parity structure is important in determining the percent of infected animals within the herd. We have seen test charts demonstrating a 15% infection, but when we applied the parity structure within the herd to
the test, the true statistical estimate of prevalence of infection has varied from 5 to 45%. Parity specific testing is the key to knowing when to begin test and removal.

By doing this type of sampling, we have demonstrated three general scenarios: infected gilts and sows; infected gilts but non-infected sows; or non-infected gilts and infected sows. When both gilts and sows test positive, the most common causes include poor disease management; poor management of the vaccine administration whether in dose, delivery, frequency, and/or timing; occurrence of recent infection; a nursery infection; and/or active infection in finishing herds.

A manager can have all the best advisors, facilities, vaccine, and genetics and continue to circulate the virus. It is very important not to leave the lowest paid employee unsupervised while vaccinating. Each of us has probably been that person at some time. It is not enough to have the tool, which in this case is the vaccine. Vaccinating properly requires constant management. For example, if the needle used during vaccination is too short (1" needle), then the vaccine might be delivered into cervical fat and thereby rendered ineffective. Positive gilts usually means virus circulation on a finishing floor; however, North Carolina has had several nursery infections that were eliminated by changes in animal flow.

A second general scenario involves infected gilts but non-infected sows. The most common cause of this situation is introduction (down pyramid) of infected replacements. This is unique to pyramid structures. It has been the most common method of initial virus spread in North Carolina. Producers have responded by testing more frequently prior to moving animals from multiple farms to commercial sow farms.

The last scenario involves non-infected (test negative) gilts and infected sows. Some common causes of this scenario are poor management of the vaccine, environmental stress, recent addition of gilts and sows that have not yet sero-converted. Conversely, this scenario could also reflect the early stages of successful cleanup efforts. This is a common picture in a herd vaccinating for years without adequate protection of replacements prior to exposing them to the sow herd. For those of you with children, it is like not properly immunizing your children prior to sending them to school. We see this in recent herd additions which have not had enough time to convert. This test scenario can also indicate early stages of an effective cleanup. As the cleanup progresses, the old infected herd will be replaced with disease-free sows. Testing to determine prevalence on infected sow farms helps measure progress and identify needed changes. These are obviously generalizations of how regular testing helps identify for managers the direction they need to take. Progress in herd cleanup can be measured by reduced prevalence within finishing floors. As previously indicated, most of North Carolina's quarantined finishing is off-site, all-in, all-out by building from vaccinated sow herds. In order to clean
MANAGEMENT TOOLS USED IN PRV CLEANUP

up heavily infected areas, we evaluated this aspect of production in 1990 and again in 1991 with the below results:

North Carolina Off-Site Finishing Study

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Pos.</th>
<th>(%)</th>
<th>Neg.</th>
<th>Swine Capacity</th>
<th>Pos.</th>
<th>Neg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>40</td>
<td>27</td>
<td>(67)</td>
<td>13</td>
<td>73,902(75)</td>
<td>24,492(25)</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>55</td>
<td>11</td>
<td>(20)</td>
<td>44</td>
<td>40,515(25)</td>
<td>121,116(75)</td>
<td></td>
</tr>
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</table>

Progress was made in North Carolina when the PRV Task Force Committee, under the chairmanship of Jim Stocker, Executive Vice-President of Murphy Farms, recognized 75% of the large finishing floors circulating virus and sought the cooperation of owners of infected herds to make some changes in management. These changes included testing quarantined finishing floors and vaccinating animals on floors positive to infection.

Based on these changes, the estimated decrease in virus circulation was:

<table>
<thead>
<tr>
<th>Quarantines</th>
<th>Total</th>
<th>Neg.1990</th>
<th>Neg.1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premises</td>
<td>243</td>
<td>79</td>
<td>194</td>
</tr>
<tr>
<td>Finishers</td>
<td>1,459,232</td>
<td>364,808</td>
<td>1,094,1424</td>
</tr>
</tbody>
</table>

**Gain – 115 Premises
- 729,616 Finishers

When applying these ratios to the total number of off-site finishers under quarantine, an estimated net gain of 115 premises and 730,000 finishers per year no longer circulating the virus. This is a key to area cleanup. Progress is not initially seen in fewer quarantines, but it is seen in no new large sow farm outbreaks of disease in the highly infected area and in a significant decrease in the prevalence of disease in finishing animals. This was accomplished not by the use of any single tool. This was accomplished by the North Carolina swine industry’s unified effort to:

1) Identify causes of disease spread. This was done through the combined efforts of the North Carolina swine industry to identify why the disease was spreading, to acknowledge that the cleanup of sow farms would necessitate reducing virus circulation in off-site finishing floors, as well as restricting the movement of diseased animals, and to strive for improved management of swine health.
Various steps were taken to improve management. Strict biosecurity was implemented, thereby limiting the access of outsiders to the farm. In the past, equipment sharing (trucking) has been found to spread PRV. Under new management guidelines, trucks are designated for movement of either clean runs or infected runs. Trucks are cleaned and disinfected daily. Personnel cannot go between farms until they have been outside of a swine facility for 24 hours.

Three site production has proven to be an effective way of moving disease-free animals off an infected farm.

Poorly managed on-farm dead animal disposal sites have been incriminated as causing disease spread. North Carolina is moving away from on-farm burial to the pick up of dead animals in sanitized leak proof containers outside of facilities.

In conclusion, we have found several management tools beneficial to North Carolina's PRV program. We issue permits and monitor the use of vaccines. A printout of herds approved to use the vaccine, but are not using it consistently, is periodically sent to practitioners. Quarantined herd files are reviewed each month and pre-addressed charts are sent to herds not tested with a listing of specific action required by State/Federal veterinarians.

North Carolina is providing to producers who wish to expand their operations 10–40 geographic reviews each week of the disease status of surrounding farms. The large investment in these new units will require hundreds of acres of land and justifies careful consideration. With this tool, North Carolina’s industry is locating new farms in areas of less risk from disease spread from neighbors.

Transportation is extremely important to multi-unit operators. Routing trucks to save time, money, and minimize disease spread is a management tool into which North Carolina is expanding.

"Some remedies are worse than the disease." Publilius Syrus. The remedies we seek in North Carolina involve better management of the tools available to us and an innovative industry ready to step up to the challenge of better health instead of hiding from it. Inconsistent, poor management makes any remedy look worse than the disease. Poor management makes many decisions seem insurmountable. Good management practices applied with vigilance produce long term economic benefits. Effective health programs eradicate many of the decisions involved in disease control and elimination.
MANAGEMENT TOOLS USED IN PRV CLEANUP

FIGURE 1

NORTH CAROLINA
SOW POPULATION

TOTAL SOWS
417,258

FIGURE 2

NORTH CAROLINA
NUMBER OF SWINE HERDS

TOTAL HERDS
8,439
SWINE IDENTIFICATION FOR SOWS AND BOARS
for
SLAUGHTER SURVEILLANCE

Donald R. Bridgewater, BS, DVM, MPVM; United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services; Englewood, Colorado 80155

Donald P. Ferlicka, BS, DVM; Administrator and State Veterinarian, Animal Health Division, Montana Department of Livestock; Helena, Montana

INTRODUCTION

A review of swine identification studies and systems is addressed, with an approach for supplementing the official swine backtag that is currently utilized for identifying sows and boars for slaughter surveillance.

SUMMARY

Can industry afford to wait until the perfect swine identification system is developed, be it the use of a better backtag, a better glue for applying the backtag, a better eartag, a better electronic device or any other device?

In the interim, this summer, the State of Montana selected a two-piece plastic bangle eartag, on a trial basis, to supplement the use of the official swine backtag for slaughter surveillance. Six Montana producers, who have a vested interest in their product, are currently applying the eartags to their sows and boars on the farm. The recovery of the eartags and blood samples was coordinated with a large packing plant in South Dakota, where 80% of Montana's sows and boars are slaughtered. This should not imply that eartags are the only suitable devices.

If a State and its swine industry select and support the use of an optional swine identification device in the interim, should it be classified as official, provided it meets pseudorabies program requirements? Some producers are already identifying their hogs with some form of identification selected from the various identification devices currently on the market.

If the producer(s) cannot apply identification to their sows and boars, would it be reasonable to expect the producer(s) to bear the cost for someone else to apply it?

To help the producers to cover the added expense of other forms of officially-approved swine identification devices, would it be reasonable to suggest that the involved State, Animal and Plant Health Inspection Service (APHIS), Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA) pay for them?

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SWINE IDENTIFICATION FOR SOWS AND BOARS

HISTORY

Prior to Skinning

The United States Department of Agriculture (USDA) developed the slap tattoo system in 1925 and recommended it for disease control activities.\(^1\)

For many years, the packing plant industry utilized an in–house slap tattoo code to identify where the hog was purchased, and to pay for a hog purchased grade and yield. Also, it was, and still is, used for in–plant quantity and quality control (grade and yield and condemnation accountability).\(^1\)

In the early 1970s, USDA, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), adopted a six-figure, coded (alphanumeric) slap tattoo system to identify eligible breeding hogs to the farm of origin if they were found to be diseased (infected with tuberculosis and/or brucellosis). Three letters on the top identified the market and/or state, and three numbers on the bottom identified the herd of origin (owner's lot). The hog was identified on the shoulder with the slap tattoo when the farmer unloaded it at the stockyard. At the packing plant, the tattoo became clearly legible on the skin after the carcass moved through the scalding and dehairing process. Necessary paperwork was maintained to establish a paper trail, so to speak, so that a diseased hog, identified with the tattoo, could be traced back to the farm of origin.\(^2\)

Prior to 1978, the swine brucellosis program was voluntary\(^3\) and was based on the coded slap tattoo, requiring sows and boars moving interstate for slaughter to be identified prior to movement, and before being mixed with swine from another source.

On May 22, 1978, 9 CFR 78.30 (identification of sows and boars) went into effect.\(^4\) This required that sows and boars moved interstate for slaughter or for sale for slaughter be identified to the herd of origin by a VS–approved tattoo code applied to the back of each swine prior to such interstate movement, and before they are mixed with swine from any other source. Upon written request of the State animal health official, the Area Veterinarian In Charge (AVIC) could authorize the use of an approved swine identification tag in lieu of the slap tattoo. Veterinary Services–approved slap tattoo codes and approved swine identification tag serial numbers were officially assigned to owners, market agencies, dealers and other persons upon application to the State officials.

This slap tattoo system worked well. It was used primarily in lieu of an approved swine identification tag because it was permanent, less costly, more easily applied to a hog in non–confined areas (such as pens and alleys), and more easily recovered at slaughter.

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BRIDGEWATER, FERLICKA

During Skinning

Even prior to the time 9 CFR 78.30 first went into effect, the swine industry, USDA, FSIS, and VS recognized that some plants were skinning, and this procedure was on the increase. With this procedure, the coded tattoo could not be recovered. This led VS, other Government agencies and Industry to look for alternative methods to identify hogs for disease control activities and in-plant quantity and quality control.

For surveillance purposes, the goal was to produce an identification system that was not expensive, could be easily applied, retained for a minimum of two weeks with a 95% retention rate, easily recovered, and not affect the hide value. Ideally, the identification system would be national in scope, with a national numbering system, and it would serve for the productive life of the animal. The ultimate goal was a computerized, electronic system.

Identification Devices

To date, many forms of identification have been considered, reviewed, or tried. These include, but are not limited to:

1) standard ear notch 2) hot brand 3) Dennison Tag Fast II (where the T-bar portion is inserted beneath the skin [subcutaneously]) 4) an external tag subcutaneously attached, i.e., ETSA - self- and non-self penetrating 5) paint branding 6) metal ear tags coded for each lot 7) steam recovery of tattoo from hide 8) ear tattoos 9) modified fat slap tattoo 10) official backtags for cattle and swine 11) modified backtags includes various types of glue 12) official and non-official metal ear tags 13) various plastic ear tags (bangle type and those that fit close to the ear) and 14) implanted electronic devices.

In 1980, Veterinary Services reported a swine identification field trial that was conducted in 1978 and 1979. Seven swine identification devices were selected for the trial, to provide a cross section of the types being used by industry. The tags were applied to sows and boars at auction markets and were recovered at the packing plant where the swine were slaughtered. The official cattle backtag, three plastic ear tags, an official cattle ear tag and two metal eartags (one was applied with a hog ring) were used in this study.

There were several variables addressed in this study. Three will be discussed: retention, ease of recovery, and cost. The variables were ranked on a numerical scale: 1 = best and 7 = poorest.

The retention of the identification applied was ranked as follows: 1) official cattle backtag = 7; 2) non-official metal ear tag (without hog ring) = 6; 3) official cattle ear tag = 5; 4) the plastic ear tags and the hog ring applied metal eartag = 1.

The ease of recovery of the identification applied was ranked as follows: 1) official and non-official metal ear tag (without hog ring) = 5; 2) plastic ear tag that attached closely to the ear = 4; 3) official cattle backtag
SWINE IDENTIFICATION FOR SOWS AND BOARS

= 3; 4) two plastic bangle tags that did not attach closely to the ear = 1; 5) hog ring applied metal eartag = insufficient data.

The cost of the identification applied was ranked as follows:
1) one plastic bangle tag that did not attach closely to the ear = 7; 2) the second plastic bangle tag that did not attach closely to the ear = 6; 3) plastic eartag that attached closely to the ear = 5; 4) non-official metal eartag (without hog ring) = 4; 5) hog ring applied metal eartag = 3; 6) official cattle eartag = 2; 7) official cattle backtag = 1.

The trial indicated that two of the seven tags, the official backtag and the non-official metal eartag, had poor retention rates or other deficiencies to discourage them from being used on a large scale.

Even though the trial considered the official backtag retention to be unacceptable, it considered the backtag to have features that made it appealing. It was not expensive, market operators would use it, it can be applied without restraint, and markets can use it as a sales tag. It indicated, however, that the retention could perhaps be augmented by changing its size, shape, place of application, type of glue, or waterproofing the tag.

Mr. M. C. Shelnut, USDA, APHIS, VS, Animal Identification Coordinator for the State of Georgia, was already in the process of developing a 2 1/2 inch round alphanumerically coded swine paper backtag. He later made it out of a stronger, waterproof material and it became the approved official swine backtag. Veterinary Services, through the years, has modified the glue that is provided for the application of both the official cattle and swine backtags.

In 1987, brucellosis general restrictions went into effect under 9 CFR 78.30. This part of the regulation placed the responsibility for identification on each person who causes the movement of swine in interstate commerce. The identification of sows and boars, which was under 9 CFR 78.30, became 9 CFR 78.33 (sows and boars).\(^{14,15}\) For swine disease surveillance purposes, identification has routinely been applied at the first point of concentration (buying station, livestock market, packing plant) rather than at the farm level. This remained in effect. Swine moving to an approved market or concentration point can be identified on arrival before they are mixed with swine from any other source.

In 1988, under 9 CFR 78.33, an official eartag or a USDA Backtag replaced the tattoo as the primary form of identification.\(^{16}\) Sows and boars, under this part, may be moved in interstate commerce for slaughter or for sale for slaughter if they are individually identified by an official eartag or a United States Department of Agriculture backtag applied before movement in interstate commerce and before they are mixed with swine from any other source. Upon request by the user or the State animal health official, the VS approved tattoo can be used, in lieu of the eartag or backtag, when the Deputy Administrator authorizes it in writing.
The backtag and the eartag, under 9 CFR 78.33, were adopted to address the skinning problem. These types of identification could be applied to eligible sows and boars and recovered from them at packing plants that skinned swine, as well as from those that scalded. Compared to the slap tattoo system (considered a permanent form of identification) these forms of identification are considered to be temporary.

Between the two forms of identification, the backtag is primarily used over the eartag, because it is more economical. It is also considered easier to apply and easier to recover at slaughter. In addition, APHIS supplies livestock markets and other approved concentration points with the backtags and glue at no charge. The tags can be used at livestock markets as a backtag sales tag.

Under the Pseudorabies Eradication State–Federal–Industry Program Standards, states that must export their sows and boars long distances (because they have very few swine packing plants and swine livestock markets) must rely on other states to sample their breeding swine population to meet sampling and traceback requirements for entry into Stage III of the program. Since the current backtag system does not work well for some of these states, they have had to revert to first-point testing or area testing to move into Stage III. For surveillance, slaughter sample testing is considered to be the least expensive, first point testing the next most expensive, and area testing the most expensive.

At the Ninety-fourth Annual Meeting of the United States Animal Health Association (USAHA), Denver, Colorado, 1990, a resolution was presented to the Committees on Pseudorabies and Livestock Identification, which addressed the optional use of a proposed numbered plastic bangle eartag as an identification device for the Market Swine Identification Program (MSI). It would be applied to sows and boars with a hog ring. The same identification code would be located on the upper and lower portions of the tag. In the packing plant, the upper part, attached to the ear, would remain with the carcass. The lower part would be cut off and collected with the blood sample. The Pseudorabies Committee requested that APHIS consult with the National Pork Producers Council, market associations, and packer organizations, with a goal to have available an optional nationally uniform eartag by March 1, 1991. The livestock identification committee supported the resolution.

At the Sow and Boar ID Task Force meeting held in Washington, DC, January 28, 1991, APHIS personnel presented a proposed swine identification field trial to study swine identification at markets. The proposal did not materialize.
SWINE IDENTIFICATION FOR SOWS AND BOARS

MONTANA'S SOLUTION

Montana State Veterinarian, Dr. Donald Ferlicka, supported ongoing studies to develop a better form of swine identification, but recognized there were commercially available plastic bangle eartags that he could use in the interim, which would supplement the use of the swine backtag. Even though more costly than a backtag, they would better serve Montana's marketing practices because they would have a greater chance of being recovered at slaughter. A backtag applied that doesn't make it to the packing plant serves no purpose, no matter how little it costs.

The State of Montana recognized that 80% of their sows and boars went to a large packing plant in Sioux Falls, South Dakota. Dr. Ferlicka did not want to apply for any stage of the Pseudorabies Program until the State could qualify for Stage III. He also wanted to assure industry that once the State obtained Stage III, it would move ahead and proceed to Stages IV and V and not remain in Stage III or slip to a lower stage. Dr. Ferlicka concluded that a plastic bangle eartag would provide this assurance, and proceeded to pursue this approach.

On May 1, 1991, the State of Montana applied for Pseudorabies Program Stage III and was granted this status on June 10, 1991.

By the summer of 1991, Dr. Ferlicka had obtained, for trial purposes, a two-piece plastic bangle eartag from a major eartag producer. The tags are Montana-identified, and alphanumerically coded to six producers. The producers, who have a vested interest in their product, agreed to apply the tags to their sows and boars on the farm. The code is located on both tag pieces.

The recovery of the tags with the blood sample, at the Sioux Falls packing plant, was coordinated with officials of the packing plant, State officials of South Dakota, and Federal officials. One portion of the tag is collected with the blood sample, and the remaining portion stays with the carcass. The carcass part can be used by the plant, and can also be used to identify the source of hogs, if diseases or drug residues are revealed. Not enough time has transpired since the trial was implemented to draw any conclusions. Dr. Ferlicka has faith that the tag will serve its purpose if everyone involved supports it.

QUESTIONS FOR CONSIDERATION

Can industry afford to wait until the perfect swine identification system is developed, be it the use of a better backtag, a better glue for applying the backtag, a better eartag, a better electronic device or any other device?

If a State and its swine industry selected and supported the use of an optional swine identification device in the interim, should it be classified
as official, provided it meets Pseudorabies Program requirements?

If the producer(s) cannot apply identification to their sows and boars, would it be reasonable to expect the producer(s) to bear the cost for someone else to apply it? Some producers are already identifying their hogs with some form of identification selected from the various identification devices currently on the market.

To help the producer cover the added expense of another form of officially-approved swine identification, would it be reasonable to suggest that the involved State, APHIS, FSIS and FDA pay for them?

REFERENCES

1. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Cattle Diseases and Surveillance Staff, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.


THE IMPLICATIONS OF THE IMPORTATION OF WILD HOGS ON THE NORTHERN UNITED STATES

A paper presented by
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St. Paul, Minnesota
at the USAHA Convention, San Diego, California
October 26 – November 1, 1991

The European wild hog is not a native wild animal species in Minnesota. The Minnesota Board of Animal Health became concerned when they recently learned that wild hogs are being brought into Minnesota for hunting purposes. We had heard of the environmental and ecological damage that wild hogs have caused in other states. If these animals should escape and become established in Minnesota’s extensive woodlands they could be very harmful to our agricultural and recreational industries.

Minnesota has several special concerns because of our large domestic swine industry and our ongoing pseudorabies and brucellosis eradication programs. Much of our heavily forested land, swamps and river bottoms are located in close proximity to our major swine raising areas.

WHAT KINDS OF DAMAGE DO WILD HOGS CAUSE?

Most biologists agree that wild hogs are very destructive animals that do extensive damage to forests, crops and wildlife. The public literature is replete with articles that describe the environmental and ecological damage caused by wild hogs in such diverse areas as North Carolina, Florida, California and Hawaii, because of their foraging activities. The following are some of the ravages attributed to wild hogs:

Rooting up large areas of soil and destroying forests, crops, gardens, lawns and golf courses in their search for food.
Killing and devouring other animals including fawn deer, game, lambs and baby calves.
Turning trout streams into mud wallows.
Destroying ground nesting birds, such as pheasant, grouse and ducks.
Spreading livestock diseases such as brucellosis, pseudorabies and leptospirosis.
Causing soil erosion and destruction of forest flora and fauna.

TO WHAT EXTENT ARE WILD HOGS BEING IMPORTED INTO MINNESOTA?

When it recently became known that wild hogs are being imported and raised in Minnesota, an investigation was begun by the Minnesota Board
IMPLICATIONS OF THE IMPORTATION OF WILD HOGS

of Animal Health to determine the extent of these activities. A superficial investigation revealed that approximately 300 head of wild hogs are being maintained on 8 farms located throughout the entire state.

FOR WHAT PURPOSES ARE WILD HOGS BEING IMPORTED INTO MINNESOTA?

Wild hogs are being imported and raised in Minnesota for a variety of reasons. The following are some of those reasons:
For sale as meat.
For wild game hunting purposes.
For exhibition on exotic animal farms.
As a hobby species.

IF WILD HOGS SHOULD ESCAPE WOULD THEY SURVIVE IN MINNESOTA'S WINTERS?

Most authorities agree that wild hogs would not only survive, but would thrive and multiply rapidly if they should escape into Minnesota's swamps and woodlands. They have lived for centuries in northern Europe and Russia so they would thrive successfully in Minnesota's winter climate. There is adequate food for them and they are extremely hearty animals.

WILD HOG SURVEY OF OTHER STATES:

In order to assess the potential hazards associated with wild hogs, the Board of Animal Health did a survey of all the other states to learn of their experiences with wild hogs. They sent a short survey to the State Veterinarian's and Chief Wildlife officials of each state. They were asked if they had loose, wild hogs in their woodlands, and if so, what was their impact on livestock, wildlife, crops and environment. They were also asked if they considered wild hogs to be a liability or an asset to their state. The following are some of the things that were learned from this survey:

50 of the 50 states responded to the Survey.
23 states said they had Wild Hogs loose in their woodlands.
They said they had in excess of two Million wild hogs.
Texas had 1,1000,000
Florida had 500,000+
California had 300,000+
Hawaii had 100,000+
Some wild hogs were located as far north as Ohio, Illinois, Vermont, and Oregon.
35 states said they allowed importation of wild hogs.
14 states said they Banned the importation of wild hogs. These include: Michigan, Massachusetts, Ohio, Maryland, W. Virginia, Washington, Idaho, Utah, Kansas, Colorado, California, N. Mexico, Kentucky, Rhode Island.

10 states said wild hogs had a negative impact on disease control.

14 states said wild hogs had a negative impact on wildlife.

14 states said wild hogs had a negative impact on the environment.

14 states said they do allow wild hog hunting. Some allowed unlimited hunting.

45 states said they considered wild hogs to be a liability to their states.

SUMMARY:

I believe there are a number of very compelling reasons why any state that does not have wild hogs should take action to keep them out:

#1. Wild hogs are very destructive to the environment and ecology.

#2. Wild hogs cannot be confined by the average hog fencing.

#3. Wild hogs spread diseases and will have a negative impact on area disease control.

#4. Wild hogs cannot be eradicated by hunting.

#5. Wild hogs would survive the northern winter climates.

It seems to me to be a prudent precaution for any state to give serious consideration to the control or elimination of wild hogs.
COMPLIANCE WITH PSEUDORABIES ERADICATION STAGE STATUS REQUIREMENTS

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W.W. Buish
Director, Northern Region
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The requirements to achieve and maintain pseudorabies (PR) eradication stage status are for the most part not particularly demanding. However, a few requirements are posing difficulties for several states. On the other hand, some states have already met the requirements to advance to the next stage but have been reluctant to apply because doing so would cause problems for their producers. The purpose of this report is to review the most significant requirements for each stage of the PR eradication program and to comment on problems encountered by states trying to meet those requirements. The information is based on a survey of states in the Northern Region of USDA/APHIS/VS in early October, 1991.

Stage I — Preparation. The requirements for Stage I are minimal. A pseudorabies advisory committee must be functioning, and a reliable method of determining prevalence must be in place. In addition, the state must have or be seeking regulatory authority relating to mandatory reporting and quarantine of infected herds, epidemiologic investigations, and vaccine use. None of the surveyed Stage I states indicated any problems with maintaining status. However, advisory committees in some of the higher status states with low or zero prevalence were not scheduling meetings with great frequency due to complacency about the need for a continued high level of program activity. In no state surveyed had the frequency of advisory committee meetings fallen below one per year.

Stage II — Control. To achieve and maintain Stage II status a state must have a surveillance system for sows and boars at slaughter, farm or first point. The movement of breeding swine, feeder pigs and slaughter hogs must be regulated, and herd cleanup is voluntary. None of the surveyed states in Stage II had trouble maintaining status. At least one Stage I state did not want to advance to Stage II status because the restrictions on imported feeder pigs would limit potential sources for that state’s swine finishers.

Stage III — Mandatory Herd Cleanup. To achieve and maintain Stage III status, states must meet the following conditions: require mandatory herd cleanup plans, trace back herd additions and trace forward herd sales with testing of source and recipient herds, circle testing of positive herds, herd
COMPLIANCE WITH PSEUDORABIES ERADICATION

prevalence rate of 1% or less, and maintenance of a surveillance index (SI) of .08 (proportion of the breeding swine surveyed annually times the proportion of successful traceback of positives). In Stage III, vaccination is only permitted as part of herd cleanup plans or in area control programs. Maintaining a SI of .08 was the only requirement mentioned as a problem for achieving and maintaining Stage III status. However, almost all of the Stage III states did report concerns and/or difficulties in meeting the required SI, and several of the Stage II states cited expected problems with fulfilling the SI requirement as a reason for not seeking Stage III status. Because many states are barely achieving a SI of .08, officials in those states do not know if their state will meet the SI requirement until the end of the reporting year and therefore have trouble planning future program activities. States differed in the reason given for having difficulty meeting the SI requirement. A common complaint was that samples were not collected with high enough frequency at slaughter, especially at other states’ slaughter plants. Officials in some states were concerned that traceback of positive animals was hampered by poorly applied or unreadable backtags and by application of backtags to wet hogs.

First point testing is not a good alternative in most states because not enough cull sows and boars are sold at markets. In addition, consistent and efficient first point testing requires a sufficiently large sale population, which is not always available at many markets, and coordination of catchers and bleeders whose other duties limit their ability to be at markets when hogs are available. Area testing is an expensive surveillance method that some states are forced to consider or adopt. Officials in states planning to test all or a random sample of herds as a means of satisfying SI requirements are concerned that PR monitored feeder pig herds will not count toward calculation of the SI, as stated in the program standards. However, this need not be a problem if the herds in the state to be sampled under area testing are chosen randomly, without regard to feeder pig monitoring test status. If a feeder pig monitored herd is randomly selected and if the last test was within a certain time period, say six months, the results from such testing should count toward computation of the SI.

Several states with relatively low numbers of swine herds mentioned inaccuracies (overestimates) in the number of breeding animals as determined by NASS. Since the SI is based on the estimated number of sows and boars in the state, overestimation of the size of the breeding population can result in an unrealistic SI target. In some cases, the excess number was enough to place the number of animals to be sampled to meet the SI requirement beyond the capacity of the state to comply. In those cases, locally derived, and presumably more accurate, estimates of the size of the breeding population were allowed to form the denominator of the SI.

Difficulties in meeting SI requirements will continue until an effective means of animal identification is developed and until the rate of sample collection
is increased in slaughter establishments. Increasing the effectiveness of both animal identification and slaughter sampling should be high priorities for federal and state regulatory officials and the swine industry.

Stage IV -- Surveillance. States in Stage IV must have been free of in-state source infection for 2 years and out-of-state source infection for 1 year before applying for status. In addition, vaccination may only be permitted as part of a herd cleanup plan. Stage IV states must restrict importation of swine from lower status states. For example, feeder pigs may only be imported from a farm or market in a Stage IV or V state or directly from a qualified negative herd.

Stage IV states must meet the same SI requirements as Stage III states and thus they report similar problems. Some Stage III states otherwise eligible for Stage IV status have elected not to apply because the restrictions on swine imports would pose a burden on producers. Buyers of feeder pigs especially would have a difficult time finding a sufficient number of animals from Stage IV or V states or from qualified negative herds. Because of the restrictions on vaccine use in Stage IV states, at least one Stage III state would not consider applying for advanced status until the prevalence rates in neighboring states was reduced. Some producers feel that there are not enough incentives to move from Stage III to Stage IV other than the "honor" of being in a higher status.

Stage V -- Free. There were no states in Stage V at the time the survey was taken.

In summary, the major problem that states face in achieving and maintaining status is meeting the SI requirement, due mainly to inadequate animal identification and slaughter sample collection. Unfortunately, these problems effect not only status requirements but also the success of the eradication program itself. Regulatory officials and the swine industry must work together to improve the effectiveness of slaughter surveillance. Attention should be given to time in market channels and mislabeling of blood samples taken in slaughter plants. Sows and boars from quarantined (and possibly qualified negative herds) must be so identified to reduce costly, unnecessary testing and tracebacks. Ideally, animals from vaccinated, negative herds should be identified as such, including the type of vaccine used.
PSEUDORABIES VIRUS ASSOCIATED DISEASES AND MECHANISMS OF VIRAL IMMUNOSUPPRESSION

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My goal in this presentation is two-fold: to review the evidence for associated diseases that are either intensified in severity or increased in frequency in PRV-positive herds and also to provide some possible mechanisms for this immunosuppression that we have been working on in the laboratory.

PRV Associated Diseases

Several observations have suggested the existence of PRV immunosuppression. Many investigators, practitioners, and particularly producers, have been frustrated by the fact that PRV does not induce complete protection. Neither vaccination nor an outbreak itself completely prevents later circulation of the virus within a herd. This is one of the biggest problems thwarting eradication. In addition to poor immunity to the viral infection itself, there have been studies that indicate that PRV-positive herds do not sustain good immunity to other vaccines. Lee et al., showed the ability to immunize against a bacterial infection, *B. bronchiseptica*, was reduced in PRV-positive herds [15]. The humoral antibody response to vaccination in a control, PRV-negative herd yielded high antibody titers which were maintained over time. Little antibody was produced in response to similar vaccination of a PRV-positive group of animals. Such PRV suppression of vaccination for hog cholera has also been reported [14].

Table 1. PRV Associated Infections

<table>
<thead>
<tr>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacillus pleuropneumoniae</td>
<td>1, 2, 5, 13</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>2, 7, 8, 22</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>2, 9</td>
</tr>
<tr>
<td>Transmissible Gastroenteritis</td>
<td>2, 9</td>
</tr>
<tr>
<td>Leptospira</td>
<td>2, 9</td>
</tr>
<tr>
<td>Swine Influenza</td>
<td>16</td>
</tr>
<tr>
<td>Salmonella</td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>2, 12</td>
</tr>
</tbody>
</table>
The different bacterial and viral diseases that have been associated with pseudorabies infection are shown in Table 1. The best documented and studied has been *Actinobacillus pleuropneumonia*, which used to be called Hemophilus. Reports of association of this bacterial infection with PRV have been made by a number of groups [1,5,8,12,16]. The frequency of herds that are infected with both this respiratory pathogen and PRV seems to be notable. Although within a herd, Hall et al. [9] found no consistent association between the pigs that were positive for PRV and the pigs that had been exposed simultaneously to Actinobacillus. Each herd had a different pattern of diseases and different degrees of association with PRV. Dr. Ralph Vinson reported at the 1986 Peoria PRV meeting that a PRV-positive herd with *A. pleuropneumonia* problems did not respond to the bacterial vaccine but was improved by PRV vaccination [2].

Work with *Pasteurella* has indicated that PRV-infected pigs are more susceptible to bacterial pneumonia. Fuentes and Pijoan have shown that PRV-infected alveolar macrophages are unable to kill the *Pasteurella* bacteria [7,8]. The frequency of other diseases (mycoplasma, TGE, leptospirosis, swine influenza, systemic salmonellosis, and respiratory streptococcosis) have all been associated with PRV in a number of field situations at one time or another (see Table 1). It is probably not so much a problem of increased susceptibility to these other agents in PRV-positive animals, but rather a situation of intensified disease. Where these other pathogens are present in a herd, there will be greater problems if that herd is also positive for pseudorabies. If the other infectious agents are also immunosuppressive, the combined respiratory disease will be intensified. So what we are seeing is a synergistic effect [22] of the respiratory form of pseudorabies with these other respiratory diseases. The reason for this appears to be based upon the ability of PRV to immunosuppress the host.

In one study, the frequency of diagnoses of a number of secondary infections was increased in various PRV-positive herds compared with negative herds [2]. An increase in the frequency of leptospirosis, TGE, hemophilus, mycoplasma, and *salmonella* was observed. There seemed to be an associated increase in the frequency of PRV positive herds that were diagnosed for these diseases. The exception was parvovirus which was lower in the PRV positive herds. Although some of these results may be skewed because positive herds were followed more closely than negative herds, nevertheless, these findings indicate that pseudorabies can increase the chance of more severe secondary respiratory infection.
Experimental Studies

Several laboratories have indicated that PRV can infect various cells within the immune system. Wittmann et al. [23] provided evidence that circulating monocytes can harbor virus. In vitro studies have shown that macrophages from a number of sources are susceptible to PRV [3,4,10,18,21]. Virus has been isolated from alveolar macrophages obtained from acutely infected pigs [10]. More direct evidence at the cellular level has shown that PRV infection of alveolar macrophages reduces the ability of these cells to phagocytize and destroy ingested material [7,11]. All immune cells interact with one another in vivo; thus, it is probable that PRV infection of macrophages could have an indirect effect on lymphocyte performance.

The susceptibility of lymphocytes also has been studied directly, but primarily in vitro. Isolated leukocytes or leukocyte subfractions were infected with PRV and examined for expression of surface antigens by flow cytometry [20,21]. About 4% of the isolated lymphocytes were positive for PRV. This fraction could be increased about 4-fold by stimulating T cells with the T-cell mitogen, PHA. Infection of isolated B cells has not been found, even after stimulation of B cells with LPS. The fraction of susceptible neutrophils was negligible, remaining at background levels. Positively staining cells were caused by viral infection, not antigen processing, because UV-irradiation of virus before infection reduced the fraction of positive cells to near background levels. The lymphocyte results, taken together with the macrophage infectivity studies described above, indicate that a number of immune cells can be infected with PRV, particularly macrophages and T cells.

Our research interest has been to find out what cellular and molecular mechanisms account for the increased immunosuppression that one sees with pseudorabies. We have quantitated the number of lymphocytes, monocytes, and total leukocytes in pigs at various times after acute infection. Neutrophil numbers were constant after challenge [17]. Monocytes were lost from the peripheral circulation almost immediately, even at a time when total white cells were increasing. The lymphocytes, on the other hand, maintained themselves up to day 4 and then drop rather precipitously on day 5. In those pigs that survived infection, lymphocyte numbers recovered and cycled. In the pigs that died, we have observed that the precipitous drop in lymphocytes did not come back but remained low. We think this condition favors subsequent severe CNS involvement and death. Infection reaching the bone marrow can reduce the supply of replacement leukocytes [19].
We were interested in determining whether various immune effector cells were susceptible to infection, and so we took leukocytes from 5 pigs and infected them \textit{in vitro} with PRV (Figure 1). The first graph (upper left) represents cells infected with Shope strain of pseudorabies which is a moderately virulent laboratory strain. The hatched bars show the percent of PRV-positive monocytes at 24 hours after infection. The cross hatched bars represent the percent of lymphocytes that were infected. Five to 25\% of the cells, both monocytes and lymphocytes, were positive for pseudorabies antigens as determined by flow cytometry. We have observed that there is quite a bit of variation from pig to pig in terms of the susceptibility of their peripheral lymphocytes and monocytes to PRV. We wanted to know whether this susceptibility of monocytes and lymphocytes to PRV was restricted just to virulent infections, so we repeated these experiments with live modified vaccines. On the upper right panel are the results of infection of the same pig cells with Norden vaccine virus. This virus was attenuated in that the number of monocytes and lymphocytes infected was low for all 5 pig samples. On the lower left, TechAmerica vaccine virus also produced very few infected cells. In distinction, the lower right graph, showing results of infection with Upjohn recombinant vaccine, Tolvid, reveals a significant number of both monocytes and lymphocytes positive for viral infection. Note that the scale here is actually different from the other three graphs and the
percent positive after viral infection of these cells with Tolvid was even higher than for our virulent strain, Shope. This does not mean that the Tolvid is a bad vaccine but indicates that there is a possibility that the vaccine virus may be immunotropic and actually may be more able to infect certain lymphoid cells than some field strains.

Table 2. Effect of PRV on Immune Cell

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Susceptibility</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage</td>
<td>YES</td>
<td>LOST</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>NO</td>
<td>OK</td>
</tr>
<tr>
<td>Resting T Cell</td>
<td>NO</td>
<td>??</td>
</tr>
<tr>
<td>Stimulated T Cell</td>
<td>YES</td>
<td>LOST</td>
</tr>
<tr>
<td>Resting B Cell</td>
<td>NO</td>
<td>??</td>
</tr>
<tr>
<td>Stimulated B Cell</td>
<td>NO ?</td>
<td>??</td>
</tr>
</tbody>
</table>

Our studies have been extended to other cell types. We looked not only at the ability of cells to be infected but also studied whether infected cells can function immunologically. A summary of our findings is shown in Table 2. In the terms of the susceptibility to PRV at the cellular level, macrophages and stimulated T-cells are particularly susceptible. Neutrophils and B cells were not susceptible in terms of viral antigen expression after infection.

We have also looked at the immune function of these various cell types. Our data indicate that macrophage function and T-cell function are lost after PRV infection. Figure 2 shows the two types of assays used to define the effect of PRV directly on immune cells. The first is called antibody-dependent cellular cytotoxicity or ADCC. Here macrophages, in the presence of antibody are able to kill virus-infected target cells. The second is a cytotoxic T-lymphocyte assay, where cells are taught to recognize and kill foreign or virus-infected cells. Both of these cell types are important components of the cellular immunity to both viral and bacterial diseases.
Antibody-Dependent Cellular Cytotoxicity

Figure 2. Functional assays for macrophage and T cell immune function. Macrophages kill virus infected cells that have been sensitized with anti-PRV antibody. Cytotoxic T cells can recognize and kill infected target cells directly.

Figure 3 shows the effects of PRV infection on the ability of macrophages to perform ADCC. In this experiment, macrophages were used to kill PRV-infected cells in the presence of small amounts of anti-viral antibody. In the noninfected control macrophages, which are shown in the left-hand, shaded bars, a slight increase in the ability to kill the infected targets was seen at 24 hr. The right-hand cross-hatched bars show the effects of infection of these macrophages. At time 0, immediately after infection, there was a decrease in the ability of the macrophages to kill the infected targets compared to non-infected controls. At 24 hours after infection of the macrophages, their ability to kill was diminished to almost background levels. This indicates that macrophages are exquisitely sensitive to PRV infection and that infection may reduce their ability to function. Other macrophage activities also have been shown to be compromised by PRV infection [7,11].

T-cell cytotoxicity
To determine whether the virus can reduce T-cytotoxic activity, we used an in vitro cytotoxic T lymphocyte system in which isolated pig lymphocytes are taught to kill mouse cells by culturing the pig white blood cells with irradiated mouse cells. At 3 to 5 days after co-cultivation, the pigs lymphocytes develop the ability to kill the stimulator mouse cell targets. When there is no co-cultivation, the pig lymphocytes are not able to kill. To show the effects of PRV infection on T-lymphocyte function, we exposed some of these T-cytotoxic cells on day 5 after co-cultivation to pseudorabies virus. At various hours after infection, we assayed for the ability of the T-cells to kill the mouse cells. Within a few hours pseudorabies infection was able to disarm the ability of these T cells to kill their targets.

In conclusion, the number of associated diseases that one might see in a chronically infected herd suggests involvement of PRV. These diseases are probably not increased in frequency, but the chances of secondary respiratory involvement, and particularly the severity of those infections is assuredly increased by pseudorabies infection if the secondary pathogen is present in the herd. The degree of PRV immunosuppression and the extent of associated infectious disease are dependent on virulence of both the virus and other pathogens and on the immune status of the host. Interactions between PRV and secondary pathogen could act synergistically. A model for viral immunosuppression can be proposed that involves local infection of lymphocytes and macrophages that predisposes pigs to secondary infection. With compromise of host immunity, both PRV infection and infection by other
pathogens is facilitated.

We have looked experimentally at the mechanisms for this immunosuppression and believe that pseudorabies is able to selectively destroy a portion of the host cellular immune mechanisms that are necessary for defense. The immune cell destruction by PRV is not as severe as in AIDS but can be telling in local tissues such as the respiratory tract. The ability of attenuated vaccine virus strains to infect various immune cells raises a cautionary note that we should investigate not only the attenuation and safety of these vaccine strains in the whole animal but also explore the activity of these viruses at the cellular level.

References


INTRODUCTION
Several pseudorabies virus (PRV) vaccines are commercially available in the United States (1). Most are modified-live-virus (MLV) preparations and several contain gene deletions that result in the failure of the vaccine virus to produce a secreted (gX) or structural (gI, gII) glycoprotein.

This feature enables the differentiation of humoral antibody responses induced by vaccination from those induced by infection with field virus. Therefore, vaccination of pigs for protection against disease can be done without interfering with serologically based regulatory activities. Likewise, within herd eradication programs using vaccination to suppress virus transmission are feasible as differential serotests can be used to assess the transmission patterns of field virus.

The following experimental studies were conducted to simultaneously evaluate several PRV vaccines with respect to serological responses, protection against clinical disease and reduction of virus shedding after challenge. Also, the effect of time interval between vaccination and challenge was evaluated. In addition, the design and conduct of PRV vaccine evaluation experiments, the vaccine licensure process, and a brief overview of PRV vaccine experiments reported in the literature will be presented.

VACCINE EVALUATION AND LICENSURE

In designing experiments to evaluate PRV vaccines, many factors are considered before reaching a final plan. These factors include selection of independent variables (experiment inputs), selection of dependent variables (experiment outcomes or measurements), experimental design (especially as it relates to vaccine licensure), and application of data generated from experimental studies to field situations.

Numerous independent variables or experiment inputs can be manipulated in designing an experiment. These include:

1) Vaccine type including modified-live-virus versus killed virus preparations, adjuvant used with a killed vaccine, and the type of gene deletion used to provide differential serodiagnostic capabilities.

2) Route of administration such as intramuscular (IM) versus intranasal, and the avoidance of subcutaneous and intra-fat deposition.
COMPARISON OF PSEUDORABIES VIRUS VACCINES

3) Vaccine dose in terms of the number of virus particles per dose, vaccine volume, and the number of vaccinations. The vaccine dose is usually determined by conducting a dose titration experiment where different amounts of vaccine virus are administered to pigs that are subsequently challenged. The minimal vaccine dose is calculated based on the results. The final vaccine dose is usually 100–1,000 times higher than the minimum dose to insure similar efficacy under a variety conditions.

4) The vaccination/challenge model including the age of pig at vaccination, the interval between vaccination and challenge, and the type and dose of challenge virus used. For vaccine licensure, the age at vaccination is selected as the minimum age for which the manufacturer is seeking a label claim (typically 3 days of age). The dose of challenge virus is usually high enough to induce >80% mortality in nonvaccinated controls.

Likewise, numerous dependent variables or experimental outcomes can be evaluated. The evaluation occurs during the post-vaccination period for vaccine safety and immunogenicity, and during the post-challenge period for efficacy against experimental challenge. Dependent variables evaluated include:

1) Clinical parameters such as rectal temperature, clinical signs (attitude, appetite, nasal discharge, central nervous system disorders), weight gain, and mortality. These clinical parameters provide the majority of information used to decide if a vaccine can be licensed.

2) Immunogenicity with respect to inducing serum neutralizing antibodies after vaccination. Development of titers is not an absolute requirement for licensure.

3) Post vaccination and post challenge virus shedding. With live virus vaccines, it is preferred that the vaccine virus is not shed after vaccination as determined by failure to isolate vaccine virus from nasal and/or throat swabs collected after vaccination, and the failure of nonvaccinated sentinel pigs to seroconvert after exposure to vaccinated pigs. In the field situation, shedding of PRV vaccine virus usually is not an important consideration because generally all pigs within a population are vaccinated.

Reducing or eliminating the shedding of virulent virus after experimental challenge is a desired feature of
PRV vaccination because of the intent to eradicate PRV from the swine population. The amount and duration of virus shedding after challenge is greatly dependent on the dose of challenge virus and in the case of licensure experiments, the >80% lethal challenge dose is most likely much greater than pigs would encounter in endemically infected herds and is similar to what may occur with an outbreak in a previously susceptible population. In our judgement, vaccine studies reported in the literature and the licensure process have not emphasized enough the ability of vaccines and vaccination strategies to reduce post challenge virus shedding. Likewise, it may be useful for these evaluations to use a non-lethal dose of challenge virus in addition to the >80% lethal dose to mimic the situation in endemically infected herds.

Development of latency and prevention of recrudescence is not evaluated for vaccine licensure. In previous studies, we have found that no commercially available vaccines completely prevent latency development. This can be expected in the field situation where: 1) the level of exposure may vary and may be great enough to induce latency in properly vaccinated pigs; and 2) individual pigs may develop inadequate immunity from a single vaccination due to such factors as operator error or temporary immune suppression from other diseases or environmental deficiencies. Reduction/prevention of latency development by vaccination is less critical if vaccination can reduce/prevent recrudescence of latent virus. This area has not been adequately investigated although one could theorize that repeated vaccination, as recommended for breeding animals, may reduce/prevent recrudescence. Latency and recrudescence data are not considered for PRV vaccine licensure.

The guidelines for federal licensure are published in the Code of Federal Regulations (CFR). The suggested experimental design is to challenge 20 vaccinated pigs and 5 nonvaccinated controls. At least 4 of the 5 controls should either exhibit CNS signs or die, and the vaccinated pigs should remain free of clinical disease. In numerous studies conducted in our laboratory and as reported below, we have never found any vaccine or vaccination strategy capable of completely preventing clinical signs following experimental challenge. Furthermore, the unbalanced number of pigs in each treatment group greatly hinders the validity of statistical analysis. As stated above, the emphasis for licensure has been reduction of clinical signs and not reduction of virus shedding after challenge. Because of the need for the reduction of virus shedding in eradication schemes, vaccines should be stringently evaluated for their ability to reduce the shedding of virus after
COMPARISON OF PSEUDORABIES VIRUS VACCINES

Experimental challenge using at least two different levels of challenge virus. A final concern is that the licensure experiments are conducted on 3–day-old pigs. In the field, pigs are rarely vaccinated at this age. Can we extrapolate data generated in 4#, 3–day-old pigs to 40#, 8–week–old nursery pigs or 500#, 2–year–old sows?

A major concern with any data generated under experimental conditions is it’s application to field situations. There are several major differences between experimental settings and field situations. Compared to field situations, experiments are conducted with lower animal densities (floor space, air space, pigs per pen, pigs per room), high health status pigs (less confounding with other diseases), fully susceptible pigs (no passive immunity), a defined challenge model (as discussed above), and careful handling and administration of vaccines. All of these factors must be reviewed and optimized in the field situation to insure the success of any vaccination program. In many instances, these factors are deficient and the vaccine is inaccurately judged to be the cause of an ineffective vaccination program.

REVIEW OF REPORTED VACCINE STUDIES

There are numerous reports of vaccine studies in the literature. It is not within the scope of this paper to review these reports in detail. Rather, there are several concepts that appear to be consistent. Intranasal vaccination appears to reduce the level of virus shedding after challenge better than IM vaccination. Likewise with intranasal vaccination, interference by passively acquired antibodies, which can occur with IM vaccination, apparently is avoided. Several companies have extended their label claims to include intranasal vaccination as well as IM administration. However, not all vaccines can be used as intranasal vaccines. It is doubtful whether killed preparations would induce sufficient immunity when administered intranasally. Likewise, some MLV’s may not be good intranasal vaccines. We have conducted studies with a glycoprotein gII deletion mutant vaccine (Omnimark as listed below) and found that it was not as effective when administered intranasally compared to IM administration.

In general, MLV’s induce better immunity than killed virus preparations. Although this is a general trend the literature, we conducted an experiment where we vaccinated pigs twice with a killed vaccine containing Omnimark and a water—in—oil emulsion adjuvant. In this experiment, virus shedding after challenge was minimal and much less when compared to other experiments where pigs were vaccinated once with live Omnimark.

Of recent interest, a report from Belgian researchers demonstrated a dramatic reduction in post challenge virus shedding in pigs that were
THACKER, MAES, HAN

vaccinated with an MLV that was administered in combination with an oil–in–water emulsion. This study along with our study indicate that the newer, oil–in–water emulsion adjuvants may be useful additions to MLVs or as a replacement for traditional adjuvants such as aluminum hydroxide in killed virus preparations.

EXPERIMENTAL STUDIES

MATERIALS AND METHODS

Challenge virus— The P2208 strain of PRV was used. The inoculating dose contained $10^6$ CCID$_{50}$ per ml and 0.5 ml was instilled into each nostril.

Vaccines— The vaccines were designated by letter code: A– Syntrovet's PRV–Marker (gX deleted); B– Upjohn's Tolvid (gX deleted); C– Fermenta Animal Health's Omnivac; D– Fermenta Animal Health's Omnimark (gIII deleted); and, E– Norden's PRV–Vac (gI deleted). Nonvaccinated control pigs were designated as group F. All vaccines were modified live virus preparations contained within a 2 ml dose. Vaccines were administered intramuscularly into the ham.

Virus isolation and serum neutralization— The Crandell-Rees feline kidney cell line was used for isolating virus from nasal swabs. Swab media (3 ml) was filtered through a .45 um millipore filter prior to inoculating cell cultures. Virus neutralization tests were done by the standard microtiter method using a swine testes cell line. Sera were tested in triplicate and titer results were reported as the reciprocal geometric mean.

Experimental design— Two groups of 5 litters of 3–day–old pigs and their dams were obtained from a PRV free herd. The pigs were vaccinated at 7 days of age and then bled and weighed every two weeks for the duration of the experiment. To avoid potential cross contamination of vaccine virus, a litter was vaccinated with only one vaccine type and several pigs were left as nonvaccinated controls to verify if contamination had occurred. The first group of pigs were challenged at 29 days after vaccination (Experiment 1) and the second group was challenged at either 70 (Experiment 2A) or 119 days of age (Experiment 2B). Nasal secretions were collected by swab from the first group after vaccination to assess if vaccine virus shedding had occurred. After challenge, nasal secretions were collected for virus isolation starting on Day 0 and continuing through Day 10. For 14 days after challenge, pigs were monitored for clinical signs. A daily clinical score was assigned based on observable signs: nasal discharge=1; depression=2; inappetence=3; nervous disorder=4; and death=5. A total clinical score for each pig was calculated by totaling each daily score. The percent gain for the 14 day post challenge period was calculated for the first experiment.
RESULTS

Serological responses by month after vaccination are listed in Table 1. All vaccinated pigs were seropositive at all bleedings after vaccination. Vaccine virus was not recovered from any of the 5 pigs per vaccine group on days 0 to 10 after vaccination.

Post challenge mortality, weight gain, clinical scores and challenge virus shedding are presented in Table 2. Clinical disease was reduced in vaccinated pigs compared to control pigs in all experiments regardless of vaccine type. As the time interval between vaccination and challenge increased, the level of protection appeared to decrease. Likewise, as the interval between vaccination and challenge increased, the amount of post challenge virus shedding increased.

DISCUSSION

All vaccines were effective in reducing the severity of clinical disease after challenge when compared to nonvaccinated control pigs. The level of protection appeared to diminish as the interval between vaccination and challenge increased. Likewise, the percentage of days during which pigs were shedding virus after challenge increased as the interval between vaccination and challenge increased. In most reported experiments and for vaccine licensure, the interval between vaccination and experimental challenge is generally 4–6 weeks. These data indicate that if the interval between vaccination and challenge is prolonged, post challenge virus shedding is much greater compared to a shorter vaccination/challenge interval. It could be anticipated that in herd eradication schemes that rely on vaccination of young pigs (6–12 weeks of age) to reduce/eliminate virus shedding, if exposure to field virus occurs soon after vaccination, the program will have a better chance of success compared to a situation where infection occurs later such as in the late finishing phase. Also, it appears that older pigs that were vaccinated at a young age may not be well protected if field virus enters the herd from an external source such as during shipping. These findings also indicate the need to determine if repeated vaccination at shorter intervals may help reduce the level of virus shedding after challenge. As discussed above, the critical benefit of vaccination in eradication schemes is the reduction/elimination of virus shedding following exposure to field virus. It is critical in developing herd plans that all aspects that may affect vaccine efficacy are considered including the level of passive immunity at the time of vaccination, the need to use intranasal vaccination versus intramuscular vaccination, the timing of infection with endemic field virus, and as indicated by our data, the decline of protective immunity, especially with respect to reducing virus shedding, as the interval between vaccination and exposure increases.

REFERENCES

Table 1 - Serum antibody titers after vaccination (Exp. 2B)

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* Reported as reciprocal geometric mean

Table 2 - Summary of challenge experiments

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* Days 2-7 post challenge
** Survivors only
HUMAN HEALTH BENEFITS OF MILK

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As part of the Nutrition Research staff at the National Dairy Council, and representing the National Dairy Promotion and Research Board, I have been asked to present some of the latest research news in the area of nutrition that is relative to the Dairy Industry – such as

THE HUMAN HEALTH BENEFITS OF MILK

However, I modified the title to:

DAIRY GOOD NEWS

Because our industry seems to be flooded at times with negative news, I took this opportunity to present some of the "good" news that is happening in the research--world of dairy that most people don't hear about.

But before highlighting some of the exciting studies funded by the dairy industry, I thought it might be beneficial to explain briefly how and why the dairy farmers are involved with funding research projects.

[SLIDE]

Dairy farmers support research efforts by their contribution of 15 cents per 100 lbs of milk, as determined by the Dairy and Tobacco Act of 1983. 5 cents make it to the National Dairy Promotion and Research Board where approximately 11 million dollars is available to fund Dairy Product and Nutrition/Health Research projects. Of this, about 4 to 5 million is allocated for Nutrition and Health Research projects which are administered by the National Dairy Council. We currently have about 50 Competitive grants, 2 Institutes, 1 Center, and a few Directed Projects.

[SLIDE]

Our mission then, is to coordinate or position nutrition and/or health-related research that will benefit the dairy industry and we do this by...

providing the scientific and technical basis for increasing the sales and utilization of dairy foods.

Now with this very brief introduction, let me begin to highlight some of the recent studies I hope you'll find interesting.

[SLIDE]

Some of you may recall this Spring when there was a flurry of media attention given to the study in Wales, where there appeared headlines like...

"Milk may help prevent heart attacks".

Everyone was calling the Dairy Council to find out more about the great news from this study. These results originated from an annual Progress report

[SLIDE]
HUMAN HEALTH BENEFITS OF MILK

from the Medical Research Committee and not from a peer-reviewed journal; Hence, our communication department couldn't promote this exciting piece of news. However, the data do look quite positive for the dairy as shown here...

[SLIDE]

These results came from a questionnaire asking 2400 men, who are part of a larger, 10-year epidemiological study, about their consumption of liquid milk. One can see quite readily that the incidence of heart disease decreased strongly as daily milk consumption increased.

As exciting and clear-cut as this information appears, it does not mean that milk drinking will lower heart attacks — as implied by the headlines, because this study was an epidemiology study and not designed to look at a “cause and effect”. So other factors may be responsible for the decreased heart attacks. For example, milk drinkers may be more affluent, exercise more, lead a healthier lifestyle or do something that makes them different from the non-milk drinkers who had more heart attacks. But, I think you can certainly say that milk didn't do any harm!

Eventually, we may be able to say that people who are not genetically at risk for heart disease do not need to modify their diets to be low-fat, low cholesterol or even replace whole milk with skim. Individualizing diets — making diet changes only for those who need it—is the type of approach we take to educate the public and health professionals and fund for research. For example,

[SLIDE]

at the "Dairy Research Institute for Genetics and Nutrition" in Berkeley, (one of our institutes funded by the NDB) much of the research focuses on the individual response to high- and low- fat diets. Results are just beginning to come out suggesting that low-dairy fat diets may actually increase the risk of CHD in some individuals!

All subjects on a high-dairy fat diet had HIGHER HDL levels—the good cholesterol carrier—compared to their HDL levels when they were on a low dairy fat diet.

The HDL levels may indicate individuals having a lower risk of having CHD, especially in light of recent findings which were published in the Sept. 19, 1991 issue of Nature that showed transgenic mice — those who had the human gene to make HDL — had increased HDL levels and less CHD risk as indicated by less fat build-up in their arteries when on a high-fat diet (compared to mice lacking the human HDL gene). This is the first direct evidence supporting HDL’s role as a protective factor in heart disease.

When these data are confirmed, we can say that low-fat diets are not beneficial for all people!

Other great news for the dairy industry is in the area of Calcium...

[SLIDE]

We are getting more and more evidence that calcium may decrease
the risk for certain diseases. For example, we know that calcium is essential all through life to build strong, dense bones so that the risk of osteoporosis can be decreased.

But, new evidence is suggesting that calcium may even be involved in reducing colon cancer risk and may also be the best treatment around for preventing PREMENSTRUAL SYNDROME (PMS)!

The dairy industry believes the colon cancer issue is worth pursuing and has begun the start-up of a new institute to study this association in great detail.

Not only is the calcium in milk viewed as a potential colon cancer inhibitor, but other compounds present in milk and cheese are being investigated as potential anticarcinogens. Two such compounds that are in the fat-soluble fraction of milk are [SLIDE]

Sphingolipids and CLA—conjugated linoleic acid

The data on sphingolipids is sparse, but their ability to inhibit colon cancer looks very promising from the data we have seen so far—we have 2 studies currently underway.

You may have heard about this other compound—CLA—because it got lots of press as being an ingredient in grilled hamburgers! The data on this anticarcinogen looks fantastic, but what's also great is that this compound occurs naturally in milk and cheese—especially processed cheese! It appears that consuming dairy products would be one of the best sources of this natural anticarcinogen and so the dairy industry has begun a directed program to further study this newly recognized compound.

[SLIDE]

One study currently funded by the dairy industry is looking at what phase CLA inhibits cancer and the dose—how low of a dose can you go?

Also, we want to find out more about what type of cancer is inhibited by CLA because only mammary cancer has been studied to date; and, what is the CLA content in dairy foods? What products are high in CLA? How does processing affect its concentration?

What effects do region and season have on CLA content in milk, and how do feeding practices influence its levels?

This type of research is not only necessary for promotional purposes—like giving consumers a new reason to consume milk; but it also helps alleviate the negative perception that the fat in dairy products is bad, especially milk.

What is the fat content in milk? Let's look.

[SLIDE]

This slide shows the fat composition of an 8-oz glass of milk.

As you can see, there are 8 grams of fat, 5 of which are saturated. However, not all saturated fats are the same or raise blood cholesterol.

For example, milkfat is unique in that 60% of its saturated fats are
HUMAN HEALTH BENEFITS OF MILK

either short chain or monounsaturated—and these kinds of fatty acids do not raise cholesterol and may even lower it!

The remaining fatty acids, lauric, myristic and palmitic, have been shown to raise cholesterol levels, but only when incorporated as oils in a synthetic diet. Even a recent article in Am. J. of Clin. Nutr. by K.C. Hayes suggested that palmitic (16:0) may not be a major contributor to plasma cholesterol.

If so, this would support many of the studies that have shown that milk—when incorporated into diets as a food—actually lowers plasma cholesterol.

[SLIDE]

Many studies have suggested that various milk products have hypcholesterolemic effects— that is, they have the ability to lower plasma cholesterol. Some of these studies include...

whole milk, fermented milk, skim milk, yogurt, buttermilk

However, even those who have confirmed the presence of a cholesterol lowering effect (and this is still debatable), are not certain what factors are responsible.

[SLIDE]

Several milk constituents have been suggested, such as

orotic acid
uric acid
calcium
lactose
the milk fat globule membrane
AND even "some bacterial fermentation product"

But, the general consensus seems to be that skim milk is the most consistent in lowering cholesterol, whole milk doesn't seem to really effect blood lipids; yet, pure butterfat does!

The reason why it's so hard to get a consensus is that many of the studies were not well-designed. Until we have good studies feeding milk products to humans over a long time, we just won't have an answer to the question of milk effects on plasma lipids.

[LAST SLIDE]

To conclude, I would like to leave you with the message that research funded by the dairy industry supports and identifies new ways of saying that milk is an essential food for a healthy diet and that

"MILK HAS SOMETHING FOR EVERY BODY!

Thank you very much.
Bovine Tuberculosis in Cervidae; Human Health Concerns

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Prepared for the Committee on Public Health and Environmental Quality
United States Animal Health Association
San Diego, CA, 1991

ABSTRACT

The United States Department of Agriculture, Animal and Plant Inspection Service is responding to an epizootic of bovine tuberculosis (Mycobacterium bovis) in its developing captive cervid industry which was revealed in January 1991. In late 1990, Agriculture Canada reported that M. bovis had been isolated from an elk that had originated in the United States. Follow-up investigation established an epidemiologic linkage between the Canadian herd and an infected elk herd in the United States. By September 30, 1991, bovine tuberculosis had been confirmed in 10 captive deer and elk herds located in 8 States. Unexpectedly high rates of tuberculin test positive humans associated with M. bovis confirmed elk herds reported in Canada suggested that tuberculous Cervidae may present a greater occupational hazard than traditional livestock species. Aerosol exposure is suggested as the primary mode of M. bovis transmission to humans on the farm and in the...
work place. Extensive tuberculosis pathology characterized in cervids by suppuration and productive liquefactive necrosis of the lymph nodes and other tissues may more readily result in aerosolization of *M. bovis* when subjected to power tools and other routine procedures during slaughtering, rendering, hide processing, and necropsy. Though not entirely conclusive, the information presented should warrant further epidemiological investigations, and a scientific risk assessment to determine the human health risks associated with tuberculous Cervidae, and to identify occupational hazards and the precautions necessary to protect workers at risk, and the public.

**INTRODUCTION**

Historically the principal mode of transmission of bovine tuberculosis from animals to man has been through the consumption of untreated milk from infected cows, or less frequently by direct association with infected animals. Infection by the latter route is usually by the inhalation of contaminated aerosol from live animals with pulmonary disease. Though disease transmission is possible by the consumption of meat from infected animals, by handling or inspecting infected animals in slaughtering establishments, or by necropsy procedures on farms, these modes of transmission of *Mycobacterium bovis* are not efficient. There are reports, however, suggesting aerosol transmission of *M. bovis* as an occupational hazard in the slaughtering industry.

Quinn in 1963 emphasized the public health considerations associated with bovine tuberculosis in Cervidae as it related to a Michigan deer park where there was close contact between infected animals and the public. Stumpff, in 1982, reported that the owner, the herd veterinarian, and a rendering plant employee associated with an *M. bovis* infected elk herd in South Dakota converted from negative to tuberculin skin test-positive status. Fanning, in 1991, reported high rates of positive skin tests in persons linked by occupation to tuberculous elk. This report deals with *M. bovis* infection in the captive cervid industry and possible human health concerns associated with cervid production, exhibition, slaughter, rendering hide processing and necropsy.

**BACKGROUND**

Bovine tuberculosis in captive deer and elk herds in the United States became an important issue during fiscal year 1991. The investigation of cervid herds for tuberculosis followed reports from Canada during late 1990 that elk from the United States may have introduced *M. bovis* infection to one or more of their captive elk herds. One possible source herd was found infected with bovine tuberculosis in the opening months of the United States investigation. By the end of fiscal year 1991, bovine tuberculosis had
been confirmed in 10 cervid herds located as follows; 3 herds in Montana, and 1 herd each in Idaho, Colorado, Oklahoma, Nebraska, Texas, Wisconsin and New York. Confirmation of *M. bovis* is also pending in 1 herd each in Montana and Washington (Figure 1). The known movements of tuberculosis exposed animals from these infected premises are extensive. In fact, the fledgling cervid industry in the United States is characterized by extensive movements of breeding animals to support its present rapid growth stage[6].

The full significance of bovine tuberculosis in captive Cervidae has not been fully assessed with respect to the epidemiology of this disease in cattle and bison, the official program species. The first known United States cattle herd infected by association with tuberculous elk was detected in Nebraska in 1991, and confirmation of disease is pending in an adjacent herd[6]. A tuberculosis outbreak in bison detected in 1984 resulted from the association of bison with tuberculous elk. This outbreak eventually involved a total of 24 infected bison herds located in 10 States including 7 with Accredited-Free status[7,8,9].

The tuberculosis situation in cervids in Canada is similar to that in the United States except that Canada is in a much better position for managing this problem. Agriculture Canada implemented a captive ungulate program in 1989 which had been in the process of development for several years[10]. By July 31, 1991, *M. bovis* had been confirmed in 15 captive ungulate herds in Canada including 8 elk herds in Alberta where additional herds are presently being investigated for having received tuberculosis exposed elk[11].

Investigations for possible spread of tuberculosis are hampered in the United States by the absence of Federal authority to govern interstate movement of tuberculosis exposed Cervidae. The absence of Federal indemnities for deer and elk destroyed because of tuberculosis and the absence of a Federal program for controlling tuberculosis in Cervidae further deter progress. Investigations are conducted entirely under State authorities which vary widely regarding quarantine, identification, testing, slaughter of reactors and indemnity allowances. As a result, investigations are ineffective in some States ending at the stage where test–positive animals must be destroyed to confirm the existence of *M. bovis*. Known movements from such herds cannot be investigated because the disease was not confirmed.

**PATHOLOGY**

Tuberculosis lesions in Cervidae have a distribution pattern similar to that found in domestic cattle. The most commonly affected organs are the lungs, pulmonary lymph nodes, tonsils, and lymph nodes of the head. Less frequently, lesions may be present in the mesenteric lymph nodes as well as on the serosal surfaces of the thoracic and abdominal cavities. The liver,
BOVINE TUBERCULOSIS IN CERVIDAE

spleen, and kidneys are rarely affected.\(^{18}\)

Partially calcified, caseous granulomas typical of tuberculosis in cattle are sometimes observed in deer and elk. However, tuberculosis lesions in cervidae often resemble large, encapsulated abscesses that are filled with watery or viscous, grayish-white exudate. Some of these pyogranulomas, particularly lesions located on the serosal surfaces of the thoracic and abdominal cavities, may be very large (up to 12 cm in diameter).\(^{18}\)

Microscopically, tubercles collected from cervidae usually contain the cellular components characteristic of pyogranulomas. Most lesions have a connective tissue capsule surrounding a peripheral mantle of lymphocytes admixed with epithelioid macrophage and modest numbers of Langhans giant cells. Central zones are characterized by large areas of caseous or liquefactive necrosis containing substantial populations of neutrophils and widely scattered foci of mineralized debris. Modest numbers of acid-fast bacilli (1–3 organisms per 25 250X fields) are typically present in the necrotic centers and within the layer of macrophage. However, some individual lesions may contain numerous organisms (>25 organisms per 25 250X fields).\(^{18}\)

BACTERIOLOGY

Animal tissues suspected of having *M. bovis* were submitted in saturated sodium borate for processing. The tissues were trimmed of excess fat and the surface decontaminated in dilute sodium hypochlorite. The tissues were macerated and decontaminated in 2 percent sodium hydroxide. Tissue sediment from this procedure was inoculated onto the following media:\(^{16}\):

1. Lowenstein–Jensen egg medium with glycerine
2. Stonebrink egg medium
3. Middlebrook 7H10 agar medium
4. Herrold egg yolk medium
   a. With malachite green and glycerine, without mycobactin
   b. With malachite green, glycerine, mycobactin
   c. Without malachite green, glycerine, mycobactin
   d. Without malachite green, glycerine, mycobactin, with 0.5 percent lysed sheep blood and 10 percent bovine serum.
5. XP–2
6. Middlebrook 7H12 (BACTEC–Becton Dickinson)

The media were incubated at 37 C for 8 weeks. They were examined weekly for mycobacterial colonies or a digital reading >100 on the BACTEC 460 reader. The average isolation time was 29 days on solid media.
and 18 days for BACTEC. The average identification time is 14 days with conventional biochemicals and 7 days for BACTEC\(^{(10)}\).

Mycobacteria were identified on the basis of colony morphology, cellular morphology, antimicrobial sensitivity patterns, and three biochemical tests. The GEN–PROBE RAPID DIAGNOSTIC SYSTEM to differentiate the *Mycobacterium tuberculosis* complex from the *Mycobacterium avium* complex was also used. Finally, the test data was also computer analyzed to demonstrate the statistical probability of the identification\(^{(10)}\).

In FY 91, 67 deer and 62 elk tissues were submitted to National Veterinary Services Laboratories for examination for mycobacteria. *M. bovis* was isolated from 10 deer and 22 elk. *M. avium* was isolated from 2 elk.

**HUMAN HEALTH CONCERNS**

In the spring of 1991, reports reached the United States from Canada of possible human health concerns associated with the handling of tuberculous cervidae on ranches, on necropsy, at rendering, and at hide processing. Preliminary findings of an investigation conducted by Tuberculosis Services, Alberta Health, of persons involved at different levels of the industry were presented at the International Conference on Bovine Tuberculosis in Cervidae, held in Denver, Colorado, in July 1991\(^{(12)}\). Because of their importance, these findings are reported here in some detail.

*M. bovis* was isolated from the sputum sample of the Canadian veterinarian who had treated a chronically sick elk. The animal was later euthanized and determined to be infected with *M. bovis*. A sputum sample was submitted by this veterinarian after he and his assistant were found to be positive to a PPD tuberculin skin test. Investigation of 446 other human contacts who were exposed to elk herds being investigated resulted in 394 being skin tested. The overall reactor rate was 21 percent (81 positive of 394 tested) of which 48 (12 percent) were found skin test positive for the first time. Twenty of these subjects had a known history of BCG vaccination. Persons found positive for the first time either had no previous history of a tuberculin skin test or were unaware of a prior positive skin test making it impossible to determine their true status prior to association with affected elk. This is an important distinction from known skin test conversion from negative to positive status which should be considered throughout the context of this report. The other 33 reactors had a previous history of positive skin tests. Of these, 11 had a known history of BCG vaccination\(^{(8)}\).

Analysis by type of exposure showed that 50 of 182 persons (27 percent) working with culture–positive elk herds were skin test–positive; 17 (9 percent) had a previous positive skin test, and 33 (18 percent) were positive for the first time. Analysis by occupation showed that 8 of 30 veterinarians and Federal inspectors (27 percent) dealing with culture–positive and reactor herds were skin test–positive. Only 1 of 20

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veterinarians and Federal inspectors (5 percent) working with nonreactor herds was test-positive. Tanners had the highest reactor rate with 23 of 36 persons (64 percent) testing positive, 15 (42 percent) for the first time. It was noted that most tanners were immigrants from countries with higher rates of tuberculosis where they may have been infected prior to their movement to Canada. The reactor rate was 20 percent (14 of 72 persons tested) for rendering establishment workers. It should be noted that the rendering plant had been used as a postmortem site for two large infected elk herds, which may have contributed significantly to exposure potential to \textit{M. bovis}. Twenty-nine percent of laboratory personnel (10 of 35 tested) were tuberculin test-positive\(^6\).

Six persons converted from skin test-negative at the onset of the investigation to skin test-positive subsequently. Two were laboratory workers, bringing to 34 percent the rate of test-positive laboratory personnel. The four additional conversions occurred in persons having significant contact with animals from culture-positive herds. Three of these worked at a rendering establishment (including one inspector), and one was a field inspector working with a severely infected herd\(^6\).\(^{11}\)

DISCUSSION

Quinn, in 1963, perceived the potential public health significance of bovine tuberculosis in deer in a commercial deer park. This was a popular tourist attraction in which the public wandered and fed the deer. Such animal paradises often have petting zoos where children and adults alike have close contacts that may include nuzzling the more gentle members of the species. Gross pathology described in these deer closely paralleled that reported here including lung cavitation, liquefactive obliteration of affected lymph nodes filled with a grayish–white flocculent exudate and tuberculous pneumonia with massive saturation of lung tissue with caseous material\(^9\).

Sawa, in 1974, remarked upon the potential public health hazard in tuberculosis infected feral Axis deer on the Hawaiian Island of Molokai\(^{14}\). In Stumpff's 1982 report of an outbreak of bovine tuberculosis involving three infected captive elk herds in South Dakota it was noted that an owner, a herd veterinarian, and a rendering plant employee converted to tuberculin skin test–positive status. The pathology observed in tuberculous elk was not reported in detail, but huge mesenteric lymph node abscesses were noted. Eight of nine animals necropsied had granulomatous lesions in the lungs. Many animals in these herds had died of a clinical disease indistinguishable

\(^{11}\)As of November 15, 1991, three additional skin test converters have been detected in a total of 467 contact persons tested, bringing to 9 the total of true converters from known negative to tuberculin skin test–positive status. These include 1 necropsy laboratory technician and 2 rendering establishment employees.
from tuberculosis. Very little skin testing of humans associated with this outbreak was done\(^4\).

Important factors have been noted which may make the handling of tuberculous members of the family Cervidae more likely sources of human infection than conventional domestic livestock species. These are the extensive pulmonary disease which characterizes tuberculosis in cervids, the productive liquefactive necrosis of lymph nodes, pleura, subcutis and other tissue\(^{13}\) and the subjectivity of these lesions to extensive aerosolization of \textit{M. bovis} during routine procedures practiced in the work place. Large abscesses have been reported especially of the mesenteric lymph nodes where up to 3 liters of pus has been found in one abscess\(^{16}\). If the disease spreads from the lungs, abscesses may develop on the diaphragm, on the pericardium and on the adjacent pleura even penetrating the thoracic wall to appear as subcutaneous swellings or fistulas\(^{4,14,16}\). Aerosol exposure may be pronounced in handling live elk and deer with pulmonary disease. High risk activities would include procedures requiring direct handling of infected animals, including close contact by visitors in deer parks and petting zoos.

Although routine skin testing of abattoir workers is not done in most countries, the long history of casual handling of tuberculous cattle at slaughter indicates the relatively low risk of transmitting bovine tuberculosis to man by manual contact or ingestion of bacilli under these circumstances. Aerosol contamination, on the other hand, has been suggested as the vehicle for transmission of \textit{M. bovis} in the abattoir\(^{13}\). Aerosols resulting from slaughtering, rendering, and necropsy of cervidae may be more infectious than those from other species. The pathology described suggests that the family Cervidae may be less resistant to \textit{M. bovis} infection than either cattle or bison. The relative absence of calcification in cervids compared to bovines, the characteristic pulmonary cavitation, the bronchopneumonia with extensive suppuration, liquefaction of lymph node parenchyma, large thin walled abscesses, all suggest little suppression of mycobacterial replication in cervidae with possibly higher bacterial counts per unit of affected tissue. The use of power tools such as circular or reciprocating saws for carcass splitting, especially of the brisket could produce a high risk aerosol of \textit{M. bovis} in animals with extensive tuberculosis of parietal pleura or peritoneum. The brisket area is anatomically significant because infectious materials tend to gravitate to the ventral aspect of the thoracic cavity. The ventral wall near the thoracic inlet can be covered by firmly attached fibrinous exudate in which extensive tubercles may be embedded.

The skinning of carcasses would result in infectious aerosols from environmental contamination if skin abscesses of \textit{M. bovis} etiology are present as common in cervidae\(^{4,14,16}\). Splashing from handling viscera, head, and carcass parts, from sending offal by chute to a collecting bin, and by hosing the work area and floor would contribute to aerosolization of \textit{M.}
BOVINE TUBERCULOSIS IN CERVIDAE

M. bovis in slaughtering and rendering plants. Recently, a volume of viscous exudate estimated at ½ liter was expelled upon opening the thoracic cavity of an elk found with tuberculosis at a slaughtering establishment\(^\text{17}\). The skinning of carcasses at rendering by inserting air under high pressure subcutaneously through a small slit in the hide was observed to facilitate the skinning procedure but would contribute to possible infectious aerosol from subcutaneous abscesses that might be present.

The diagnostic laboratory appeared to be a high-risk area for persons doing necropsies of tuberculous cervids. All four pathologists conducting necropsies of affected elk in one laboratory were skin test positive, two of which were true converters. High pressure hosing of the necropsy area was believed to cause aerosolization of the bacillus.

Agriculture Canada is responding to this potential occupational health issue by developing guidelines for the safe handling of tuberculosis affected or exposed cervidae at all levels of the captive cervid industry including animal production and husbandry, necropsy, slaughtering and rendering. Procedures considered in developing guidelines include: (1) minimizing aerosol by avoiding power sawing, reducing high-pressure hosing, and eliminating chuting where possible, (2) area disinfection prior to final low-pressure hosing, (3) protective clothing and devices such as face masks of appropriate filter size, (4) improved ventilation (5) precautions during necropsy, and safe disposal of carcasses, (6) education and training of workers in disease prevention, and (7) notification of affected agencies and personnel for skin testing with appropriate followup.

CONCLUSIONS

The higher than expected rate of tuberculin positive persons working with M. bovis infected elk herds, (including six true converters from negative to positive skin test status) suggests that the human exposure potential may be greater from tuberculous Cervidae than traditional livestock species. The liquefactive and suppurative nature of tuberculosis lesions in Cervidae may render infective particles more subject to aerosolization under conditions found in industry, and this is proposed as the most likely mechanism for bacillar transmission to humans. Further epidemiological investigation and scientific risk assessment are needed to determine the human health risks associated with tuberculous cervids, and to identify occupational hazards and precautions necessary to protect workers at risk and the public.

REFERENCES
10. Agriculture Canada, Disease Control, Captive Wild Ungulates, September 1989.
Bovine Tuberculosis in Cervidae

FY 1991

- Infected herds 10
- Pending 2
REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND ENVIRONMENTAL QUALITY

Chairman: Dr. Stanley L. Diesch, St. Paul, MN
Vice Chairman: Dr. D.F. Schwindaman, Littleton, CO

F.J. Alderink, MD; R.D. Anderson, NV; A.W. Bailey, OK; G.W. Beran, IA; T.C. Bunting, IL; C.R. Dorn, OH; S.L. Hendricks, MN; W.T. Hubbert, MD; W.E. Jennings, TX; J.C. Leightly, MD; A. MacKenzie, CAN; F.V McCasland, TX; E.L. Menning, Washington, D.C.; W.R. Miller, AL; T.G. Murnane, TX; J.C. New, TN; M.E. Potter, GA; J.C. Prucha, MD; M.M. Pullen, MN; S.K. Scott, IA; L.D. Shipman, PA; T.P. Siburt, VA; R.H. Singer, KY; C.D. Stumpff, KS; L.P. Thomas, WV; J. Webb, DC; S. Williams, MD; L.D. Woodson, KS.

The Committee on Public Health and Environmental Quality was called to order with 8 members and 23 guests in attendance.

Chairman Stanley L. Diesch made opening remarks and introduced the first speaker, Dr. Conrad Bögel, World Health Organization, Geneva, Switzerland, who spoke on the interrelationship of the health triad in the management of zoonoses. Dr. Bögel stated that the health triad is constituted by the direct relational effects of people, animals, and the environment. Such a model is the most effective method of merging management and epidemiologic principles in dealing with zoonoses. Dr. Bögel contends that traditional zoonoses management programs do not include environmental considerations as an integral part of the program. However, in addressing population veterinary medicine, we must involve all three elements of the health triad and get away from the regular principles of epidemiology which deal only with people and animals. Dr. Bögel feels that the concept of the health triad should be the model for a tool in managing zoonotic problems.

The Committee members were pleased to have Dr. Susan Rockway of the National Dairy Council provide an opportunity for discussion of her presentation on the Human Health Benefits of Milk. Dr. Rockway's paper will be published in its entirety elsewhere in these proceedings. The stimulating discussion included the National Dairy Council's strategy for getting the results of such important research to the public. Dr. Rockway stated that research findings undergo a peer review similar to the National Institutes of Health review system before publication in appropriate scientific journals. It was suggested that practicing physicians should be targeted so the seemingly routine advice to patients that consuming animal fats is bad for the heart should be modified to meet individual patient's medical history and needs. Dr. Rockway discussed the National Dairy Council's method of making research grants. The Council's procedure parallels the National Institutes of Health by requiring detailed written proposals with an internal
review before awarding the grant to an investigator. The Council encourages the submission of research proposals to compete for the grants.

Dr. James Steele, University of Texas School of Public Health, led a participatory discussion on his general session presentation of Food Irradiation Hygiene. Dr. Steele's paper is also published elsewhere in these proceedings. Dr. Steele's paper presents the value and advantages of using irradiation as a method for the reduction or elimination of living food-borne bacteria and parasites that cause disease. Irradiation also maintains the freshness of food by inhibiting enzyme activity that breaks down cellular integrity. The radiation used is called "ionizing radiation" because it produces electrical particles. Only radionuclides that produce gamma rays (e.g., cobalt-60) and machines that produce x-rays and electrons at energy levels insufficient to induce radioactivity are the radiation sources that are used for food treatment. One of the points brought out in the stimulating and positive discussion was the need to apply a different descriptive term to the food irradiation process to alleviate the public fear of being exposed to food contaminated by radioactive particles. Public fears are generated by media coverage of nuclear accidents such as Chernobyl in the Soviet Union and Three Mile Island in Pennsylvania. It is the responsibility of all public health professionals including private and public veterinarians to educate the food chain consumers.

Dr. Walter Mackey, Assistant State Veterinarian, Minnesota, made an interesting presentation on the negative impact that feral swine have on the environment. Dr. Mackey's literature research shows that wild hogs cause much destruction such as rooting up soil which destroys gardens and crops; killing young deer, game, lambs, and chickens; making mud wallows out of trout streams; and many other behaviors that destroy the environment. However, Dr. Mackey's primary concern is the transmission of several diseases, especially pseudorabies, from feral swine to domestic swine. He also presented the results of a mail survey by respondents of wildlife or animal health officials in all 50 States. In addition to providing estimated population numbers and other information, a majority of the respondents considered feral swine a detrimental species to their States. Additional information exists in other Committee Reports.

Dr. Diesch, Minnesota, presented background and information on the International Society on Animal Hygiene. This Society, developed in Europe, has been primarily European since 1973. Its definition of Animal Hygiene is, "The science regarding the influence on animal health and disease by factors in the animal environment and measures taken with such factors in order to promote health and prevent disease. The science thus comprises research regarding the influence on animal health by factors such as buildings, pasture, waste and feed handling methods, climate, noise, light, dust, straw, etc., in combination with microorganisms or not. It comprises research regarding the influence on the environment by animal husbandry."
Finally, it comprises preventive measures against man–made diseases and thus is one of the most important sectors of preventive veterinary medicine."

At the 7th International Congress in Leipzig, Germany, Dr. Diesch, Minnesota, USA, was elected President of the Society. The 8th International Congress on Animal Hygiene will be presented in the USA, St. Paul, Minnesota, September 12–17, 1994. It will be sponsored by the University of Minnesota and co-sponsored by Washington State University. The theme of the Congress and its 30 member countries will be Environmental and Management Systems for Total Animal Health Care.

After further discussion and deliberation, the Committee is made the following recommendation to the Executive Committee of the USAHA. It states:

The Public Health and Environmental Quality Committee hereby recommends that the United States Animal Health Association commend and support use of food irradiation. This technique is a safe, economic, and hygienic means of food protection and preservation. The widespread use of food irradiation will prevent many food–borne illnesses and deaths and extend the shelf life of foods. The USAHA should assist in improving levels of understanding for health protection of all levels of customers in the food chain. The USAHA should support application of scientific research findings in food irradiation and efforts aimed at education of the public.

Dr. Diesch announced that he will appoint two subcommittees to enhance the Committee's activities. The first subcommittee will be charged with the responsibility to identify current and emerging public health and environmental quality issues and concerns which could be addressed by the Committee. The second Committee will formalize a continuing interest in addressing the problem of cysticercosis in humans Taenia solium in swine and Taenia saginata in cattle.

At the request of USAHA President Pat Smith, the members developed a Committee purpose and objectives document. The meeting was then adjourned.
SALMONELLA SEROTYPES FROM ANIMALS AND RELATED SOURCES REPORTED DURING JULY 1990 – JUNE 1991

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SUMMARY

Serotyping results for 32,813 salmonella cultures from animal disease cases and epidemiologically related sources are reported for July 1, 1990, through June 30, 1991. Of these, 30,270 (92%) cultures were serotyped at the National Veterinary Services Laboratories. The most frequently identified serotypes were Salmonella enteritidis, S. typhimurium, S. heidelberg, S. hadar, and S. cholerasuis var. kunzendorf.

INTRODUCTION

Data for this report were accumulated by the National Veterinary Services Laboratories (NVSL). The data, with the exception of serotyping results, were provided by the many laboratories who requested serotyping services. The data were screened for obvious errors, but it was not possible to verify each entry. Accuracy of the data reflects the commitment of referring laboratories to a quality report. This report also contains information submitted to the NVSL by several laboratories that serotype salmonellae. We are grateful to these laboratories for submitting serotyping results to be included in this report. This enabled 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 serotype distribution and frequency 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 to easily compare data from previous years. 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.
SALMONELLA SEROTYPES

DISCUSSION

This report includes serotype information for 32,813 salmonella isolates from animals and related sources from 47 states and the District of Columbia. This represents an increase of 11,936 isolates (57%) over last year's total of 20,877.

Two hundred fifty-six serotypes were identified (Table 6). The ten most common serotypes (Table 12) accounted for 52% of the total isolates identified. One hundred fifty-one serotypes were identified five times or less (Tables 3 and 6).

Salmonella cerro was among the ten most frequently identified serotypes (Table 12) for the first time. Isolations of this serotype increased 154% from 266 in 1990 to 676 this year. The majority of isolates were from research projects involving chickens (71%) from Georgia (65%).

Eighteen percent of the isolates (6,038) were not completely serotyped, but were identified as "NOT SE" (Tables 1, 2, and 3). These isolates were from environmental swabs of chicken flocks designated as "study" flocks by the Salmonella Task Force and from samples of liquid eggs. The isolates were determined to be either Salmonella enteritidis or not Salmonella enteritidis (NOT SE).

There was an increase of over 220% in the number of isolates of Salmonella enteritidis from 1,499 in 1990 to 4,824 in 1991 (Table 12). S. enteritidis is listed as the most common serotype from chickens (Table 8), and 55% of the isolates were from surveillance or research submissions. Although S. enteritidis isolates were received from 38 states (Tables 1 and 2), 72% were from four states: Indiana (40%), Maine (7%), Maryland (6%), and Pennsylvania (19%). The majority of isolates were from chickens (51%) and environmental sources (37%) (Table 3).

Isolates identified as 9,12:NM continued to increase from 108 in last year's report to 161 this year (Table 3). Eighty-four percent of the isolates were from cattle.

Isolates identified as S. dublin decreased from 660 last year to 574 this year (Table 3).

Isolations of S. pullorum decreased from 252 last year to 179 this year (Table 3).

Considering all serotypes, 29% were recovered from chickens, 13% from turkeys, 8% from cattle, 5% from swine, 5% from avian sources, and 26% from environmental sources (Table 3).

REFERENCES

| SEROTYPE | AL | AR | CT | SC | FL | GA | IL | IN | KY | LA | MD | MA | MS | NJ | NY | NC | OH | PA | SC | TN | VA | WI | ME |
|----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 4.12/158  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 4.5/1/158  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 4.5/1/10     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 5/1/10       | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 7             | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 8/1/8         | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 9             | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 22            | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 25/18/38     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 41            | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 56            | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 66            | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 106           | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 157           | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 303           | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |

**FERRIS, MILLER**

**Table 1. Distribution of Salmonella Serotypes by State (1/7/63 through 4/24/64) - Eastern States (A)**

<p>| ORDER | ALABAMA | ARKANSAS | CALIFORNIA | COLORADO | CONNECTICUT | DELAWARE | DISTRICT OF COLUMBIA | FLORIDA | GEORGIA | HAWAII | ILLINOIS | INDIANA | IOWA | KANSAS | KENTUCKY | LOUISIANA | MARYLAND | MASSACHUSETTS | MICHIGAN | MINNESOTA | MISSISSIPPI | MISSOURI | MONTANA | NEBRASKA | NEVADA | NEW HAMPSHIRE | NEW JERSEY | NEW MEXICO | NEW YORK | NORTH CAROLINA | OHIO | OKLAHOMA | OREGON | RHODE ISLAND | SOUTH CAROLINA | SOUTH DAKOTA | TENNESSEE | TEXAS | UTAH | VERMONT | VIRGINIA | WASHINGTON | WEST VIRGINIA | WISCONSIN | WYOMING |
|-------|---------|----------|------------|----------|-------------|----------|----------------------|---------|----------|--------|----------|---------|-------|---------|-----------|-----------|----------|----------------|-----------|-------------|-------------|-----------|----------|-----------|---------|---------------|-------------|----------------|----------|---------------|--------|-------------|--------|---------------|----------|----------------|----------|--------------|---------|-------------|---------|----------------|----------|---------------|----------|---------|--------|---------|---------|----------|----------|----------|</p>
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**FERRIS, MILLER**

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(C) VAR. COPENHAGEN
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TABLE 7.  TURKEY--MOST FREQUENTLY IDENTIFIED SEROTYPES FROM 07/90 THROUGH 06/91

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TABLE 8.  CHICKEN--MOST FREQUENTLY IDENTIFIED SEROTYPES FROM 07/90 THROUGH 06/91

TUES IDENTIFIED BY CASE TYPE

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<tr>
<th>SEROTYPE</th>
<th>CLINICAL</th>
<th>SURV/RESEARCH</th>
<th>ENVIRONMENT</th>
<th>UNKNOWN</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENTERITIDIS</td>
<td>33</td>
<td>1345</td>
<td>357</td>
<td>731</td>
<td>2466</td>
</tr>
<tr>
<td>HEIDELBERG</td>
<td>71</td>
<td>893</td>
<td>383</td>
<td>128</td>
<td>1475</td>
</tr>
<tr>
<td>HADAR</td>
<td>27</td>
<td>590</td>
<td>33</td>
<td>80</td>
<td>730</td>
</tr>
<tr>
<td>CERRO</td>
<td>0</td>
<td>461</td>
<td>11</td>
<td>5</td>
<td>477</td>
</tr>
<tr>
<td>INDIANA</td>
<td>2</td>
<td>343</td>
<td>2</td>
<td>4</td>
<td>351</td>
</tr>
<tr>
<td>KENTUCKY</td>
<td>12</td>
<td>242</td>
<td>46</td>
<td>45</td>
<td>345</td>
</tr>
<tr>
<td>MONTEVIDEO</td>
<td>16</td>
<td>254</td>
<td>40</td>
<td>17</td>
<td>327</td>
</tr>
<tr>
<td>INFANTIS</td>
<td>6</td>
<td>260</td>
<td>23</td>
<td>8</td>
<td>297</td>
</tr>
<tr>
<td>TYPHIMURUM</td>
<td>13</td>
<td>188</td>
<td>29</td>
<td>18</td>
<td>248</td>
</tr>
<tr>
<td>ANATUM</td>
<td>0</td>
<td>220</td>
<td>26</td>
<td>2</td>
<td>248</td>
</tr>
<tr>
<td>ALL OTHERS</td>
<td>157</td>
<td>1733</td>
<td>323</td>
<td>352</td>
<td>2565</td>
</tr>
<tr>
<td>TOTAL</td>
<td>337</td>
<td>6529</td>
<td>1273</td>
<td>1390</td>
<td>9529</td>
</tr>
</tbody>
</table>
### TABLE 9. CATTLE—MOST FREQUENTLY IDENTIFIED SEROTYPES FROM 07/90 THROUGH 06/91

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Clinical</th>
<th>Surv/Research</th>
<th>Environment</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>755</td>
<td>33</td>
<td>7</td>
<td>67</td>
<td>862</td>
</tr>
<tr>
<td>Dublin</td>
<td>452</td>
<td>26</td>
<td>2</td>
<td>52</td>
<td>532</td>
</tr>
<tr>
<td>Typhimurium (Copenhagen)</td>
<td>263</td>
<td>13</td>
<td>4</td>
<td>6</td>
<td>286</td>
</tr>
<tr>
<td>Sal 9,12:Nonmotile</td>
<td>122</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>136</td>
</tr>
<tr>
<td>Cerro</td>
<td>86</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td>Anatum</td>
<td>66</td>
<td>3</td>
<td>0</td>
<td>10</td>
<td>79</td>
</tr>
<tr>
<td>Newport</td>
<td>99</td>
<td>11</td>
<td>0</td>
<td>6</td>
<td>76</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>58</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td>Montevideo</td>
<td>46</td>
<td>9</td>
<td>0</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>Agona</td>
<td>42</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>53</td>
</tr>
<tr>
<td>All Others</td>
<td>459</td>
<td>33</td>
<td>4</td>
<td>49</td>
<td>545</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2408</td>
<td>142</td>
<td>19</td>
<td>214</td>
<td>2783</td>
</tr>
</tbody>
</table>

### TABLE 10. SWINE—MOST FREQUENTLY IDENTIFIED SEROTYPES FROM 07/90 THROUGH 06/91

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Clinical</th>
<th>Surv/Research</th>
<th>Environment</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholerasuis (Kunzendorf)</td>
<td>889</td>
<td>33</td>
<td>0</td>
<td>56</td>
<td>978</td>
</tr>
<tr>
<td>Cholerasuis</td>
<td>7</td>
<td>12</td>
<td>0</td>
<td>248</td>
<td>267</td>
</tr>
<tr>
<td>Derby</td>
<td>109</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>126</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>78</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>85</td>
</tr>
<tr>
<td>Agona</td>
<td>49</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td>Typhimurium (Copenhagen)</td>
<td>34</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>Anatum</td>
<td>25</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>18</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Infantis</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Brandenburg</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>All Others</td>
<td>95</td>
<td>14</td>
<td>0</td>
<td>17</td>
<td>126</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1330</td>
<td>78</td>
<td>3</td>
<td>360</td>
<td>1771</td>
</tr>
</tbody>
</table>
### Table 11. Horse--Most Frequently Identified Serotypes from 07/90 Through 06/91 Times Identified by Case Type

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>CLINICAL</th>
<th>SURV/RESEARCH</th>
<th>ENVIRONMENT</th>
<th>UNKNOWN</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>141</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>150</td>
</tr>
<tr>
<td>Agona</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>88</td>
</tr>
<tr>
<td>Anatum</td>
<td>63</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>Newport</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Javiana</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Typhimurium (Copenhagen)</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Rubislaw</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
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<tr>
<td>Muenchen</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Krefeld</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Infantis</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>All Others</td>
<td>105</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>113</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>479</td>
<td>8</td>
<td>4</td>
<td>22</td>
<td>513</td>
</tr>
</tbody>
</table>

### Table 12. Salmonella Serotypes Identified Most Frequently from July 1, 1990 through June 30, 1991 with Comparison Data for 5 Years (All Sources)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteritidis</td>
<td>4826</td>
<td>(1)</td>
<td>1499</td>
<td>(3)</td>
<td>488</td>
<td>(13)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>3137</td>
<td>(2)</td>
<td>2500</td>
<td>(2)</td>
<td>2926</td>
<td>(1)</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>2507</td>
<td>(3)</td>
<td>3038</td>
<td>(1)</td>
<td>2600</td>
<td>(2)</td>
</tr>
<tr>
<td>Habar</td>
<td>1576</td>
<td>(4)</td>
<td>873</td>
<td>(6)</td>
<td>1031</td>
<td>(4)</td>
</tr>
<tr>
<td>Cholerasuis</td>
<td>1007</td>
<td>(5)</td>
<td>1150</td>
<td>(4)</td>
<td>1050</td>
<td>(3)</td>
</tr>
<tr>
<td>(Kunzenendorf)</td>
<td>1007</td>
<td>(5)</td>
<td>1150</td>
<td>(4)</td>
<td>1050</td>
<td>(3)</td>
</tr>
<tr>
<td>Montevideo</td>
<td>954</td>
<td>(6)</td>
<td>730</td>
<td>(8)</td>
<td>539</td>
<td>(12)</td>
</tr>
<tr>
<td>Reading</td>
<td>802</td>
<td>(7)</td>
<td>870</td>
<td>(7)</td>
<td>614</td>
<td>(9)</td>
</tr>
<tr>
<td>Anatum</td>
<td>790</td>
<td>(8)</td>
<td>547</td>
<td>(11)</td>
<td>679</td>
<td>(6)</td>
</tr>
<tr>
<td>Agona</td>
<td>714</td>
<td>(9)</td>
<td>647</td>
<td>(10)</td>
<td>622</td>
<td>(8)</td>
</tr>
<tr>
<td>Cerro</td>
<td>676</td>
<td>(10)</td>
<td>266</td>
<td>(18)</td>
<td>262</td>
<td>(19)</td>
</tr>
</tbody>
</table>

* Number of times serotype was identified

** Rank beginning with the most common

*** Includes S. typhimurium and S. typhimurium var copenhagen

454
PULLORUM CONTROL IN THE UNITED STATES

Irvin L. Peterson, D.V.M.
Senior Staff Veterinarian—
Poultry Diseases
VS, APHIS, USDA
Hyattsville, Maryland

The greatest single factor preventing the early expansion of the poultry industry was the disease known as "White Bacillary Diarrhea" caused by Salmonella pullorum. This disease, later called pullorum disease, caused very high mortality—often approaching 100 percent. This was especially true with the advent of large forced-air hatcheries. When the causative organism was described by Leo Retiger in 1899 and when a diagnostic test was developed by F. S. Jones in 1913, a method of controlling this scourge was available. Individual poultrymen started to test their birds; and a number of States initiated Statewide pullorum testing programs.

When the national testing program under the National Poultry Improvement Plan (NPIP) became effective in 1935, the reactor-rate was 3 percent and in some States was as high as 10 percent. There were many officials who were very skeptical of the program and predicted its failure. By 1961, all turkey, and by 1967, all chicken hatcheries were classified as "U.S. Pullorum–Typhoid Clean." By the early 1960's, pullorum was almost eliminated from commercial poultry flocks. With this near elimination, justification for testing multiplier breeding flocks was challenged. This led to sample testing and eventual removal of the testing requirement for parent or multiplier breeding flocks under certain conditions.

In 1974, the U.S. Department of Agriculture made available the "U.S. Pullorum–Typhoid Clean State" classification or status for States meeting certain prerequisites. When a State qualified for this classification, sample testing of primary breeding flocks of a certain size was allowed. When no problems arose from extraneous sources of pullorum spreading to untested multiplier or parent flocks, certain primary flocks were allowed to be classified without testing as long as all of their products would be used only for parent or multiplier breeding flocks.

Nearly all of our outbreaks during the last 20 years have originated in the central portion of the United States. These outbreaks have involved small breeding flocks usually associated with small shipments usually by mail–order shipments. Some of these outbreaks have been extensive and involved many shipments into 20 or more States. Luckily, most of these shipments are terminal products that are used to produce eggs or meat and do not move in commercial poultry channels. Occasionally, however, these outbreaks do involve small breeding flocks which contaminate some hatcheries involved in considerable commerce. In two such outbreaks, other hatcheries have become involved in outbreaks which were not detected, due
to other disease programs or a lack of concern, until infected products had been shipped into many States. An outbreak last year was not detected until over 1,000 shipments into 17 States had occurred. To make matters worse, the infection rate was very high in these shipments. In another State, an outbreak occurred in a commercial operation in the mid 1970's and again in an integrated broiler operation in 1983 and 1984. In this latter outbreak, the State and the integrator managed the outbreak effectively and no known spread occurred outside of the integrator's operation; and the broiler performance was reported to be above normal. The sources of these outbreaks could not be determined. For the record, these were not NPIP participating hatcheries.

The latest outbreak, in an integrated broiler/roaster operation in the South Atlantic States, is unique and interesting as the infection involved a grandparent male-line flock and the infection was not detected until their progeny were well into their breeding period. This resulted in the marketing or depopulation of 20 implicated or infected parent flocks.

Because the implicated grandparent male-line flock was not detected until after it had been marketed, another distressed replacement flock was placed in the same houses without cleaning. Pullorum was not detected in this flock on the first 100-percent test at 28 weeks but was detected at a second 100-percent test a couple of months later. All of the offspring or progeny from this flock were marketed as broilers. However, they had been hatched with other female-line chicks for about 9 weeks with possible exposure due to hatchery transmission. All of these parent flocks were considered to be high-risk flocks and were tested accordingly. Evidence indicates that hatchery transmission did occur on two occasions during this period resulting with infection occurring in three parent flocks.

There was evidence of horizontal spread during this outbreak outside of the integrator's operation. The most likely explanation of how this occurred is that cleanup equipment used in cleaning an infected roaster growout house was used to clean out a breeding house of another integrator without proper cleaning and disinfection. This infection was not detected in the breeding flock but was detected in two widely separated progeny broiler/roaster flocks. No known spread occurred from this infection even though other breeding flocks were located on the same premises.

What did we learn from this outbreak?

1. A couple of states were not aware of nonparticipating flocks and hatcheries in their State.
2. In two flocks a 100-percent test at 28 weeks did not detect flocks that were found to be infected 2 months later.
3. A sample test of 500 birds per flock was unable to detect three flocks that were later found to be infected with pullorum disease.
4. States and industry were responsive; and except for one incident, no known horizontal spread occurred.
5. Laboratories and diagnosticians were not familiar with pullorum disease and were not following laboratory procedures for the isolation of *S. pullorum* as required by the NPIP provisions.
6. Indiscriminate use of new males in flocks without a sound biosecurity program resulted in two flock infections.
7. Infection or contamination of breeding flocks by other serotypes of *Salmonella* and medication programs can complicate the detection of pullorum disease.
8. Careful scrutiny of progeny flocks at 2 to 3 weeks of age proved to be a sensitive method of detecting low levels of infection.

How can we prevent similar outbreaks from occurring again?

1. Pullorum is a serious and costly disease. States and industry should not become apathetic toward this disease.
2. All primary meat-type chicken breeding flocks should be tested—The level of testing may be debatable.
3. Male-line meat-type chicken grandparent flocks should be tested at the 100-percent level.
4. Flock supervisors should examine flocks closely for stunted or other birds showing signs of pullorum at 2 to 3 weeks of age and submit them to a laboratory for necropsy.
5. Laboratories and flock managers should closely follow the provisions of the NPIP.
6. In every State, at least one person should be well informed on the poultry breeding industry in that State and with the provisions of the NPIP.
The committee met at 1:30 p.m., Monday, October 28, 1991. Thirty seven members and 22 guests were present.

Eight papers were presented and three subcommittee reports were considered:

1. Overview of the Symposium on Diagnosis and Control of Salmonella. Dr. Brad Smith, Chair, Symposium Planning Committee, reported on the Symposium that was held on Tuesday, October 29, 1991. There were 21 papers and seven posters presented. A separate proceedings will be published and distributed to those registered for the Symposium. Additional copies of the proceedings may be purchased from the USAHA office.


The authors reported that 32,813 salmonella isolates were serotyped from animals and related sources from 47 states and the District of Columbia. This represented an increase of 11,936 isolates (57%) over last year's total of 20,877. A complete report will be published in the proceedings of this meeting.

3. Current status of Salmonella food-borne outbreaks in the U.S.
Dr. Morris E. Potter, CDC–DHHS, Atlanta, Ga.

Dr. Potter reported that the most commonly reported serotype of Salmonella isolated from human clinical specimens in CY 1990 was enteritidis (21.1%) followed by typhimurium (20.8%), heidelberg (9.2%), newport (4.3%), and hadar (4.2%). A total of 40,778 human isolates of Salmonella were reported in 1990.

The most recent data available from the CDC Foodborne Disease Outbreak Surveillance System are for CY 1988. In 1988, 57 foodborne outbreaks of salmonellosis were reported, compared to an average of 53 per year for the preceding 15 years. Food vehicles were identified for 36 (63%) of the outbreaks reported in 1988. As in previous years, foods of animal origin, including beef, turkey, chicken, eggs, and pork were important vehicles for foodborne salmonellosis in 1988.

Information from a third CDC data source, the Salmonella enteritidis (SE) outbreak reporting system, is available for CY 1990. In 1990, 65 outbreaks of SE infections were reported to CDC, down from 77 in 1989. For the first time since reporting began in 1985, more than half (52%) of the outbreaks reported in 1990 were from sites outside New England and the mid-Atlantic states. As in past years, the mean number of cases per reported outbreak (30–40) and the proportion of outbreaks with identified vehicle that were egg related (80+%) remained the same, indicating that surveillance procedures were likely constant over time. A decline in SE outbreaks was observed in health institutions (e.g., hospitals and nursing homes) in 1990; this accounted for the sharp drop (0.097% vs 0.51% for 1985–1989) in the proportion of outbreak–related cases that were fatal. SE outbreak reporting thus far in 1991 is behind the same period last year.

Table 1 reports the 10 most frequently reported Salmonella serotypes from humans and non–human sources reported to CDC and USDA in 1990.
TABLE 1

THE 10 MOST FREQUENTLY REPORTED SALMONELLA SEROTYPES FROM HUMAN SOURCES REPORTED TO THE CDC IN 1990 AND FROM NON-HUMAN SOURCES REPORTED TO THE CDC AND USDA IN 1990

<table>
<thead>
<tr>
<th>HUMAN 1990</th>
<th></th>
<th>NON-HUMAN 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANK</td>
<td>SEROTYPE</td>
<td>NUMBER</td>
</tr>
<tr>
<td>1</td>
<td>ENTERITIDIS</td>
<td>8591</td>
</tr>
<tr>
<td>2</td>
<td>TYPHIMURIUM *</td>
<td>8489</td>
</tr>
<tr>
<td>3</td>
<td>HEIDELBERG</td>
<td>3746</td>
</tr>
<tr>
<td>4</td>
<td>NEWPORT</td>
<td>1749</td>
</tr>
<tr>
<td>5</td>
<td>HADAR</td>
<td>1733</td>
</tr>
<tr>
<td>6</td>
<td>MONTEVIDEO</td>
<td>899</td>
</tr>
<tr>
<td>7</td>
<td>AGONA</td>
<td>873</td>
</tr>
<tr>
<td>8</td>
<td>THOMPSON</td>
<td>732</td>
</tr>
<tr>
<td>9</td>
<td>BRAENDERUP</td>
<td>715</td>
</tr>
<tr>
<td>10</td>
<td>JAVIANA</td>
<td>696</td>
</tr>
</tbody>
</table>

| SUB TOTAL | 28223 | 69.2 | | 14921 | 65.5 |
| TOTAL ISOLATES | 40778 | | | 22774 | |

* TYPHIMURIUM INCLUDES VAR. COPENHAGEN
** CHOLERAESUIS INCLUDES VAR. KUNZENDORF
REPORT OF THE COMMITTEE


Dr. John Mason, USDA-APHIS-VS, Hyattsville, Md.

Dr. Mason reported on the activities of the task force since the initiation of the SE program early in 1990. Detailed reports on these activities will be included in the paper published in the proceedings USDA/AAVLD sponsored Symposium on the Diagnosis and Control of Salmonella held on October 29, 1991. He also reviewed proposed changes in the program and requested comments of these changes. APHIS intends to encourage voluntary SE control efforts within the table egg industry.

5. Current status of the Salmonella control program in Canada. Dr. John Kellar, Associate Director of Disease Control, Agriculture Canada.

Dr. Kellar outlined a very comprehensive Salmonella reduction program for Canada, which would include 14 multidiscipline teams composed of an epidemiologist, bacteriologist, program designer and operational supervisor.

The program identified 17 elements in the production chain which required effective control efforts to achieve the goal of significant reduction in Salmonella contamination of the end product by the year 2000.


Dr. Earl Grass, Chair, reported a summary of opinions of members of the committee:

A. The present format of the Quarterly Salmonella Report (QSR), while not perfect, is probably as good as we can get. Reliable data is not provided from veterinary practitioners and their clients. Many cases are not reported and several states do their own serotyping.

B. While there is value in reporting "outbreaks" of salmonellosis, we need to distinguish between outbreaks and endemic situations. When does an endemic situation become an outbreak, and will information be given freely if it has an adverse economic effect? Few states have the manpower to do an in-depth investigation of salmonella cases unless CDC is involved.

C. A means of gathering information to be dispersed with the QSR was presented to the committee members. This may become more feasible because NVSL is proposing a quarterly newsletter on it's activities. Someone needs to provide accurate data for such a report in the newsletter.

D. The consensus was that the subcommittee should continue, although there was a question whether there is a favorable
cost/benefit ratio may become more important when NVSL imposes a fee for serotyping as implied in the 1992 budget.

7. *S. dublin* infections in cattle.

Dr. Cliff Wray, Central Veterinary Laboratory, Weybridge, England.

Dr. Wray reviewed the incidence and characteristics of *S. dublin* infections in cattle in the U.K. The incidences of *S. dublin* infection were equally divided between calves and adult cattle. Abortions from *S. dublin* are the second most frequent bacterial cause.

Dr. Brad Smith commented on the somewhat different expression of the infection in cattle in California. The major clinical problem in California is calves. Non-motile forms of *S. dublin* are frequently isolated in the United States and the United Kingdom. Wray indicated that virulence of non-motile strains was undiminished.

Dr. R. A. Robinson reported approximately 40 outbreaks per year in Minnesota, primarily in calves and no human cases. Non-motile forms of *S. dublin* were encountered.

8. Report on Salmonella pullorum–typhoid status in U.S.

Dr. I. L. Peterson, USDA–APHIS–NPIP, Hyattsville, Md.

Dr. Peterson reported that, during calendar year 1990, there were 91 outbreaks/isolations of *Salmonella pullorum* and no isolation of *S. gallinarum*. more isolations or diagnoses of pullorum were reported; however, many were involved in the same case or same outbreak situation. The total number of isolations or diagnoses reported was 155 from 22 states. An initial infection in a commercial integrated operation resulted in 52 of these reports. Five states reported 59 outbreaks/isolations. Pullorum was reported in turkeys twice and in a duck once.

In 1991 up to October 1, twelve states reported 92 isolations of pullorum and no fowl typhoid. one state reported 52 isolations or diagnoses of pullorum disease based on epidemiologic information. The commercial broiler/roaster operation was involved in 20 of the reported isolations. Seventy of the reports involved flocks with less than 100 birds. Fifty-five of the reports are believed to have originated from an outbreak involving a mail-order hatchery. Montana became the 40th state to be recognized as a "U.S. Pullorum–Typhoid Clean State" under the National Poultry Improvement Plan. Only eight of the 48 contiguous states remain unclassified. All are located in the western region of the United States.

Dr. Fred Bisplinghoff, National Renderers Association, reviewed the progress of the APPI program and indicated that 190 plants participated in the program in the spring of 1991. The incidence of salmonella was 21% in the samples submitted. This is an improvement over the 1990 results. Information on the plants participating in the program may be obtained from the National Renderers Association. The protein blenders have the most serious problem with 51% of the samples positive.

10. Proposed Goals of the Committee on Salmonella.

Chairman Pomeroy reviewed the proposed goals of the committee on Salmonella. It was moved, seconded and carried that proposed rules be accepted and forwarded to the executive committee. The proposed rules are attached to the report.


Dr. E. T. Mallinson, Chair, reported that a major effort by the subcommittee has resulted in the completion of a document, Salmonella Risk Reduction, Integrated Guidelines for Table Egg Producers, and is ready for publication in the proceedings of the annual meeting of USAHA. The committee approved the document on a voice vote.

The subcommittee held two fact-finding meetings with representatives of post-processing distributors, warehousers and retailers, which included need for educational efforts regarding salmonella control in these industries. The subcommittee recognized the need for developing effective and practical methods for sanitizing poultry transport crates to prevent dissemination of pathogens.

Dr. Mallinson presented preliminary data demonstrating the great difficulty in destroying salmonella on wood surfaces by various germicides and the progressive improvement in results of these treatments on contaminated fiberglass and galvanized steel.

SUBCOMMITTEE ASSIGNMENTS FOR 1991

A. Program Committee–Symposium on Salmonella
   Dr. Bradford P. Smith, Chairman, E. S. Bryant, W. H. Dubbert, G. A. Mitchell, Lonnie King, B. S. Pomeroy, G. H. Snoeyenbos, Max Van Buskirk, Jr., Ex officio, P. L. Smith, Ella R. Blanton

B. Diagnostics, Data Collection and Epidemiology

C. Education and Information
SALMONELLA


SALMONELLA RISK REDUCTION

Integrated Guidelines for Table Egg Producers

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INTRODUCTION

This document represents a response to industry concerns about Salmonella. With growing public awareness concerning egg–associated salmonellosis, and with potential egg production losses, the table egg industry is looking for guidelines. The following is a compilation of some of the most current information available on tackling the problem.

The intent of the authors in preparing this paper is to assist the table egg production industry in the reduction of Salmonella as a matter of good business practice and public health policy. This paper is intended to provide general informational guidelines and not specific legal, fiscal or business advice. The integrated sanitation, biosecurity and hygienic procedures outlined here provide no guarantee or warranty of eliminating Salmonella.

These guidelines are not exhaustive of the subject. While a number of pertinent federal (EPA, FDA, OSHA, USDA), state and local regulations relate to items mentioned herein, the reader should be aware that they are subject to change. Producers are encouraged to work closely with regulatory officials, veterinarians, trade associations, and university faculty in order to
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maintain an awareness of significant developments in the industry. Producers are also advised to consult with appropriate professional specialists regarding specific situations.

Appendix A provides additional background on the marketing, fiscal and legal implications of the salmonella problem.

ACKNOWLEDGMENTS

The numerous valuable contributions of members of the USAHA Committee on Salmonellosis and many other veterinarians, poultry producers and allied industrymen are very gratefully acknowledged.

EXECUTIVE SUMMARY

This section provides a broad overview and a roadmap showing where to find key topics. The reader should carefully consider the details of all sections being researched.

1. Develop a source of chicks and pullets that are on a salmonella risk reduction program. Refer to following sections on Chick and Pullet Replacements, Bacteriological Monitoring and Appendix B.

2. Schedule chick/pullet placements with sufficient time allowances to accommodate longer-than-usual layer house down times. Positive monitoring results at the end of lay, or as may occur after initial house disinfection attempts, can lead to lengthy disinfection or re-disinfection operations.

3. Locate and use live-haul services which ensure that all pullet hauling utilizes biosecure personnel and thoroughly cleaned and disinfected coops and trucks. Transport coops are frequently contaminated with highly resistant salmonellae. Unsanitary live-haul practices and equipment have a long history of spreading laryngotracheitis and avian influenza to new and distant locations. The situation is very likely the same with Salmonella.

4. Two or more weeks before layer house depopulation, monitor for Salmonella. Refer to section on Bacteriological Monitoring and Appendix B (Monitoring Plans and Schedules).

5. Be prepared to start your empty-house rodent killing program at the same time as your birds are being removed. Rats promptly leave empty houses, but they and their salmonellae soon return when you restock your new house. Mice remain and wait. Both are believed to be major amplifiers of S. enteritidis infection on contaminated farms (Henzler and Opitz, 1991).

6. Promptly remove feed and continue rodent baiting program,
ban all pets and institute your beetle-and fly-killing and wild bird control programs. Continue these vector control programs throughout the life of your flock. Refer to section on Vector Control and Appendix C (Rodent Control Details).

7. Dry- and wet-clean, repair and service your cages, equipment, the layer or pullet house, and all adjacent work, break and restrooms. Refer to section on Decontamination of Facilities and Appendix D (Decontamination Step-By-Step).

8. Obtain a third-party visual inspection for thoroughness of vector control, dry- and wet-cleaning and repair operations before proceeding with disinfection.

9. Disinfect all building and equipment surfaces using procedures approved for their safety and efficacy. Refer to section on Decontamination of Facilities and Appendix C.

10. Evaluate the results of your disinfection program using a qualified laboratory. The bacteriological evaluation of salmonellae should be negative before new birds are placed. Refer to section on Bacteriological Monitoring and Appendix B.

11. Consider flock bacteriologic monitoring during chick/pullet grow-out and during lay. The legal and fiscal implications of monitoring or not monitoring for Salmonella, during grow-out or lay, are extremely important. A paper, An Overview of Salmonella Litigation Liability (Pakuris, 1990) and a magazine article, Choosing Between the Breaking Plant and the Slaughter Plant (Bender and Ebel, 1991) provide information on these issues. To locate copies, refer to Appendix H (Educational Resources – Economic/Legal heading).

12. Review your quality control procedures for egg collection and handling. Make repairs and corrections and plan for long-term improvement. Refer to section on Egg Handling and Appendix E (Why Refrigerate).

13. Be especially prudent about eggs marketed to mass feeding establishments (camps, fast food outlets, hospitals, hotels, institutions, nursing homes, etc.). Some producers/distributors have preferred to provide such potentially high-risk establishments only with liquid pasteurized eggs.

14. Review the quality control procedures used in production and storage of your feed supplies. Correct problem areas and make plans for long-term improvement. Refer to section on Feed.

15. Consider nipple drinkers or chlorination of your poultry drinking water. Refer to Appendix D. Part VII–H.

16. Review your biosecurity program and the standards of hygiene expected from your employees. It is essential to establish, maintain and continuously improve barriers that work to keep
SALMONELLA RISK REDUCTION

Salmonella from (a) being introduced onto your farm and, (b) if introduced, from gaining a foothold in your operation and in your products. Refer to section on Biosecurity and Appendix F (Biosecurity/Hygiene Check Lists) and Exhibits A and B (Proposed Georgia SE Program for Layers and Pullets).

17. Keep learning and train and retrain your old and new employees in the elements of disease prevention and salmonella risk reduction. You first must personally set the example. Refer to Appendix H.

18. Hire and promote workers who consistently exhibit a "mind set" for cleanliness and disease prevention.

19. Be alert for and use "quality control management systems" that promote orderly, ongoing, verified implementation of risk reduction practices such as those outlined here.

20. Documentation that employee training has been done and that safe procedures were carried out on a farm will be very valuable if the matter is reviewed by farm owners, health agencies or the courts.

CHICK AND PULLET REPLACEMENTS

Chickens have been found to be especially susceptible to salmonellosis from 1 to 14 days of age. Increased susceptibility also may recur when pullets are relocated to laying houses. Consequently, extra effort to reduce potential salmonella exposure and enhance bird vigor and resistance (optimal nutrition and husbandry) is highly advisable at these two critical ages.

I. Purchase your chicks from hatcheries participating in the "U.S. Sanitation Monitored" program. Obtain your pullets from sources with an acceptable salmonella prevention and control program.

II. Rid your chick and pullet facilities of all rodents, wild birds, insects and pets. Refer to section on Vector Control and Appendix C.

III. Clean, wash and disinfect your chick and pullet houses, cages and other equipment. Refer to section on Decontamination of Facilities and Appendix D.

IV. Monitor for successful pullet house decontamination. Monitor replacement chicks on arrival and pullets before movement to your layer facilities. Refer to section on Bacteriological Monitoring and Appendix B.

V. Attend to feed quality control and proper feed storage. Use of well-formulated, properly pelletized/crumbled feeds has been recommended at times of extra susceptibility to salmonellosis (e.g.,
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days 1 to 14 and during early maturity or transport stress). Refer to section on Feeds.

VI. Know your pullet hauler's reputation. Insist that your pullets are always transported in decontaminated coops and trucks. Undisinfection coops frequently carry live salmonellae.

VII. Adhere to strict, continuing vector control and biosecurity standards throughout chick/pullet grow-out. One diseased pullet can permanently ruin the health status of an entire multiple-age or in-line complex. Refer to sections on Vector Control and Biosecurity and Appendix F (Biosecurity Check Lists).

VIII. Vaccines. Historically, vaccination has been used in conjunction with biosecurity to attempt to reduce the effects of disease exposure when the risks of exposure appear high. Vaccination results vary with different diseases and methods of vaccine preparation. Vertical transmission of Salmonella has been successfully halted with bacterin usage in turkeys. Salmonella vaccination research is underway at the Universities of Maine and Minnesota and other locations. Preliminary indications suggest that bacterins also reduce fecal and egg transmission of Salmonella in chickens.

IX. Competitive Exclusion (CE). Immediate post-hatch establishment of CE intestinal microflora, in combination with a sanitary environment, reportedly may help reduce the risks of salmonella colonization in the gut of typically susceptible young fowl. CE cultures appear to be a way to help speed the maturation of a potentially protective intestinal microflora. Probiotics, such as defined cultures of Lactobacilli, do not appear to be the same as CE cultures. CE research is being conducted at various locations, including Texas A&M and USDA's Russell Research Center in Athens, Georgia. An assay for CE efficacy has been published. Refer to Appendix H under the heading, "Evaluating Products."

VECTOR CONTROL

Vector control goes beyond preparation of depopulated houses prior to cleaning and disinfection. It also is an absolutely essential risk reduction practice for the entire life of your chicks, pullets and layers.

Contracting routine, professional rodent and insect detection/extermination services is suggested. Be sure that personnel practice strict biosecurity procedures for their clothing, equipment and vehicles and that the service provider has a good vector control record with poultry operations. A well illustrated, detailed publication, Integrated Pest Management for Poultry, (Arends and Stingham) is available free of charge. To order, refer to Appendix H. Purina Mills has produced a film
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Illustrating unique techniques for rodent monitoring and control.

I. Rodents

Rodent feces have been found to contain infectious doses of *Salmonella* (Henzler and Opitz, 1991). Consequently, mouse pellets commonly deposited in feed troughs are now believed to amplify salmonella contamination in poultry. Beyond their salmonella amplification role, rodents appear to carry infection to both nearby and distant houses and farms.

Consequently, salmonella risk reduction should include (1) making all facilities rodent-proof, (2) proper selection of baits and bait placement, (3) prompt, secure disposal of any dead birds, broken eggs or unused or spilled feed, and (4) regularly repeated professional rodent inspections, baiting and trapping. Cats are not recommended. They spread fowl cholera and *Salmonella*. A detailed rodent control program is provided in Appendix C.

II. Insects

A. Basic control strategy

The control of flies and beetles, which also may be salmonella vectors, requires use of a variety of practices. This reduces the selection pressure encountered with any single method. For example:

1. **Keep manure well ventilated and dry.**
2. **Prevent water leaks and remove any wet areas.**
3. **If possible, use biological control methods (fly parasites and predators).**
4. **Use different classes of insecticides.** Alternate, for example, between organic phosphates (Malathion), carbamates (Sevin) and pyrethrins.

B. Insecticide Application

1. After the floor is dry following cleaning and disinfection, apply an approved insecticide to the floor, support poles and walls to control beetles and other insect pests.
2. **Synergized pyrethrins** (pyrethrin + piperonyl butoxide) are among the few insecticides that can be utilized in automatic spray systems inside poultry.
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houses. Their advantages are: ability for quick "knockdown" of flying insects; short residual times; and low mammalian toxicity. The rate of application of such insecticides, particularly when applied by an automatic spray system, should be no more frequent than twice a week.

3. Follow all manufacturer-recommended safety precautions when applying insecticides.

III. Wild Birds and Pets

If a facility is impervious to mice, it will probably be inaccessible to wild birds. Use of 3/4" or smaller wire mesh has generally been recommended. Avoid feed spills outside buildings and clean up immediately if they occur. Buildings should be constructed not only to exclude wild birds but to avoid birds perching under eaves or on blinds. Pets should be banned from pullet and layer houses.

DECONTAMINATION OF FACILITIES

To reduce the risks of a flock-to-flock build-up of Salmonella and other infectious agents, a between-flock pullet and layer house decontamination program is necessary.

If any of your facilities tested salmonella-positive, the facility needs to be decontaminated promptly after bird removal to prevent residual contamination from infecting your replacements.

Successful decontamination programs require thorough, systematic implementation, proper equipment and professional training. Consider contracting for this service or obtaining guidance from a provider with a good record against Salmonella.

Decontamination of conventional facilities presents the table egg industry with a serious challenge due to facility size and complexity. Additionally, the common use of wooden construction materials, which are porous, appear to provide bacteria protection from the killing effects of disinfectants. Plastic and fibrous egg handling surfaces also appear to be more difficult to disinfect than non-porous metal surfaces. This problem will diminish the effectiveness of the decontamination procedures outlined in these guidelines.

Formaldehyde has been widely used in the past to help disinfect porous materials. Although particularly effective against salmonellae, its use appears in jeopardy because of human safety concerns, product availability, and regulatory policies. Application of alternative fumigants, heat-enhanced disinfectants, high-pressure sprays or disinfectant foams and use of sealants to reduce wood porosity may need to be further assessed as possible aids.
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in disinfecting porous surfaces.

Facility decontamination needs to include the following basic considerations and precautions. Appendix D provides step-by-step decontamination procedures and the names and properties of various commercial disinfectants.

Basic Considerations

I. Invest in vigorous thorough dry cleaning with scrupulous removal of all caked, layered or loose debris. This simplifies future cleanup operations and reduces the expense of wet cleaning.

II. Disinfectants and fumigants are effective only on clean surfaces. Cleaning and disinfection programs, when not fully effective at first, have been reported to have increased efficacy when they were improved and redone or repeated from flock to flock (Bryant, 1990). Opitz and Henzler, 1999 reported a significantly improved probability for maintaining salmonella-negative replacements when they were placed in facilities that had previously passed both visual inspections and post-disinfection laboratory tests.

III. Bacteria can multiply on damp surfaces; therefore, disinfection should be started soon after washing/rinsing, and preferably within 24 hours.

IV. Drying of the facility immediately after application of disinfectants is advisable. Dryness is an impediment to salmonella multiplication.

V. Carefully follow all disinfectant safety, dilution and application directions provided by the manufacturer. Consult with the suppliers of both your equipment and disinfectants for the best and safest procedures for such items as egg elevators and belts, feeders, waterers and cages.

BACTERIOLOGICAL MONITORING

I. Purpose

A visual inspection may not be enough. Salmonella are invisible. Birds, pets, pests or surfaces that "look O.K." can, nonetheless, be contaminated. Consequently, properly executed bacteriologic monitoring at a qualified laboratory is necessary to complete your quality control program.

II. A Matter of Choice

Like a compass, monitoring lets you know where you are on the sea of risk reduction. Lawyers have offered that knowledge of a problem may be preferable to being blind to it. (Pakuris, 1990). Refer to Appendix B for
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examples of monitoring times and purposes and Appendix H for sources of further guidance under the Economics/Legal heading. Sampling and testing services are available from both private and public laboratories.

III. Sample Collection Sites and Procedures

Sampling often requires on-the-spot judgements. It is impossible to sample everything. Collection technique and care to sample the most vulnerable or suspect items is more important than the number of samples collected. Limited sampling and improper laboratory technique can result in a false negative reading. Choose a laboratory that follows recommended salmonella culture techniques. A laboratory manual for the isolation and identification of avian pathogens, including salmonellae, has been published by the American Association of Avian Pathologists. For copies, refer to Appendix H.

A. Sampling the Building and Its Equipment

1. Surfaces of ceilings, walls, floors, fan housings and blades, cages, waterers, feed troughs, manure scrapers, egg belts, rollers and sponges may be sampled. In pre- and post-disinfection monitoring, a total of 20 samples from the above-mentioned areas is generally considered a minimal sampling volume, provided proper collection procedures are employed.

2. Collection procedures. Use of cotton-tipped swabs is limited to sampling only hard-to-reach locations. For most other areas, three-by-three or 4 x 4 inch multiple-ply lightly-moistened gauze pads are preferred. They accommodate the forceful wiping of large (sometimes 2 x 2 foot) areas of a variety of surfaces. Pad moistness (e.g., sterile buffered peptone water or double-strength skim milk) enhance adherence of particulate matter. Sample collectors should wear sterile disposable gloves. Samples should be promptly refrigerated at 35 to 38 F.

B. Sampling Manure/Litter Surfaces with Drag Swabs

1. Drag swabs, which typically contact the surfaces of many hundreds of freshly-voided droppings, are useful because they have tended to produce results
that reflect the salmonella intestinal carrier or organ infection status of chickens. (Kingston, Mallinson, USDA–SE Task Force).

2. Collection procedures. Using 2 gauze pads (the same type described for environmental samples) connected to a cord of proper length and strength, draw the pads over the freshly-voided droppings accumulating under the full length of each row; or representative areas of floor pen litter. Ordinarily in caged pullet or layer houses, one 2-pad drag swab set is used per row, with all rows being similarly sampled. Usually two or three 2-pad drag swab sets are drawn over the litter surface for flocks maintained on litter. Swabs should be drawn over litter surfaces at both pen ends and sides and the center. Total elapsed litter-exposure time for each 2-pad drag swab set should be at least 5 to 6 minutes.

Note on Manure Pit Hazards: The National Institute for Occupational Safety and Health (NIOSH) has issued warnings of farm worker deaths in manure pits. For additional information, see the NIOSH Alert on manure pits (DHHS–NIOSH–90–103) or call 1–800–35–NIOSH.

C. Sampling of rodents appears to be a prime sampling strategy, with collection of 30 mice per flock for salmonella culture being suggested (Opitz, 1991).

IV. Monitoring Research

Salmonella monitoring of a) culled eggs (bloods and meat spots), and b) blood samples from hens is being evaluated at the Pennsylvania State University and the University of Minnesota, respectively. These techniques may offer additional or alternative monitoring strategies.

EGG HANDLING

Rationales behind current refrigeration recommendations are discussed in Appendix E (Why Refrigerate). USDA and regional extension booklets detailing egg washing and sanitation practices have been published. To obtain copies, refer to Appendix H.
Basic Considerations

I. Protect eggshell strength. Weak shells contribute to salmonella penetration. Proper feed formulation at all ages (e.g., adequate calcium supplementation and vitamin D₃ levels) is highly advisable as is broad, strong vaccinal protection from the shell-weakening effects of infectious bronchitis and Newcastle disease.

II. Gather eggs frequently.

III. Empty egg belts at the end of each day. Eggs held overnight in today's modern cage plants can be exposed to temperatures (80-90 F) that promote bacterial multiplication and increased salmonella risk.

IV. For in-line washing systems, wash the eggs and cool to 45 F or less. Use only potable water with a maximum iron content of 2 parts per million and a minimum wash water temperature of 90 F.

V. For nest run systems, cool eggs immediately to 60 F, until they are washed to avoid thermal cracks during washing. If the temperature difference between the eggs and the wash water is 50 F or more, it may be necessary to pre-warm the eggs before washing. Wash and rinse solutions should be 10-15 F warmer than the eggs. Sanitizers should be used according to manufacturer's recommendations. After washing, cool the eggs to 45 F or less if sweating can be controlled.

VI. The storage and transport temperature of eggs should remain at 45 F or less.

VII. Rotation of product in coolers should be given top priority at all levels of distribution, warehousing, sale, and home or institutional use. Such inventory control decreases the likelihood of salmonella amplification in the product.

VIII. Advise customers on egg carton labels that eggs are a perishable food requiring the same total care, including cooking, as any other food of animal origin. Better informed consumers work to the benefit of both producers and their customers. Eggs should be stored in their original carton in the main section of the refrigerator and not in the door shelf where temperatures can reach 60 F.

IX. Fiber flats and egg cartons should not be reused. Although an economy, their reuse could lead to further spread of contamination. The use of plastic flats is encouraged. They must be disinfected after each use.

FEED

Many salmonella serotypes have been found in feed and feed ingredients. Salmonella contamination after manufacturing also needs to be prevented. Care should be exercised in selecting feed suppliers and in shipping, storing feed.
I. Feed and Feed Ingredient Suppliers


B. It is strongly recommended that producers use animal protein ingredients originating from rendering plants participating in the Animal Protein Producers Industry (APPI) Salmonella Reduction Education Program. Refer to Appendix H to locate a listing of renderers participating in the APPI Program.

C. Dryness of feed ingredients and finished feed at all stages of manufacture and storage is a critically important risk reduction step.

II. Feed Protection and Quality Control

A. Proper pelleting of feeds (e.g., 15% moisture and a come-up temperature of 180°F) may eliminate, or very significantly reduce Salmonella. Crumbled (crushed pellet) feeds are most appropriate for chicks from at least 1 to 14 days of age.

B. Prevent contamination of feed in storage with an aggressive rodent control program and monthly cleaning and disinfecting of storage bins.

C. Prevent feed from getting wet. Condensation control and maintenance of the moisture barriers in storage bins is essential.

D. Seek advice from your nutritionist and/or veterinarian before using anti-salmonella feed additives. They vary in effectiveness and mode of action and may be subject to regulatory control by FDA.

E. Establish your own bank of feed samples. Feed banks and testing promote quality control. Take about 12 tablespoons of feed (for a total of about 1/2 lb.) from different, widely scattered areas of the load of feed, using technique that precludes cross-contamination between lots. This may include use of sterile feed probes or grab samples using sterile bags inverted over the collector's hand during collection and pulled back afterwards. Store samples in a clean, dry location at room temperature.
Biosecurity practices for the prevention of most virus, mycoplasma, and various bacterial diseases are equally appropriate in an integrated salmonella risk reduction program.

Salmonellae best gain a foothold when a virus or other infectious agent weakens your flock’s natural defenses. Research has demonstrated that bursal disease, coccidiosis, mycoplasmolisis, infectious bronchitis, mycotoxicosis, and even antibiotic medication may increase susceptibility to salmonellosis. Consequently, every step in biosecurity (human traffic control, cleaning and disinfection of all materials moving between flocks, proper building location and construction, and much more) adds up to an investment in survival. Look not at biosecurity as an expense – Look at it instead as an insurance premium helping to ensure a more predictable future.

Humans can also carry Salmonella to your chickens and their eggs. Consequently, the personal hygiene of all farm workers is an essential consideration. Provide enough clean, operable toilets, with hand washing and drying facilities, in locations and numbers to serve all employees. Pullet and layer buildings are closer to nurseries and kitchens than many fully appreciate! Suggested Biosecurity/Hygiene Check Lists are provided. Refer to Appendix F.

Additional materials (videotapes, pamphlets, etc.) to inspire, train, and retrain everyone in your operation are available for use at all levels, from the owner to the hired hand. Refer to Appendix H. Review them regularly. If you cannot adopt all recommended practices, adopt some of them. Then add more every year until you have built a solid defense.

APPENDIX A
SALMONELLA CAN COST YOU IN MORE WAYS THAN ONE
F. E. Bender and E. D. Ebel

Salmonella have been with us for as long as we have been producing poultry and eggs. But the economic consequences are only now being documented.

Each firm producing eggs in the United States is well aware of the adverse effects on effective demand for eggs as a result of adverse publicity concerning outbreaks of Salmonella enteritidis (SE) among consumers. This adverse effect on effective demand is translated immediately to all firms in the industry through a reduced market price. Consequently, the presence of SE or similarly dangerous salmonellae in a competitor firm can have a direct adverse effect on you, even if your flocks are free of such problems. As a result, it is in your economic interest to reduce the presence of Salmonella in the table egg market.
SALMONELLA RISK REDUCTION

In addition, if SE or other salmonellae are present in your flocks and an outbreak occurs among consumers that can be traced back to your flock, you may well face serious liability and devastating lawsuits in the event of major illness or death due to a salmonella outbreak. In such a circumstance, the very survival of the firm is at stake. Consequently, it is in the vital interests of each firm to be free of such bacteria.

But even if there were no adverse effects on demand as a result of an outbreak of salmonellosis among consumers or if firms could be protected completely from lawsuits as a result of such outbreaks, there is evidence to support the idea that most salmonellae cost you money. Bender and Mallinson (1991) reported that broiler flocks where drag swab cultures are salmonella–positive incur a higher cost of production than birds produced in an environment where drag swab cultures are salmonella–negative. The difference in cost is approximately 0.4 cents per pound and is statistically significant at the 1% level. It is possible to measure this economic cost in the production of broilers because of the way that records are kept in broiler grow out operations. It is much more difficult to quantify the cost of salmonellosis in pullet and egg producing operations. However, the evidence in broiler production is strong enough to suggest that a similar cost differential exists in pullet or egg production as well.

In summary, the presence of salmonella contamination in any firm in the industry that results in an outbreak of salmonellosis among consumers that becomes widely publicized has a direct adverse effect on every firm in the industry through lower market prices. Within our current society, which seems to settle every dispute with a lawsuit, an outbreak of salmonellosis among consumers that can be traced to the presence of the same salmonella strain within a specific firm can result in ruinous litigation.

And finally, even if no one knows that salmonellae are present, the evidence developed by studying the broiler industry indicates that their presence in an egg laying flock will result in an increase in production costs that are great enough that it is in the economic interest of the firm to reduce and, if possible, eliminate their presence.

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The opinions expressed in this appendix are those of the authors and do not necessarily reflect the policies of the US Department of Agriculture or the University of Maryland.

References:
State laws, regulations and policies vary on the confidentiality of voluntary monitoring to facilitate the acquisition of research/epidemiologic and/or in-house quality control data. Positive results at any of the bacteriological monitoring times presented below may present complex fiscal, legal and ethical issues. The same may be true for not monitoring. Professional guidance (legal, underwriter and veterinary) is essential in developing monitoring programs and choosing from the following examples for pullet and layer flocks.

Table 1. Examples of CHICK/PULLET Monitoring Times, Locations and Purposes.

<table>
<thead>
<tr>
<th>Time/Age</th>
<th>Location</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1 day(^a,b)</td>
<td>Chick transport papers, meconium, cull and dead chicks</td>
<td>Detection of breeder or hatchery transmitted Salmonella</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks(^c)</td>
<td>Dropping boards (cage-reared) or litter surfaces (floor reared)</td>
<td>Detection of infection after period of high susceptibility</td>
</tr>
<tr>
<td>14–16 weeks</td>
<td>Droppings or drag swabs of manure (litter) surfaces</td>
<td>Detection of infection prior to movement to layer facilities</td>
</tr>
<tr>
<td>2–3 days after decontamination (C&amp;D) of pullet facility</td>
<td>Building/equipment surfaces, fan blades, etc.</td>
<td>Evaluation of C&amp;D operation prior to housing new chicks</td>
</tr>
</tbody>
</table>

\(^a\)A laboratory manual detailing sampling and culture procedures and a magazine update on culture media improvements have recently been published. For more information refer to Appendix H.

\(^b\)An additional test for Salmonella in one-day-old hatchlings is described in the mentioned laboratory manual (Chapter 1, page 5).

\(^c\)At any age, bacteriological examination of culls, fresh deads, and trapped mice especially, are used to enhance detection efficiency.
SALMONELLA RISK REDUCTION

Table 2. Examples of LAYER Monitoring Times, Locations and Purposes.

<table>
<thead>
<tr>
<th>Time/Age</th>
<th>Location</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks prior to depopulation of layer house&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Droppings or drag swabs of manure (litter) and other building/equipment surfaces (e.g. egg belts and elevators, fan blades, cages, walls, etc.)</td>
<td>Detection of infection with adequate time for decontamination (C&amp;D)</td>
</tr>
<tr>
<td>2–3 days after C&amp;D</td>
<td>Building/equipment surfaces as listed above</td>
<td>Evaluation of C&amp;D operation prior to housing new pullets</td>
</tr>
<tr>
<td>10 months old&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>Droppings, or drag swabs of manure, and building/equipment surfaces</td>
<td>Detection of outbreaks during egg production</td>
</tr>
</tbody>
</table>

<sup>a</sup>A laboratory manual detailing sampling and culture procedures and a magazine update on culture media improvements have recently been published. For more information refer to Appendix H.

<sup>b</sup>Use of cull eggs and/or blood (serum) samples are currently being evaluated as additional or alternative monitoring tools.

<sup>c</sup>At any age, bacteriological examination of culls, fresh deads, and trapped mice especially, are used to enhance detection efficiency.

<sup>d</sup>More frequent monitoring during lay has also been suggested to increase the likelihood of prompt detection of contamination.

APPENDIX C

RODENT CONTROL DETAILS<sup>†</sup>

I. Rodent Proofing
   A. Eliminate potential rodent harborage inside and outside the poultry house (e.g. high grass, shrubs, garbage, broken equipment, construction debris, burrows under the foundation, cardboard boxes, old filler flats).
   B. Install rodent barriers around the perimeter of poultry houses, (e.g., a one-foot-wide zone of crushed stone or blacktop). Install a metal rodent guard strip between the
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foundation and siding.

C. Seal all entrance holes (3/8" wide or wider) inside and outside the building. Repair siding and close siding sheet seams. Doors and door frames should fit snugly.

D. Seal holes and broken cement in manure pits.

E. Hiding in reused fiber filler flats, rodents can be brought onto the farms by egg delivery trucks and equipment. Guard against this source of salmonella contamination.

F. Feed bins and sheds should be secured at night. Clean up dead birds and broken eggs daily and place in tightly sealed containers.

II. Preparations for Baiting

A. After house depopulation, all feed should be immediately removed from feeders so that rodents will promptly go to the bait.

B. Remove all alternative food sources for rodents, (e.g., spilled feed, broken eggs and dead birds).

III. Bait Selection and Placement

A. Warfarin, diphacinone, or pival are "multiple-dose" type anticoagulant rodenticides. They require continuous feeding over several days to have any lethal effect. They are best used as a routine every two-week baiting program. Newer anticoagulants contain brodifacoum and bromadiolone which may cause death 3-5 days after a single feeding. Such "single-dose" rodenticides can be used at any time and are especially appropriate immediately upon house depopulation.

B. Purchasers of rodenticides often underestimate the long-term need. Secure an adequate inventory.

C. Save bait by baiting only active holes. Fill all rodent holes with dirt or paper and then bait only those that have been reopened.

D. Place bait according to directions supplied by the manufacturer of your rodenticide.

E. Control of attic rodents is facilitated by construction of a hatch for attic access and at least once yearly baiting with high wax, single-dose bait.

F. Once control has been achieved, inspection and service of permanent bait sites is essential every two weeks.

G. Caution: All baits are poisonous to rodents, chickens,
animals and people. Place baits carefully to prevent contamination of feed and eggs or accidental access by poultry and humans. Baits should not be placed loosely on the ground in areas frequented by people who might carry it on shoe soles and contaminate sensitive areas.


APPENDIX D
DECONTAMINATION STEP-BY-STEP

Examples of commonly used disinfectants and their properties are provided at the end of this appendix.

Additional detail on the problems of decontamination in today's industry is provided in Cleaning and Disinfecting Problems in Cage Layer Houses and in Constructing Cage Layer Houses for Cleaning Ease (Graves). To order, refer to Appendix H.

I. Bird Removal
A. Remove all dead and live birds from the building; this includes all escaped birds in the deep pit or outside.
B. Immediately begin vector control procedures during bird removal. Refer to guideline section on Vector Control.

II. Dry Cleaning
A. Clean fans and other air inlets from the outside.
B. On the inside, brush, sweep, vacuum and wipe dust and other dirt from ceilings, light fixtures, beams, ledges, walls, cages, fan parts, air inlets and walkways. Move from top to bottom. Crusted areas should be hand scraped and wire brushed until they are spotless.
C. Promptly open feeder lines and remove feed from trough, all line corners and all other points of feed accumulation. Use shop vacuums, air blowers and wire brushes. A wood block fitted with abrasive material has reportedly been installed under the chain and run until all caked feed had been loosened.
D. Open egg conveyance equipment at the front of the building and remove all dust and egg debris. Remove all broken parts and all soiled items that can not be cleaned.
E. Remove as much manure from dropping boards as possible.
Manual scraping, in addition to low speed mechanical scraping, may be helpful.

F. Remove all litter and manure from floor or cage houses, including all corners, augers and pit ends. Refer to section on Bacteriological Monitoring, Part III B 2, for NIOSH alert on manure pit hazards.

Hard surface (concrete) floors can be cleaned faster and more easily than clay or earthen floors. Completely remove all manure. Hand sweeping and shoveling will be necessary around the perimeter, doorways, walkways, support poles, and corners of most houses to do a satisfactory job. If possible, fill trailers with manure inside the house and cover before moving it to a disposal or composting site.

G. Remove egg belts and sweep away all debris accumulating on both the top- and under-sides of the belt.

H. Rid pullet or layer houses, storage and egg rooms, egg coolers, hallways and stairways of all debris and non-essential items. Repeat for adjacent break, wash and restrooms or portable toilets.

I. Turn off power to electrical equipment prior to dry or wet cleaning. Non-removable motors, switches, etc., have been dry cleaned with compressed air or brushing. Extreme care should be taken about not getting any sprays inside electric motors. Duct tape can be used to cover the slots in motor housings prior to wet cleaning and disinfection. The tape must be removed after wet cleaning and disinfection is completed.

III. Wet Cleaning

Wet cleaning includes soaking, washing and rinsing steps. Use of hot water is preferred. Detergents and other surfactants are often added to washing solution to loosen debris and films and allow better penetration of cleaning agents.

Salmonella can multiply to high numbers in the presence of debris and moisture. Therefore, the following steps, although done thoroughly, should also be executed without interim waiting periods.

A. Soften dirt in heavily soiled areas. A low pressure (200 to 300 psi) sprayer, delivering 10–30 gallons/minute, has been considered adequate.

B. Washing. Professional contractors use a systematic spray technique. They start at the back and work toward the front of the building, spraying the ceiling first, then the walls, and
SALMONELLA RISK REDUCTION

finally the floor.
Use sprayer attachments and nozzles that permit washing of hard-to-reach areas.

1. Wash ceilings, walls, walkways, steps and cross-over platforms, egg rollers, all egg conveyors, cross belts, floors under conveyors, stairs to pit, outside stairs and concrete pit floors – clean everything completely.
Although use of pressure sprays ranging from 200–2000 psi have been reported, washing pressures of 750–2000 psi appear to be preferred. At high pressure, however, special care and safety garments are needed. Pressure sprayers can cut human skin like a knife. Care must be exercised to follow the manufacturer’s instructions for the use of this equipment.

2. Pay special attention not only to the top, but also to the underneath sides of troughs and obvious and hidden surfaces of all chains and augers.

3. Extreme care is needed for the egg elevator. Check for cleanliness from every angle possible – From underneath in the pit and from behind rollers. Remove all traces of egg breakage and spillage.

4. Wash storage and egg rooms, egg coolers, hallways, break, wash and restrooms.

5. Manually clean any areas that have resisted prior cleaning.

C. Rinsing

1. A final rinse is suggested to obtain a truly clean building and to reduce residues of cleaning chemicals.

2. Immediately remove all puddles. They are bacterial breeding grounds.

IV. Repairs

All repairs should be made at this point (i.e., floor cracks filled, door frames repaired, damaged panels replaced, etc.). Repair manure and egg handling and other equipment.

V. Dirt Floors
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In houses with dirt floor pits, a 3 to 6 inch layer of clean soil has reportedly been applied to decrease the risks of recontaminating new stock. The value of this procedure in salmonella prevention is unclear. Concrete floors are generally viewed as preferable.

VI. Inspection

Third-party visual inspection for completeness of the wet cleaning and repair operations is recommended. This may be done by an outside authority or by an in-house unbiased employee responsible for quality control.

VII. Disinfection

Disinfection should be started within 24 hours of rinsing. Because disinfectants are effective only on clean surfaces, do not begin disinfection until the house has passed its inspection for proper cleaning.

A. Heat Enhancement
All disinfectants, whether sprays, foams, aerosols, or fumigants, work best at temperatures above 65 F. Temperatures for chlorine- and iodine-based disinfectants should not exceed 110 F.

B. Dangerous Mixtures
Each disinfectant is the result of careful formulation, and any addition of detergents, surfactants or insecticides to a disinfectant without the approval of its manufacturer could dangerously reduce the efficacy of one or more of the products in the mixture. For economy, efficacy and human and flock safety, manufacturer's label instructions must be followed carefully.

C. Evaluation of Products
There has been an influx of claims for new salmonella disinfectants and control systems. Appendix G provides guidance on evaluating efficacy of various products.

D. One gallon of diluted disinfectant is ordinarily applied to approximately 100–150 square feet of surface area (USDA–SE Task Force). A calculation for the total amount of disinfectant solution needed has been developed. Determine the total surface area of the floor, ceiling and walls. Add 30% to this area to allow for cage surfaces.

E. Follow application instructions of the manufacturer. Use of
pressure sprays is advisable to help force disinfectants into wood pores, cracks and crevices that protect salmonellae. Spray pressures of 500–1000 psi have been suggested (USDA, SE Task Force). Move from back to front and from top to bottom.

F. Dirt floors are virtually impossible to fully disinfect. In situations where dirt floors could not be concreted, disinfectant has been applied to the floor at 1 gallon diluted disinfectant per 10 square feet. Kumar, 1991 reported favorable results with Clorox and formaldehyde. Note formaldehyde warning below in Section VII–I.

G. Disinfect egg handling equipment (elevators, egg belts, etc.) in accordance with recommendations provided by equipment and disinfectant manufacturers. The use of steam, vats of water at pasteurization temperatures, or soaking in disinfectant to disinfect egg belts has been suggested but not fully evaluated for efficacy or adverse effects on the belt.

H. Decontaminate feed bins, boots, augers, hoppers and carts. Sanitize waterlines. Waterline and feed system directions have been published in Biosecurity for Poultry (Brunet). Refer to Appendix H for source. Be careful – metal and non-metallic components of watering systems can be damaged and lines plugged from improper use of sanitizing agents. Check with the manufacturer of farm water handling equipment before implementing any specific chlorine or other sanitation treatments of your wells or water lines. Routine chlorination of poultry drinking water to a minimum 1 to 1.5 ppm free chlorine level has been reported to reduce the spread of salmonellae (Bentley, 1984).

I. In the past, direct application of formaldehyde solutions (formalin) has been used as a topical disinfectant for Salmonella. Formaldehyde fumigation also has been used as a final crack- and pore-penetrating step in salmonella disinfection, provided proper levels of relative humidity (at least 70%) and temperature (at least 70 F) were maintained. Such applications may soon be unlawful.

WARNING: Formaldehyde and formalin are dangerous chemicals and present serious health and safety hazards. Consequently, contact state/federal (EPA, OSHA, FDA) authorities and licensed professionals before considering use! Gas masks, protective clothing and rescue plans are essential.

J. Promptly dry the building. Bullet space heaters have been used to speed drying in cold or damp climates.
K. *Salmonella* are invisible. Verify decontamination success by laboratory procedures. Refer to section on Bacteriological Monitoring and Appendix B. Laboratory tests of your pullet or layer facility should be negative before you place either chicks or ready-to-lay hens.

**VIII. Preparations for Restarting***

A. Replace disposable parts with new ones (e.g., sponges on egg conveyer equipment).

B. To reduce cracked or broken eggs, repair and adjust your egg handling and conveyance system from hen to cooler.

C. Remove old water filters. Clean and disinfect casing and install new filters.

D. Restock restrooms and portable toilets with soap and paper towels or sealed hand washing packets.

E. Remove coverings and tape used to protect electrical circuits and motors and make sure that all electrical equipment, time clocks, feed and water lines, egg- and manure-handling devices, brooder stoves, etc. operate properly.

F. All decontamination equipment such as rakes, shovels, scrapers, brushes, trucks, manure spreaders, bucket loaders and spray/disinfection devices also should be cleaned and disinfected after use and stored in a secure location.

*Adapted from* Biosecurity for Poultry. 1987. (Brunet).
## Table 3. Properties and Examples of Common Disinfectants

<table>
<thead>
<tr>
<th>Special Properties</th>
<th>Hypochlorites</th>
<th>Chloramines</th>
<th>Iodophors</th>
<th>Cresols</th>
<th>Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active against</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Salmonella, E. coli, etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resistance to organic debris</strong></td>
<td>Poor</td>
<td>Poor to fair</td>
<td></td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td><strong>Effect of hard water</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Detrimental effect of heat</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Residual activity</strong></td>
<td></td>
<td>Yes</td>
<td></td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>Most effective pH range</strong></td>
<td>Acid</td>
<td>Acid</td>
<td>Acid</td>
<td></td>
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</tr>
<tr>
<td><strong>Compatibility with anionic surfactants (soaps)</strong></td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Compatibility with non-ionic surfactants</strong></td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
<td>No</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Common Brands and Names*</th>
<th>Chloramine-T</th>
<th>Betadine</th>
<th>Crel-400</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorox</strong></td>
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<tr>
<td><strong>Halazone</strong></td>
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<tr>
<td><strong>Bio-Dyne</strong></td>
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<tr>
<td><strong>Iofec</strong></td>
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<tr>
<td><strong>Isodyne</strong></td>
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<tr>
<td><strong>Liosan</strong></td>
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<td></td>
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<tr>
<td><strong>R.I.D.</strong></td>
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<td></td>
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<tr>
<td><strong>Tamed</strong></td>
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<tr>
<td><strong>Iodone</strong></td>
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<td></td>
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<tr>
<td><strong>Environ-D</strong></td>
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<tr>
<td><strong>LpH-AG</strong></td>
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<tr>
<td><strong>Lysol</strong></td>
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<td></td>
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<tr>
<td><strong>Orthophenol</strong></td>
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<tr>
<td><strong>yphenols</strong></td>
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<td><strong>PD 256</strong></td>
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</tr>
<tr>
<td><strong>Tek-Trol</strong></td>
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</tbody>
</table>

*Modified from Biosecurity for Poultry (Brunet) and Selection and Use of Disinfectants in Disease Prevention (Meyerholz and Gaskin). *Where product types or names appear, no discrimination is intended and no endorsement over other products not mentioned is implied by the U.S. Animal Health Association (USAHA). Mention of a trade name does not constitute a guarantee or warranty of the product by the USAHA.

*Unless hard water is alkaline.

*Use at less than 110 F, active principal driven off by heat.

*Hypochlorites: No, chloramines: Yes

*Products listed are intended as examples; many other products are not listed. New quaternary ammonium disinfectants exist.
APPENDIX E
WHY REFRIGERATE

It is possible for the egg shell surface to become contaminated with salmonella organisms before, during, or after lay. In some instances, Salmonella may contaminate the inside of the egg, either by contaminating the egg before it is fully formed, or by penetrating the shell. According to Humphrey (1990), such cases of contamination result in very few Salmonella in the egg—probably fewer than 10 bacteria per egg. Nonetheless, this low number can also lead to trouble.

The egg producer's goal is to eliminate or reduce unknown Salmonella from the surface or the interior of the eggs produced. Proper egg washing and sanitizing has performed well in delivering a clean egg to the consumer. The question to address now is, "What about salmonella bacteria inside the egg?"

The goal of an egg handling program in addressing the possibility of internal contamination is to prevent penetrating salmonella organisms from multiplying. We have only two tools to accomplish this: the natural antibacterial properties of egg white, and low temperatures to prevent multiplication.

Egg white contains natural antibacterial products that help to kill or inhibit the growth of bacteria. In nature, this protective mechanism helps a chick hatch from an egg that is a good growth medium for bacteria as well as chicks. These natural products decline in effectiveness as the egg white ages, but they help prevent bacterial growth during the time the egg is cooling. Cool temperatures help retard the aging of egg white, and thus help maintain its antibacterial properties.

Cold temperatures alone can also prevent or reduce the growth of salmonella organisms. Research (Kim, et al, 1989) has shown that when Salmonella enteritidis was experimentally inoculated into eggs, it did not multiply at 40 F, but did multiply at 50 F. Therefore, reducing egg temperature to 45 F or lower can be used to reduce the risk of salmonella multiplication.

References:


SALMONELLA RISK REDUCTION

I. Suggested Check Lists for Flock Caretakers and Farm Managers

A. Flock Caretakers. This list could be posted in all houses. Consider printing large, obvious posters.
   1. Watch for, correct and immediately report any rodent, beetle, fly, wild bird or pet control problems. Consider rats and mice as "Poultry Enemy Number One!"
   2. Daily patrol for prompt, secure removal of all dead and dying birds. Never return escaped birds of any age to cages.
   3. Thoroughly cull all flocks on a weekly basis. Salmonella thrive in weak birds.
   4. Wash and dry hands thoroughly before handling eggs or chickens of any age, especially baby chicks.
   5. Keep egg belts, elevators, etc. in proper adjustment. Regularly clean and sanitize.
   6. Ventilate to ensure rapid drying of droppings.
   7. Vacuum areas of dust accumulation.
   8. Wear clean clothing.

B. Farm Managers

1. Specify in contracts and check that all pullet deliveries are made in clean and disinfected coops and trucks.
2. Keep dead bird pickups at least 50 yards away from any poultry buildings.
3. Insist that biosecurity garments are worn by all visitors, farm executives and anyone else.
4. Absolutely prohibit caretakers from maintaining any home flocks of poultry.

II. Proposed Georgia Biosecurity Check List and Inspection Forms

Exhibits A (layers) and B (pullets) are examples of the use of biosecurity check lists and third party inspections as a requirement for participation in an officially-recognized risk reduction program being presented to industry. For information on the proposed Georgia program, refer to Appendix H under the Biosecurity heading.
Many products are being promoted for eliminating or preventing bacterial contamination. Fortunately, there is also interest in testing these claims. This section's purpose is to provide some "food for thought" for those considering such trials.

Tried and proven evaluation systems are available at your state university’s Agricultural Experiment Station, Cooperative Extension Service, official state and federal laboratories and other diagnostic/research units. Seek the direct involvement of persons well-recognized and experienced in setting up and carrying out meaningful investigations.

Evaluating farms for successful salmonella disinfection requires the use of technology that is appropriate for farms; this is different from other types of medical testing systems. A system for farms (El-Assaad, 1990) has been developed at the University of Maryland. It attempts to duplicate the salmonella disinfection difficulties common to manure-stained, porous, semi-porous and non-porous materials used in poultry house construction and equipment fabrication. For more information refer to Appendix H.

A World Health Organization publication on decontamination and standard efficacy tests for disinfectants has also been published. Its source is provided in Appendix H.

APPENDIX H
EDUCATIONAL RESOURCES

<table>
<thead>
<tr>
<th>Title</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosecurity for Poultry–Stomp</td>
<td>Mr. T. Milton Nelson</td>
</tr>
<tr>
<td>Title</td>
<td>Author(s)</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>Diseases of Poultry. Ninth Edition</td>
<td>Eds. Calnek, Barnes, Beard, Reid and Yoder</td>
</tr>
<tr>
<td>Proposed Georgia SE Prevention Program for Pullets and Layers</td>
<td></td>
</tr>
</tbody>
</table>

**Cleaning and Disinfection**

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Address</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. D.C. Johnson</td>
<td>Area Veterinarian in Charge</td>
<td>USDA, APHIS, VS</td>
</tr>
<tr>
<td>Dr. E.T. Mallinson</td>
<td>Gudelsky Veterinary Center</td>
<td>University of Maryland</td>
</tr>
<tr>
<td>Dr. Robert E. Graves</td>
<td>201 Agricultural Engineering Bldg., The Pennsylvania State University, University Park, PA 16802</td>
<td>(814-865-7155)</td>
</tr>
</tbody>
</table>

Agricultural Publications

Distribution Center, 112 Agricultural Building, The Pennsylvania State University

University Park, PA 16802

No charge for single copy.
SALMONELLA RISK REDUCTION

Economics/Legal


Mr. Walter Stephens Watt Publishing Company 122 S. Wesley Avenue Mount Morris, IL 61054 (815-734-4171)

Egg Sanitation

Regulations Governing the Grading of Shell Eggs etc. (7CFR Part 56). May 1991. Refer to Section 56.76, Minimum facility and operating requirements (page 9).

Agricultural Marketing Service Poultry Division USDA Washington, DC 20250


Mr. T. Milton Nelson Information and Publications Cooperative Extension Service Room 0128, Symons Hall University of Maryland, College Park, MD 20742 30 cents per copy (301-405-4596)

Evaluating Products


Dr. Larry E. Stewart Department of Agricultural Engineering, University of Maryland, College Park, MD 20742 (301-405-2223)


Chief, Veterinary Public Health Division of Communicable Diseases, World Health Organization, 1211 Geneva 27,
SALMONELLA RISK REDUCTION

Koulikovskii. 62 pp. Switzerland No charge for one copy.


Laboratory Methods


American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, PA 19348–1692 $26 per copy (215–444–4282)


Feedstuffs 12400 Whitewater Drive Suite 160 Minnetonka, MN 55343

Renderers

List of 190 APPI participants with city and state addresses.

Dr. F.D. Bisplinghoff 7150 Estero Boulevard Fort Myers, FL 33931 (813–765–1950)

Vectors


Department of Entomology Agricultural Extension, Box 7613, North Carolina State University, Raleigh, NC 27592–7613 (919–515–2703) No charge
**EXHIBIT A**

**PROPOSAL**

Georgia Se Prevention Program

Table Egg **Laying Flock** Participant

**Inspection Form**

<table>
<thead>
<tr>
<th>Field</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owner Name</td>
<td></td>
</tr>
<tr>
<td>Owner Address</td>
<td></td>
</tr>
<tr>
<td>Owner City State Zip</td>
<td></td>
</tr>
<tr>
<td>Owner Phone Number</td>
<td></td>
</tr>
<tr>
<td>Laying Houses (No.)</td>
<td></td>
</tr>
<tr>
<td>House Type</td>
<td></td>
</tr>
<tr>
<td>Length Width</td>
<td></td>
</tr>
<tr>
<td># of Cage Banks # of Tiers</td>
<td></td>
</tr>
<tr>
<td>Source of Pullets</td>
<td></td>
</tr>
<tr>
<td>Source Address</td>
<td></td>
</tr>
<tr>
<td>Previous Inspection</td>
<td></td>
</tr>
<tr>
<td>Inspection Date</td>
<td></td>
</tr>
<tr>
<td>Premises Address</td>
<td></td>
</tr>
<tr>
<td>Premises City State Zip</td>
<td></td>
</tr>
<tr>
<td>Premises Phone Number</td>
<td></td>
</tr>
<tr>
<td>Flock size Strain</td>
<td></td>
</tr>
<tr>
<td>Age of Birds Wks. Molted:</td>
<td>Yes No</td>
</tr>
<tr>
<td>Other poultry on premises</td>
<td>Yes No</td>
</tr>
<tr>
<td>Explain</td>
<td></td>
</tr>
<tr>
<td>Precondition for Qualification</td>
<td></td>
</tr>
<tr>
<td>Product Labeling &amp; Identification:</td>
<td>Must be able to identify eggs to flock of origin to qualify.</td>
</tr>
<tr>
<td>Premises Inspection:</td>
<td>Must score 80 or above to qualify.</td>
</tr>
<tr>
<td>Environmental Sampling:</td>
<td>Must be environmental culture negative to qualify.</td>
</tr>
<tr>
<td>Culture Results:</td>
<td></td>
</tr>
<tr>
<td>Total Score:</td>
<td>100</td>
</tr>
</tbody>
</table>

This flock qualifies: __________ This flock does not qualify: __________

"Eggs produced by a participant in the Georgia Salmonella enteritidis prevention program"
# SALMONELLA RISK REDUCTION

## PROPOSAL

**SE - 1**

**Page 2**

**Georgia Se Prevention Program**

**Laying Table Egg Laying Flock**

**Participant**

**Flock**

**Inspection Form**

### A. Vector Control

<table>
<thead>
<tr>
<th>Control Type:</th>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>(contract exterminator, bait, etc.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Rodents (rats/mice)

   - Effectiveness: Signs of rodent infestations
     - None ____ (20) Few ____ (5-15) Many ____ (0)

2. Insects (flies)

   - Control Type: (bait, spray, biological, etc.)
   - Effectiveness: Signs of insect infestations
     - Few ____ (1-5) Many ____ (0)

3. Wild Birds - present in house:

   - None ____ (5) Few ____ (3) Many ____ (0)

4. Pets allowed in house (dogs/cats):

   - No ____ (5) Yes ____ (0)

   **Comments:**

### B. Biosecurity

<table>
<thead>
<tr>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
</table>

1. House is kept locked: Yes ____ (5) No ____ (0)

2. Visitor policy: overall rating ____ (0-5)

   (restricted, clothes change, footwear required, etc.)

   **Explain:**

3. Repair/maintenance personnel policy: ____ (0-5)

   **Explain:**

   **Total** 15

### C. House Condition

<table>
<thead>
<tr>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
</table>

1. Dust and cobwebs:

   Minimum to Moderate ____ (1-5) Severe ____ (0)

2. C & D of house and equipment:

   - Total removal between flocks: Yes ____ NO ____
   - Disinfectant used: ________________________________
   - Frequency: ________________________________

   **Overall quality of C&D:**

   Excellent ____ (15) Good ____ (10) Poor ____ (0)

3. Condition of pit:

   Dry ____ Wet ____

4. Air quality (ammonia):

   Good ____ Poor ____

   **Comments:**

   **Total** 20

---

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### D. Feed

1. Source: 

   ![Image](image-url)

2. Bacteriological monitoring: Yes (2) No (0)

3. Storage bins:
   - Overall rating: (0-4)
     - Free of rodent and bird droppings: Yes No
     - Lid covers fit tight: Yes No
     - Feed of caked moldy feed: Yes No

4. Augers:
   - Overall rating: (0-2)
     - Free of caked moldy feed: Yes No

5. Chains, troughs, and pans:
   - Overall rating: (0-2)
     - Free of rodent and bird droppings: Yes No
     - Feed of caked moldy feed: Yes No

6. Antibiotics:
   - Name of antibiotic:
   - How much:
   - Comments: Total 10

### E. Egg handling facilities and practices

1. Room Sanitation
   - Floor is clean: Yes No
   - Walls are clean: Yes No
   - Light fixtures are clean: Yes No
   - Ceiling (beans, etc.): Yes No
   - Room sanitation: Overall rating: (0-5)
     - Excellent Good Poor
   - Comments:

2. Egg Belt: Cleanliness and maintenance (0-3)

3. Egg Grader:
   - Overall rating: (0-2)
   - Washer works properly: Yes No
   - Checks & cracked eggs removed: Yes No

4. Cooler:
   - Pre-processing holding room temperature:
     - temperature <=65 (10) >65 (0)
   - Post-processing room temperature:
     - temperature <=45 (10) >45 (0)

5. Egg pickup per week:
   - Once/wk ___ Twice/wk ___ Three/wk ___ Daily ___
   - Total 20

Inspector: _______________________________ Date: _______________________________
SALMONELLA RISK REDUCTION

EXHIBIT B

PROPOSAL

Georgia Se Prevention Program

Table Egg Layer Pullet Participant

Inspection Form

Premises Inspection: Must score 80 or above to qualify.

Premises inspection score greater than 80: Yes _____ No _____

Environmental Sampling: Must be environmental culture negative to qualify.

Culture Results: ___________________________ Date ____________

This flock qualifies: ______ This flock does not qualify: ________ to use the label

"Pullets were produced by a participant in the Georgia Salmonella enteritidis prevention program"

Program Administrator ___________________________ Date ____________

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## SalmoneLla Risk Reduction

### Proposal

**Table Egg Layer Pullet Participant Inspection Form**

<table>
<thead>
<tr>
<th>A. Vector Control</th>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Rodents (rats/mice)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Type:</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(contract exterminator, bait, etc.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness: Signs of rodent infestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>(20)</td>
<td>Few</td>
</tr>
<tr>
<td><strong>2. Insects (flies)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Type:</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(baits, spray, biological, etc.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness: Signs of insect infestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Few</td>
<td>(1-4)</td>
<td>Many</td>
</tr>
<tr>
<td><strong>3. Wild Birds present in house:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>(4)</td>
<td>Few</td>
</tr>
<tr>
<td><strong>4. Pets allowed in house (dogs/cats):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(2)</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Comments:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Biosecurity</th>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. House is kept locked:</strong></td>
<td>Yes</td>
<td>(4)</td>
</tr>
<tr>
<td><strong>2. Visitor policy:</strong></td>
<td>overall rating</td>
<td>(8-0)</td>
</tr>
<tr>
<td>(restricted, clothes change, footwear required, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Explain:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3. Repair/maintenance personnel policy:</strong></td>
<td></td>
<td>(0-8)</td>
</tr>
<tr>
<td><strong>Explain:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. House Condition</th>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Dust and cobwebs:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum to Moderate</td>
<td>(1-10)</td>
<td>Severe</td>
</tr>
<tr>
<td><strong>2. C &amp; D of house and equipment:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total removal between flocks:</td>
<td>Yes</td>
<td>NO</td>
</tr>
<tr>
<td>Disinfectant used:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall quality of C&amp;D:</td>
<td>Excellent</td>
<td>(10)</td>
</tr>
<tr>
<td><strong>3. Litter condition:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of litter:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition of pit:</td>
<td>Dry</td>
<td>(1-10)</td>
</tr>
<tr>
<td><strong>4. Air quality (ammonia):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comments:</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>
### Table Egg Layer Pullet Participant Inspection Form

<table>
<thead>
<tr>
<th>D. Feed</th>
<th>Maximum Points</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Source: ________________________________</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Bacteriological monitoring: Yes ___ (2) No ___ (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Storage bins: Overall rating: ___ (0-9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free of rodent and bird droppings: Yes ___ No ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lid covers fit tight: Yes ___ No ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free of caked moldy feed: Yes ___ No ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Augers: Overall rating: ___ (0-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free of caked moldy feed: Yes ___ No ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Chains, troughs, and pans: Overall rating: ___ (0-6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free of rodent and bird droppings: Yes ___ No ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free of caked moldy feed: Yes ___ No ___</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Antibiotics:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name of antibiotic: ______________________</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How much: _______________________________</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comments: _______________________________</td>
<td>Total 20</td>
<td></td>
</tr>
</tbody>
</table>

Inspector: _______________________________ Date: ____________
SCRAPIE CERTIFICATION – A NEW CONSENSUS FOR ANIMAL HEALTH

Lonnie J. King, MS, MPA
Deputy Administrator, Veterinary Services
Animal and Plant Health Inspection Service
United States Department of Agriculture

Background:

On February 26, 1990, APHIS established a Scrapie Negotiated Rulemaking advisory committee. The negotiated rulemaking process brought together sheep producers, allied industry representatives, State Veterinarians, USAHA, and officials of the Animal and Plant Health Inspection Service (APHIS) to negotiate the text of a proposed rule for the control of scrapie and to improve the existing program.

The advisory committee set up a series of meetings to obtain maximum input from industry interests and to allow all concerned parties to exchange views. The committee focused on finding constructive and creative solutions that the industry could support, resolving conflicts between diverse factions, and developing a user-based program for controlling and reducing the risk of scrapie.

Ground rules included agreement among all committee participants that a consensus had to be reached on every decision and that APHIS would use consensus-based language in any proposed rule.

The following organizations were represented on the Scrapie Negotiated Rulemaking Committee:

American Association of Small Ruminant Practitioners
American Farm Bureau
American Hampshire Association
American Meat Institute
American Polypay Association
American Sheep Industry, Inc.
American Suffolk Society
Animal and Plant Health Inspection Service
Continental Dorset Club
National Assembly of Chief Livestock Health Officials
National Renderers Association
National Suffolk Association
United States Animal Health Association

Outcome:

In January 1991, after meeting eight times within nine months, the
SCRAPIE CERTIFICATION

advisory committee suggested a core program for scrapie that included these facets:
* A scrapie flock certification program
* A one-time limited depopulation with indemnification for infected and source flocks, if funds could be secured.
* An identification requirement for the interstate movement of sheep from scrapie infected or source flocks.

Scrapie Flock Certification Program:

The intent of the Flock Certification Program is to monitor flocks over a period of 5 years or more and to identify flocks that are clinically free of scrapie. Since there is no live-animal test and scrapie has a long incubation period, a flock is considered free of the disease if no sheep have been diagnosed with scrapie and there is no clinical evidence over an extended period of time. While this is not an eradication program at this time, the program does focus on risk reduction and places the industry in an advantageous position to pursue total eradication when research efforts give us the appropriate diagnostic tools.

The program will provide participating owners with the opportunity not only to protect their sheep from scrapie but to enhance the marketability of their animals. The creation of a favorable marketing niche for sheep in the certification program, especially for breeding sheep, could be an important driving force in determining future producer participation. The control effort focuses on risk reduction and sound husbandry practices. Since each advancing phase represents a lower risk of scrapie in the flock, the economic value of the animals is progressively increased, especially after completing the 5-year program and attaining "certified" status. This program will also have implications for exporting breeding stock to other countries.

Members of the National Oversight Committee and State Certification Board will be producers, allied industry representatives, State animal health officials, and APHIS officials.

Any owner of a flock may apply to enter the Voluntary Scrapie Flock Certification Program by sending a written request to the State Scrapie Certification Board.

When participating in the program, the owner must:
* Agree to report scrapie-suspect animals to the proper animal health official immediately. Such animals must not be sold for breeding or slaughter.
* Officially identify all animals within a flock that are 1 year of age or older. Animals less than 1 year old must be identified whenever a change of ownership occurs, except for those in slaughter channels.
* Maintain required records as specified by the program. Records must be kept a minimum of 5 years after an animal dies or is removed from the flock.
KING

* Allow breed associations and registries, livestock markets, and packers to disclose records to APHIS and/or State animal health officials.
* After reasonable prior notice, allow inspection of animals and records by APHIS, State animal health officials, and State Scrapie Certification Board members.
* Provide necessary facilities and personnel to assist in inspections, including:
  -- checking animals for official identification and signs of scrapie; and
  -- checking records for completeness and accuracy.

Owners must account for all acquisitions, departures, births, and deaths.
* Submit to an official laboratory, tissues from scrapie-suspect animals and from animals suspected of other neurologic and chronic debilitation illnesses.

The requirements of the individual phases are as follows:

Phase 1 – Certifiable Class C

Animals must be officially identified
Each animal must have a record containing:
* Official identification number and any secondary identification
* Sex
* Breed
* Date of acquisition and source (if animal was not born in flock)
* Disposition – date and cause of death, if known, or date of removal and destination.

There will be annual inspections
Suspect animals must be submitted for diagnostic purposes
Minimum time requirement 1 year

Phase 2 – Certifiable Class B

The flock has:
* No evidence of scrapie for 1 year
* Not been found to be a source flock in the last year

The flock owner has:
* Met requirements of the Certifiable Class C phase
* The owner has agreed to follow the provisions for Certifiable Class B.

The flock has:
* All animals over 1 year of age with official identification
* Been inspected every 6 months
* Records that also include the sire, dam, and progeny of each animal.
* Reported all suspect animals to the State animal health official or to the Area Veterinarian in Charge
SCRAPIE CERTIFICATION

*Met the minimum time requirement of 2 years

**Phase 3 – Certifiable Class A**

The flock owner has:
* Met requirements of the Certifiable Class B phase
* Agreed to the provisions of Certifiable Class A

The flock has:
* no evidence of scrapie in the last three years
* not been found to be a source flock in the last three years

The requirements in this phase are the same as in Certifiable Class B

**Minimum time requirement 2 years**

**Phase 4 – Certified**

To enter the certified phase, a flock must not have been found to be infected or a source flock in the last five years. The owner must have followed all the provisions of Certifiable Class A and agree to follow the provisions of the Certified Class.

In this phase, the official identification and records will be the same as Class A, inspections will be performed annually and a flock may maintain this status indefinitely as long as all provisions are met.

All animal acquisitions must be from the same or higher class status. If an acquisition is made from a flock in a lower status, the higher level flock will be returned to the status of the flock from which the acquisition was made. Flock status will be jeopardized if animals commingle with animals from a flock in a lower phase.

If infection is found in a flock, an epidemiological investigation will be conducted. This investigation will identify trace and source flocks and exposed animals. A flock plan will be developed and implemented.

The flock plan may include depopulation of high-risk animals, reduction of risk associated with spread of infection, and facility cleanup and disinfection.

If one or more animals in a flock is diagnosed with scrapie or if the flock is identified as a source flock, the flock status will be "pending". After the flock plan is developed and implemented, the flock will return to Certifiable Class C.

**Indemnification Program**

Most agree that in order to better confront the scrapie problem at the onset of the certification program, it would make epidemiological sense to depopulate as many known infected and source flocks as possible. Not only
would this move the program forward more quickly, it also represents a type of insurance policy for BSE prevention. Hence it was proposed to have a six month sign up period in which owners of infected or source flocks could apply for indemnity. Indemnification could be generated from either private sources, or public sources through State government or the Federal Government.

In the FY 1992 APHIS appropriation, there were no funds designated for scrapie indemnification. However, there was language from the congressional conferees for the Department to consider the use of CCC funds to start the program and initiate a one-time limited indemnification. Legal, budgetary, and political considerations will soon determine the feasibility of this approach.

Pending available monies, indemnity could be paid for:
*Infected or source flocks that were known before this rule went into effect. It was felt that these flocks should be given first priority to receive funds because they have been complying with the regulations to date.
*Infected or source flocks that were identified after this rule goes into effect
*Animals destroyed for diagnostic purposes

There would be no appraisals. The amount of indemnity would be a set rate of $150 for a registered animal and $50 for a nonregistered animal.

To help prevent the possibility of fraud, there would be no indemnity payments if:
1. the owner fails to provide the requested records
2. the owner acquires animals other than by birth within 6 months prior to the date of application
3. the owner has not been a shepherd for at least a year prior to the date of application.

Identification Requirement

A permanent, highly visible form of identification will be needed for interstate movement of sheep that are of high risk to perpetuate scrapie, including: all animals from nonparticipating scrapie-infected or scrapie source flocks; and high risk animals from flocks participating in the voluntary certification program except for animals less than 1 year of age moving in slaughter channels. This was viewed as an important aspect of the scrapie program, especially by the slaughtering, processing, and rendering industries.

The Negotiated Rulemaking Process generated three documents:
1. A certification rule which grants APHIS the authority to implement the flock certification program and establishes the interstate identification regulation.
2. The one time indemnity rule which is pending relative to identifying a
specific funding source.
3. The Scrapie Flock Certification Program Uniform Methods and Rules which outlines the procedures of the program.

The certification rule was published in the Federal Register on July 16, 1991. The comment period closed on September 16, 1991. A response to the comments is now being prepared. During Fiscal Year (FY), 1992, Veterinary Services will lay the ground work to implement the Scrapie Flock Certification Program. Although scrapie is a very challenging and often frustrating disease to deal with, we look forward to cooperating with our state counterparts, the sheep industry, and veterinary practitioners in these efforts to control the disease. This national certification scheme, although voluntary, is the right message to send to international trading partners, other food-animal commodity groups, and the general public. The program’s adoption is an affirmation that the sheep industry is progressive, responsible, and is capable of assuming a leadership role in insuring the health status of this important livestock commodity.
The Sheep and Goat Committee met at 1:30 p.m. Monday, October 28, 1991. There were 22 members and 50 guests for a total of 72 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:

Dr. Ramesh Akkina, Colorado State University, reported the results of a study which compared the intracellular polypeptides of cells infected with bovine and ovine pestiviruses. Border disease of sheep and bovine viral diarrhea are caused by two serologically related viruses of the pestivirus genus of the Flaviviridae family. The two viruses can cross infect their respective hosts and are difficult to distinguish by serological methods. Using biochemical analysis, Dr. Akkina found clear differences between the intracellular polypeptides of the two viruses. Polypeptide heterogeneity, among various isolates of border disease virus, indicated that strain variation occurred with this virus. Using sequence specific antisera to various regions of the viral polyprotein he identified several new pestiviral polypeptides in infected cells. In the case of noncytopathic strains of bovine viral diarrhea virus, additional defects in the processing of viral proteins were detected. Dr. Akkina has assembled a complete genomic map of the pestiviruses which shows the relative positions of the various polypeptides.

Dr. Naba Das, Center for Veterinary Medicine of the Food and Drug Administration, summarized the work of the Center for Veterinary Medicine to approve new drugs for minor species as part of the minor species program approved in 1983. Since then, a total of 28 animal drug projects have been completed. Seventeen public master files have been published in the Federal Register providing notice of drug availability. Data contained in these public master files provide for the effectiveness, target animal safety, human food safety and environmental information for 13 new animal drugs in 10 minor species. Eight public master files have been completed and are under review by the Center. One investigational new animal drug
REPORT OF THE COMMITTEE

Submission is under review by the Center and 2 public master files are in preparation. There are 20 ongoing animal drug projects involving the cooperation of 12 universities and government agencies to study 17 animal drugs in 13 minor species.

Sheep are still considered a major species as far as human food safety is concerned. However, the Center for Veterinary Medicine is actively reviewing this status and will determine, in the near future, whether or not sheep should still be considered a major species. Whenever possible, scientifically and legally, the FDA intends to extrapolate results obtained from tests demonstrating human safety of major use drugs to support the approval of minor use of these drugs. In some cases, human food safety data may not be necessary if the sponsor can demonstrate that no residue of concern will be present in the edible tissues of treated animals when they are made available for human consumption.

Dr. Marie Bulgin, University of Idaho, reported that bluetongue continues to be an endemic problem along the Snake River from Twin Falls, Idaho, to Baker, Oregon. Sporadic cases begin in late July, build up in August, peak during September and October and die out as soon as temperatures drop consistently below 45 degrees. Although 4 serotypes have been reported in Idaho, types 17 and 11 seem to predominate. Type 17 tends to be associated with higher mortality than type 11. As many as 10% of infected lambs die, usually from pulmonary edema. More typical signs of bluetongue are seen in sheep infected with type 11 and less mortality has been reported. These recurring outbreaks of bluetongue, which tend to cycle about every 5 years, cannot be controlled without the use of a vaccine directed specifically to these serotypes. The only bluetongue vaccine that is licensed for use in this area is one containing serotype 10 virus. However, there are vaccines available for use only in California and contain serotypes 10, 11 and 17. Dr. Bulgin proposed that the committee support a resolution to request that USAHA ask the USDA to permit the California vaccine to be used in this endemic area of Idaho and Oregon until a federally licensed product is available.

Dr. Konrad Eugster, Director of the Texas Veterinary Medical Diagnostic Laboratory System, discussed the problems related to the international movement of animals whereby cattle are assumed to be persistently infected with bluetongue virus and serve as the source of new outbreaks of the disease. Dr. Eugster is convinced that the disease is limited by the range of the vector and that the movement of cattle is insignificant in the process. Because of recent studies that tend to support the idea that cattle do not become persistently infected with the virus and that import testing does not limit the introduction of new serotypes to the US, Dr. Eugster suggested that import requirements i.e., serologic testing for antibody, could be eliminated, thereby facilitating the import and export of animals. He also suggested that bluetongue be removed from the OIE list.
SHEEP AND GOATS

of reportable diseases. Various concerns were discussed by the committee; there was general agreement with the proposal to remove the virus from the list of reportable diseases, but no agreement was reached on the feasibility of his proposal to discontinue serologic testing for importation.

Dr. John Glenn, Director, Small Ruminant Collaborative Research Support Program, outlined the program to the committee. Started in 1978 and funded by a grant from the U.S. Agency for International Development, it has involved several different U.S. institutions responsible for the conduct of collaborative research projects at a number of overseas sites. The two basic objectives of the program are to add to the knowledge and efficiency of small ruminant production and to provide research training opportunities for U.S. and host country scientists. Although the research has focused on production constraints of small producers, with limited resources in lesser developed countries, much of the technology developed has been directly applicable to the U.S. sheep industry. In the area of animal health, examples include development of a vaccine and diagnostic test for Contagious Caprine Pleuropneumonia; work on viral vector vaccines and internal parasite vaccines and use of genetic markers to identify animals with greater resistance to internal parasites.

Dr. Al Smith, Oregon State University, described studies to produce antibodies against a recombinant submolecular unit of pilin protein which binds to bacteroides pili. Current vaccines for ovine footrot are polyvalent whole cell bacterins incorporating 10 strains (8 serotypes) of B. nodosus. Their efficacy ranges from 0-80% depending upon the serotype. Problems with these vaccines, in addition to their poor efficacy, include tissue reaction and abscess formation at the sites of injection.

Furthermore, they tend to become less effective over time as the "fit" between vaccine antigens and field strains of the organism drift apart. There are new approaches to vaccines for footrot which include genetically engineered purified pili used as a polyvalent vaccine but these too can be expected to share many of the disadvantages seen with current polyvalent vaccines. The objective was to identify a common epitope or epitopes shared by all or most serotypes, identify the genetic code for this antigenic region, express it in a vector system and use this expression product as an antigen to produce antipili antibodies. To this end Dr. Smith reported that they have expressed a TrpE fusion protein with 50 amino acids selected from the B. nodosus pilin protein, purified this product, used it to immunize rabbits and have shown that the antibody produced binds to B. nodosus pili. Immunoelectron microscopy showed pili to be heavily coated with antibody and to clump in a much more random way than the pili cords which have been reported. Their rationale is that such antibodies will change the B. nodosus pili function and will in this way prevent ovine footrot.

Dr. J. Glenn Songer, University of Arizona, discussed the pathogenesis, diagnosis and immunity of caseous lymphadenitis. The
pathogenesis is mediated, at least in part, by phospholipase D (PLD) and exotoxin produced by the organism. The PLD produces direct cell membrane effects, kills ovine neutrophils and inhibits neutrophil chemotaxis. PLD, produced as recombinant protein in E. coli, is useful as a diagnostic antigen in an ELISA test for caseous lymphadenitis. The test has high sensitivity, specificity, and repeatability and holds promise as a tool for use in eliminating the disease from flocks. Another tool in management of the disease is a recently licensed vaccine. Used in conjunction with the ELISA it should be possible to control the disease in production flocks and eliminate it from purebred flocks and dairies.

Dr. Linda Detweiler, Veterinary Services, USDA, APHIS, reported on the outcome of the negotiated rulemaking committee for scrapie. The committee was established in 1990 and met 8 times over a 9 month period. In January, 1991, the committee passed by full consensus the following control measures for scrapie: 1) a one time indemnification program for scrapie infected and source flocks; 2) a voluntary flock certification program and 3) permanent, highly visible identification for the interstate movement of sheep from scrapie infected or source flocks. Dr. Detweiler also introduced a resolution, which was supported by the members of the sheep and goat committee, recommending that USAHA support the use of immunoblotting as a supplemental test for scrapie.

Dr. Jonathan B. Katz, Veterinary Services, USDA, APHIS, gave the outcome of an assessment of western immunoblotting for the confirmatory diagnosis of scrapie. Results of a year long study at the National Veterinary Services Laboratories showed that the immunoblotting technique was a valuable tool to detect prion proteins in the diagnosis of scrapie.

John Wortman, Director, New Mexico Livestock Board, described the occurrence of Club Lamb Fungus. The disease, which is caused by Trichophyton sp., has been confirmed in show lambs in many western states and continues to be a problem. The New Mexico Livestock Board, in collaboration with the University of Wyoming, Colorado State University and the New Mexico Diagnostic Laboratory found fewer infected animals this year than in the past. Strict rules governing the shearing of lambs prior to arrival at Fairs, individual animal inspection by a Veterinarian, and on site testing to augment visual assessments contributed to the success of reducing the incidence of the disease at the Shows, Fairs and Jackpots.

Mr. Paul Rodgers, Director of Producer Services, American Sheep Industry Association, reported on electronic identification of sheep. A ceramic coated microchip has been developed that can be inserted with an implant gun through a 12 gauge needle. The 10 digit chip has the capability of 35 billion individually unique identifications. Several sites for insertion are being investigated. With the use of electronic identification, the potential applications in production management, quality assurance, disease surveillance, etc. are unlimited. The application of the device is simple with
very little stress to the animal and minimal tissue response. When properly applied, the implant is permanent and secure and can be easily removed at slaughter.

Dr. Warren Foote, Utah State University, reported on progress of work conducted under the Cooperative Agreements on Scrapie with USDA,APHIS, Veterinary services. Scrapie has not been transmitted using embryo transfer from naturally or experimentally infected donor females or by artificial insemination using semen from experimentally infected donor rams. Research underway includes transmissibility of scrapie from contaminated premises, the use of DNA markers to identify sheep with alleles for long and short scrapie incubation periods, and the earliest age at which scrapie can be detected in mesenteric lymph nodes using Prp analysis. Approval for importation of embryos and semen is contingent upon the results of these studies, expected to be published in 1992.

The chairman read a letter to the committee from the American Association of Small Ruminant Practitioners requesting that llamas be changed from the Committee on Infectious Diseases of Cattle, Bison, and Llama to the Committee on Sheep and Goats. After discussion, the committee voted to recommend that llamas not be added to the Committee on Sheep and Goats, which already has a full agenda, because there would not be enough time to address the concerns of all three species.

The committee passed three resolutions and made one recommendation. The resolutions were: 1) the conditional use of the California bluetongue vaccines in bluetongue endemic areas of Idaho and Oregon; 2) the establishment of a protocol to permit controlled importation of sheep genetic material and live animals into the U.S. and 3) the use of immunoblotting as a supplemental test for Scrapie. The recommendation was that llamas not be added to the Committee on Sheep and Goats.
TUBERCULOSIS IN A CALIFORNIA BULL FIGHTING HERD OF MEXICAN ORIGIN

Dorothy Davidson-York, D.V.M.
D.F.A.–Animal Health Branch
State of California

The California Department of Food & Agriculture Animal Health Branch has in recent years been involved with two tuberculosis infected breeding herds directly related to the importation of Mexican origin cattle. In February, 1989, a breeding Brahma herd consisting of 66 animals was tuberculosis tested and subsequently found infected with tuberculosis. These cattle had been exposed to thousands of Mexican stocker steers on the ranch during the preceding four years. Three of these stockers had previously been found infected with tuberculosis at slaughter. This case strongly implicated Mexican origin steers as a source of M. bovis infection in native cattle. Dr. Dennis Thompson presented this case before this Tuberculosis Committee meeting in 1989.

Our most recent case involves a cow-calf herd of approximately 150 Mexican fighting cattle, or "Ganado de Lydia," the name derived from the region of Spain where the breed originated. They are also known as "Saltillo" cattle, named for a region in Mexico known for fighting cattle. "Bloodless" bull fights are becoming a very popular spectator sport in California, especially within the Portuguese ethnic group, and bulls for this sport are in increasing demand. Presently, there are ten known breeding Lydia herds in California, eight of which are owned by dairymen and are kept on the same premises as the dairy cows. Two of these herds are located in Tulare County, home of the largest population of dairy cows in California, numbering nearly one-half million.

In July 1990, 47 yearling and 2 year-old Lydia bulls were routinely caudal fold tested in Madera, California for sale to Canada. Two yearling bulls reacted to the caudal fold test, and the subsequent comparative cervical test also yielded reactor responses. Upon slaughter, both were found to have lesions in the lungs and bronchial lymph nodes compatible with mycobacteriosis and culture attempts yielded M. bovis. These two bulls had been purchased one month previously as a lot of 23 yearlings from a breeding herd in New Cuyama, California.

Fortunately, the New Cuyama herd is not housed on a dairy. The owner breeds and trains Spanish Andalusion horses, grows alfalfa, and maintains a herd of approximately 140 Lydia cattle on an isolated 3000 acre pasture. He began importing Lydia cattle in 1986 from a single ranch in Chihuahua, Mexico. Between 1986-1990, sixty head of breeding stock were imported through El Paso, Texas and shipped to New Cuyama. Federal documents (VS 17-30) confirm that these cattle were tested for tuberculosis at the border quarantine station, although none entered California with
permits, and existing State and Federal regulations do not require a tuberculosis retest after entry. Eight head had also been purchased in 1988 from a Lydia breeder/dairyman from Tulare, California. No other purchases were confirmed and the only sale had been the 23 yearling bulls.

Due to the poor facilities and the temperament of the cattle it was decided to initially use the caudal fold test for the New Cuyama herd. In October, 1990, 138 head were tested, yielding 9 reactors. Six of these had histologic lesions typical of tuberculosis, and M. bovis was isolated in three. Three of these reactors were purchased from the Tulare herd in 1988, five were of Mexican origin and one was born in New Cuyama. Simultaneously, the Tulare herd of 94 head and the Madera herd of 67 head were tested, also using the caudal fold method. No reactors were discovered. The New Cuyama herd was quarantined.

We then instituted the single cervical test in November, 1990, testing 158 head. Thirty-one reactors were removed, seven with histologic lesions typical of Tuberculosis. M. bovis was isolated from six of the reactors, four of which had no histologic lesions. This point is significant, due to the fact that these animals would not have been detected as tuberculosis lesioned animals during normal slaughter house inspection procedures. One of the reactors, cow #3800, bearing Mexican eartag #CHB241720, and brand AB LH, had by far the most extensive lesions of any of the reactors to date. It is believed that this cow was imported from Mexico in 1988.

In December, 1990, 130 head were tested and 12 reactors were removed. One had histologic lesions but all were culture negative. The 4th test was in February, 1991, 120 head were tested no reactors found. Due to weather conditions and logistics, the 5th test was postponed until July, 1991. One hundred and twenty-seven head were tested with six reactors, no lesions and no culture positives. The 6th test was performed in September, 1991 with one reactor and no histologic lesions. Culture results are pending. The next test is scheduled for November 12, 1991.

It is now felt that the infected cattle have been removed successfully from this herd. The last lesioned animal was removed in December, 1990 and the last culture positive animal was removed in November, 1990. The owner will not consider depopulation at this time, although one-third of his herd has been destroyed over the last 12 months. At least two more negative herd tests will be required before this herd will be released. This cautious approach is considered appropriate because there is essentially no culling occurring and because of the high mobility of the fighting bulls once the quarantine is ended.

The evidence overwhelmingly implicates the Mexican origin Lydia cattle as the source of this infection:

1) The Tulare-purchased herd additions were from a negative herd and subsequently became infected in New Cuyama.

2) No sales had been made from the New Cuyama herd except the
23 yearling bulls to the Madera herd. The Madera herd tested negative.

3) The New Cuyama herd has had no contact with other cattle.

There is a clear need to modify in some way tuberculosis test requirements to minimize further introduction of tuberculosis through Mexican cattle, especially when there is a dangerous potential of transmission to the California dairy population.
Predictions in the FY 1990 status report regarding recurrence of tuberculosis in large El Paso milkshed dairy herds were borne out in 1991. Of the five tuberculosis-infected dairy herds not depopulated in 1985, but released from quarantine in 1987, all were again quarantined for tuberculosis during FY 1991. One of these herds has been found for the third time to be still infected. This outcome emphasizes the need for infected herd depopulation and raises the question of why depopulation cannot be achieved. Funds needed to depopulate these large dairy herds are substantially greater than in the past, but even if such funds were available it is doubtful that owners would depopulate because the allowable rates for indemnity are insufficient to meet the cost of replacement dairy animals. As the result, approximately 18,000 tuberculosis exposed cattle in 10 dairy herds remain under a test and slaughter program, whereas only 636 cattle in 4 beef herds were depopulated in FY 1991. It would appear the most realistic solution to this problem is for industry to assume a more substantial role in financially supporting the bovine tuberculosis eradication program.

Bovine tuberculosis in captive Cervidae became an important issue in the United States in 1991. Tuberculosis investigations of captive deer and elk herds began following reports from Canada that certain United States herds may have been sources of tuberculosis in Canadian herds. Bovine tuberculosis has since been confirmed in a United States captive elk herd epidemiologically linked with an infected Canadian elk herd. Efforts continue to determine the extent of the problem in the United States and to find other possible sources of infection. The tuberculin testing procedure was modified for cervids in December 1990 making the single strength cervical test the initial test in lieu of the caudal fold tuberculin test.

The Animal and Plant Health Inspection Service (APHIS) hosted an International Conference on Bovine Tuberculosis in Cervidae in Denver, Colorado, July 16-17, 1991. Recommendations were formulated for the management of the disease in the United States captive cervid industry. These resulted in the tuberculosis testing procedure being further modified and APHIS joining Agriculture Canada in an effort to evaluate the Blood Tuberculosis Test (BTB) in cervids. The BTB test is a product of the Deer Research Laboratory located in Dunedin, New Zealand, reportedly having high specificity and sensitivity for the detection of tuberculosis in these species.

The United States had its first tuberculosis infected cattle herds
resulting apparently from association with tuberculous elk. Tuberculous elk had been shown to be the source of the 1985 bovine tuberculosis outbreak in bison, which resulted in 24 infected bison herds located in 10 States. These events clearly show the potential role of captive Cervidae in the epidemiology of bovine tuberculosis in the United States involving not only domestic livestock, but also wildlife.

Little activity can be reported for the United States–Mexico joint effort for bovine tuberculosis eradication. After 2-years of effort, the first consignment of laboratory equipment obtained through the General Services Administration excess property system is scheduled to be officially transferred to the Mexican Government October 1, 1991. With this breakthrough, we anticipate that subsequent shipments will move more freely. The original goal of equipping 2 animal health laboratories for tuberculosis examinations has been extended to equip one or more laboratories of Mexico's national university system.

There were 243 slaughter traceback cases of feedlot cattle completed during the fiscal year of which 188 (77 percent) traced to Mexico by "M" brand reported, eartag recovered or traceback information developed. This is significantly higher that all other previous years in which the annual average has been 66 percent since 1982 (Figure 6). This coincides with a high of 1,126,883 "M" branded steers imported from Mexico during 1990. Steer importations again exceeded the previous high with the importation of 1,185,676 steers in FY 1991.

Food Safety and Inspection Service (FSIS) continues to support the Mexican tuberculosis eradication program by providing identification; making possible tracebacks to Mexican herds of origin. During FY 1991, a total of 34 official Mexican eartags were recovered from steers at slaughter with lesions confirmed tuberculous by laboratory examination. These cases were forwarded directly to Mexico for investigation. In all, 119 official Mexican eartags were collected from suspicious tuberculosis cases detected at slaughter inspection in FY 1991.

A new test for the detection of bovine tuberculosis in cattle and bison has been developed by IDEXX Corporation. This is a hematological assay for the preferential production of gamma–interferon with reportedly greater sensitivity and specificity than the standard tuberculin skin tests. The gamma–interferon test kit is currently being field evaluated for possible use in the national program. Other than tuberculin skin tests, this is the first test in program history to be considered for official tuberculosis program status.

Bovine tuberculosis was confirmed for the first time in a llama herd managed under agricultural conditions, i.e., farmed llamas. A small M. bovis infected llama herd in eastern Iowa was acquired by the International Llama Association for a joint developmental study with APHIS on the pathogenesis of bovine tuberculosis in farmed llamas naturally exposed to M. bovis. The study contributes scientific information from which methods can be developed
for the management of bovine tuberculosis outbreaks in farmed llamas.

Figure 1. Forty States plus the Virgin Islands have Accredited–Free status meaning they have had no M. bovis confirmed herds in at least 5 years and meet or exceed all tuberculosis program standards. Nine States plus Puerto Rico have Modified Accredited status. Nebraska's Free status was suspended following the discovery of a single M. bovis confirmed herd. Nebraska's Free status will be reinstated if a complete investigation of this outbreak reveals no evidence of spread.

Figure 2. There were 16 tuberculous herds (13 infected and 3 exposed) recorded in the United States during FY 1991. These included 10 herds newly detected in FY 1991 and 6 herds carried over from the previous fiscal year. The carryover herds included 2 dairy herds each in Texas and Puerto Rico, 1 dairy herd in New Mexico, and 1 beef herd in Oklahoma.

A cluster of 8 infected dairies in the El Paso area of Texas and New Mexico was the focus of activity for the year with regard to funds, time, and effort expended. Five of these dairies are undergoing test and slaughter procedures for the second or third time since being found infected in 1985. Tuberculosis was confirmed in a California beef herd tested for movement of bulls to Canada. The breeding cattle in this herd were imported from Mexico about 10 years ago for the purpose of raising rodeo type bulls. Two infected beef herds were found in North Carolina as the result of a traceback from slaughter and subsequent investigations. The 2 herds in Nebraska were found as the result of investigation following the detection of a tuberculous cow on regular slaughter inspection. One of these herds was apparently infected by association with tuberculous elk formerly located on the premises. The other herd which is adjacent to the infected herd is considered exposed. In 1988 these elk were moved to Montana where they may have been the source of infection for another farmed elk herd. The elk from the Nebraska herd were eventually traced to Colorado where tuberculosis was confirmed and the herd depopulated in June 1991. Most of these elk had tuberculosis lesions that in general were much more extensive than are usually seen in cattle.

Figure 3. shows how the 10 new herds were detected in FY 1991. Two herds were detected by direct traceback from lesioned cattle found on regular slaughter inspection and three additional infected herds were found by subsequent epidemiological investigations. Three herds were found infected as the result of annual post-quarantine retests of formerly infected herds. Two herds were detected as the result of routine testing for change of ownership.

Figure 4. depicts the 10-year history of tuberculosis infected and exposed herd detection by comparing epidemiologic methods to routine testing.
procedures. Of the 123 tuberculous herds detected during this period, 118 (96 percent) were detected epidemiologically; i.e., through slaughter traceback, tracing exposed cattle, testing possible source herds, adjacent and contact herd testing, post-quarantine annual retesting and designated high-risk area testing. Only 5 herds during this 10 year period were detected by routine testing such as to comply with milk ordinance requirements or for movement or sale. This is a clear illustration of our national surveillance as it has evolved since 1965 from area ("down the road") testing, to slaughter surveillance coupled with epidemiological investigations.

Figure 5. A total of 258 slaughter traceback cases were completed in FY 1991. Fifteen (6 percent) of these involved adult cattle. Two adult cases successfully led to herds not previously known to be infected. One adult bull led to a known infected herd. Three of these lesions proved to be caused by mycobacteria other than M. bovis. i.e., M. avium was isolated from 2 lesioned adult slaughter cattle and 1 cow had subcutaneous lesions that proved to be the injection site of M. paratuberculosis bacterin. Also, 1 adult case was traced to Canada. Realistically, only 8 of the 15 adult cases (53 percent) were actually unsuccessful. Unsuccessful cases are deeply disturbing in that these are the cases most extensively investigated, sometimes taking years before closing. Important program goals for reducing unsuccessful cases include improving the collection of identification devices at slaughter and correctly correlating these with the affected carcass, and improving our investigating and herd evaluation skills.

Feedlot cattle traceback from slaughter made up 243 (94 percent) of the 258 cases closed in FY 1991. Of these, 224 cases (94 percent) were successful traceback in that they led to a specific feedlot group of origin which were then known to be tuberculosis exposed. Tuberculosis exposed feedlot groups are investigated for possible epidemiologic linkages and move directly to slaughter under permit.

Figure 7. Only 4 herds were depopulated in FY 1991 of the 16 herds reported. All were small beef herds having a total of 650 animals. The deterrents to depopulation are almost entirely economic as discussed in the opening paragraphs of this report. Although 25 percent of infected herds were depopulated, they contained only 4 percent of the total tuberculosis exposed cattle.

Figure 8. gives the 10-year history of herds depopulated. Between 1982 and 1991 there were 123 tuberculosis infected and exposed herds discovered. Of these, 99 (80 percent) were depopulated leaving 24 herds not depopulated.
During this same period in which 24 herds escaped depopulation, 11 herds formerly infected were found again to be infected 2 or more years following their release from quarantine. The recrudescence rate for bovine tuberculosis has been estimated at about 33 percent for many years. The recent advent of large infected dairy herds has contributed to increasing the recrudescence rate to almost 50 percent and can only increase this rate further with time.

Slaughter surveillance improved in FY 1991 over last year with 3,833 lesions submitted, of which 293 (7.6 percent) were found tuberculous on laboratory examination. This is the greatest number of positive cases detected on slaughter in a single year in the history of the program.

The history of slaughter surveillance performance is shown from 1965, the year that the program emphasis changed from area testing to slaughter surveillance, to the present. The APHIS–FSIS, bovine tuberculosis incentive awards program was established in 1975, whereby meat inspection personnel qualify for modest cash awards for submitting tissues found tuberculous. If an infected herd is found, the meat inspectors qualify for a second, more substantial award. The submission rates since 1989, though impressive, are still substantially lower than deemed optimum for that level of surveillance needed to achieve the eradication of tuberculous. The target rate is 1 granulomatous lesion submission per 2,500 cattle inspected. APHIS and FSIS continue to work closely together toward this goal.

gives the location of captive cervid herds in which *M. bovis* has been confirmed or results are pending. The focus of tuberculosis activity in elk lies in Montana with 3 confirmed herds and 1 elk herd pending. Numerous movements of tuberculosis exposed deer and elk are being traced in Montana and several other States. Seven additional infected cervid herds were located; 1 each in Idaho, Colorado, Nebraska, Oklahoma, Texas, Wisconsin, and New York. One herd in Washington is pending confirmation. There are no federal authorities for restricting the interstate movement of Cervidae with tuberculosis. Quarantine, testing, indemnities, and movement restrictions for Cervidae are entirely under State authorities.

Issues deterring eradication of bovine tuberculosis in the United States, though formidable, are considered manageable. Program emphasis and improvements are needed for managing tuberculosis exposed animals and controlling their movements both interstate and internationally. Improved investigations and strict application of program policies and principles will be required. Finally, the issue of bovine tuberculosis in Mexico must be dealt with realistically. Though immediate measures are deemed necessary to reduce exposure potential in the interim period, the real solution lies in joining with Mexico for the eradication of bovine tuberculosis from both countries.
Tuberculosis Eradication

Bovine Tuberculosis State Status
September 30, 1991

- Accredited free states (40) plus Virgin Islands
- Modified accredited states (9) plus Puerto Rico
- Free status suspended (1)

Location of 16 Tuberculous Herds
FY 1991

- INFECTED (13 HERDS)
- E EXPOSED (3 HERDS)
- Carried over from FY 1990
**Tuberculosis Eradication**

**Methods of Locating 10 Newly Detected Tuberculous* Herds FY 1991**

- **Traceback of regular kill (2) cattle**
- **Epidemiologic tracing (3)**
- **High-risk herd test (3)**
- **Routine test (2)**

*infected and exposed

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**Tuberculosis Eradication**

**Detecting Methods; Epidemiologic vs. Routine Testing**

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**Total herds found - 123**

- Found by epidemiologic methods - 118 (96%)
- Found by routine testing - 5 (4%)

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521
Tuberculosis Eradication

Results of 258 Slaughter Traceback Investigations Completed in FY 1991

15 Adult Cattle Cases
- Traced to Canada (1)
- Traced, M. paraTB (1)
- Traced, M. avium (2)
- Traced, herd known (1)

243 Feedlot Cattle Cases
- 53% Unsuccessful (8 cases)
- 94% Successful (229 cases)
- 6% Unsuccessful (14 cases)

*Successful adult investigation:
- Traced to adult herd not previously known to be infected

Successful feedlot investigation:
- Traced to specific feedlot group of origin

Reasons for Closing 243 Feedlot Cattle Cases FY 1991

Traced to Mexican origin
188 (77%)

Other reasons for closure
55 (23%)
**Tuberculosis Eradication**

**Proportion of Tuberculous* Herds Depopulated FY 1991**

4 Herds depopulated
16 Tuberculous herds

* Infected and exposed

**Tuberculosis Eradication**

**Tuberculous* Herds Newly Detected vs. Herds Depopulated**

Fiscal Year 1982-1991

![Graph showing the number of herds detected and depopulated from 1982 to 1991.]

* Infected and exposed
**Tuberculosis Eradication**

**Tuberculous Herds Not Depopulated vs. Former Herds Found Still Infected**

**FY 1982-1991**

- Herds not depopulated - 24
- Herds formerly tuberculous again found infected - 11 (48%)

**Suspicious Lesions Submitted From Regular Slaughter**

**FY 1982 - FY 1991**

- Total lesions submitted
- Lesions of tuberculosis
Tuberculosis Eradication

Suspicious Lesions Submitted From Regular Slaughter
Fiscal Year 1966-1991

Thousands

- Onset of APHIS, FSIS bovine TB Incentive awards program
- Lesions of TB
- Total Lesions Sub.

Bovine Tuberculosis in Cervidae
FY 1991

- Infected herds 10
- Pending 2
PAST AND PRESENT MEASURES TO PREVENT TRANSMISSION OF BOVINE TUBERCULOSIS FROM MEXICAN CATTLE

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Animal Health Branch
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State of California

I'll start with a quote by Dr. P. L. Smith, former Chairman of this Committee. In a letter to Dr. LaBranche in May, 1991 he said "The focus of attention in tuberculosis lately has been the threat of infection in species other than cattle. I do not want to minimize the potential danger to cattle by exposure to cervidae, bison, game farm ungulates, private zoological collections, etc. These are a threat and deserve our collective concern. We are concerned they may expose our domestic livestock and that we do not have the testing knowledge or legal authority to deal with the problem. I believe we have identified the problem and are paying it the proper attention. The bigger threat to the successful eradication of bovine tuberculosis is the ex-Mexican cattle situation. This is a situation where exposure of domesticated livestock does occur (not may occur). We have the testing knowledge to cope with eradicating the disease.....However, we are not giving the problem the proper attention...........In order to achieve the eradication of bovine tuberculosis, we cannot detour around the most significant detriment to that accomplishment".

Mexican cattle have been recognized as a major obstacle to eradicating bovine TB for over 10 years. Yet these problems still loom as big or bigger than 10 years ago. As one more effort to address this problem Dr. P. L. Smith sent a letter to all State Veterinarians and some industry organizations. Most of you have received a copy of that letter and copies are available here also.

I'd like to briefly describe the response we received to that letter and to review some of the past activities of USAHA as a means of providing background information for the resolution being proposed.

We received replies from 37 states and six organizations. The organizations were the American Association of Bovine Practitioners, American Farm Bureau Federation, American Veterinary Medical Association, Holstein Association, National Cattlemen's Association and the USAHA.

Some examples of responses from organizations were: The AFBF expressed their concern, and a willingness to sponsor a meeting at the border to view and discuss problems. The AABP urged adoption of the regulations proposed in the letter. The NCA urged that a blood test be adopted for testing imported cattle and that lots containing positive cattle be rejected. The NCA also said decisions should be based upon science not politics or economics. The Holstein Association supported the proposed
PREVENT TRANSMISSION OF BOVINE TUBERCULOSIS

measures. The AVMA stated that increased requirements are needed but they couldn't comment on the specific proposals at this time.

Replies from the states expressed a general feeling that increased requirements are needed, and that Mexican cattle are a threat to the United States. Many states pointed out that except for roping steers they don't receive Mexican cattle but do receive cattle that could have been exposed to Mexican cattle elsewhere. This threatens their state and the national program and therefore they thought more should be done on a national basis. Arizona was an exception and felt that each state should protect itself. Some states such as Montana and Minnesota expressed support but questioned the administrative feasibility of the proposals. Dr. Hartin, from Oklahoma, expressed strong support and reminded us of his introducing USAHA Resolution No. 40 in 1989 that urged U.S.D.A. to provide specific information about all imported Mexican cattle to State Veterinarians.

A few states (Oklahoma, Louisiana et. al.) expressed concerns that the threat from Mexican cattle would increase with a Free Trade Agreement. Dr. England of New Mexico thought the proposed requirements could be a major step forward but would still only be "a band aid approach where major surgery is needed". Georgia pointed out that it already has a retest requirement for all Mexican cattle imported there.

I'd now like to very briefly highlight some of the past actions of USAHA to provide a context as to why the proposed Resolution is worded as presented.

In 1982 a report by Doctors Essey and Searles stated that the percentage of positive carcasses found at slaughter and originating from feedlots increased from 32% of positive carcasses in 1978 to 96 percent in 1982. The Committee recommended that a post entry retest be required and that the ultimate solution would be to eradicate Bovine Tuberculosis from Mexico.

In 1983 USAHA passed Resolution #7 which called for U.S.D.A. and USAHA to create a special committee to formulate requirements to safeguard the U.S. cattle population. Drs. Essey, Smith and Searles reported that Mexican Cattle were the primary source of tuberculous carcasses from 1978 through 83. They recommended that import regulations be strengthened and stated that the true solution is to eradicate TB from Mexico.

In 1984 a subcommittee recommended that a joint Committee with Mexico work to eradicate TB from Mexico.

In 1985 the committee unanimously urged U.S.D.A. to meet and work with Mexico to eradicate TB from Mexico. USAHA passed Resolution 21 which recommended requiring an M. brand and Resolution 22 urged that Mexican cattle be tested by U.S. officials and then retested in six months. M. bovis was reported for the first time in steers of Mexican origin tested for international shipment from the United States.

In 1986 USAHA passed Resolution 36 which asked U.S.D.A. to
establish a joint U.S./Mexico meeting aimed at decreasing the threat to U.S. cattle and urged U.S.D.A. to implement Resolution 21 from the previous year (1985).

In 1987 the USAHA passed Resolution 40 which urged U.S.D.A. to implement Resolution 36 from the previous year (1986) and report to USAHA about that. A requirement to brand cattle imported from Mexico with an M on the jaw was incorporated into the CFR in March, 1987. It was reported that from 1983 to 1987, 66% of the compatible tissues from feedlot cattle were of Mexican origin.

In 1988 a report about a joint U.S./Mexico meeting described a Mexican proposal for a 12 year plan to eradicate M. bovis. The proposal was stymied because it was based upon the U.S. paying for 80% of the program.

In 1989 Resolution 40 from the Import/Export Committee asked that U.S.D.A. provide State Veterinarians with specific details about shipments into their state prior to shipment. The Committee heard a report of the first confirmed transmission of M. bovis from cattle of Mexican origin to native U.S. cattle. It also heard a report of the third joint U.S./Mexico meeting which stated that the true solution to the problem in the U.S. is eradication in Mexico. The Committee was informed that in FY 1989 suspicious samples from slaughter jumped 89% to a new recorded high of almost 4500. An M brand was reported on only 202 of these (4.5%). Only 23 of the 270 positive samples had an M brand reported despite the fact that 167 of the 270 were traced to Mexico. Mexican eartags were reported from only 21 of the 167 positive carcasses (13%) traced to Mexico.

In 1990 a report by Doctors Essey and Meyer stated that only 7 (6%) of 120 positive carcasses from feedlots had Mexican eartags and only 13% of the 53 positives already traced to Mexico had Mexican eartags. Resolution 32 was passed and asked U.S.D.A. to enact and enforce requirements for Mexican eartags on all imported Mexican cattle.

As of now we have not had a joint U.S./Mexico meeting since 1989. Very little progress is being made to eradicate bovine tuberculosis from Mexico. The M brands and Mexican eartags are not effectively enabling rapid tracing of positive carcasses. State Veterinarians are not being provided adequate information about shipments into their states. Despite ten years of repeated resolutions by this Committee and USAHA, little progress has been made to increase requirements for importing Mexican cattle.

Clearly efforts by this Committee and USAHA have not been successful enough to protect the United States. For that reason, the proposed Resolution asks that U.S.D.A. form a Task Force Committee that includes representatives from many more segments of the livestock industry, than just USAHA. This resolution is proposed with the hope that representatives of the dairy industry and cow-calf operations will express their concerns about Mexican cattle and have more effect on the importers and U.S.D.A. than USAHA has had in the past. By involving several
respected and influential organizations as well as people that stand to be severely affected by the spread of bovine tuberculosis, maybe importers and U.S.D.A. will agree to some of the changes that are needed.
REPORT OF THE COMMITTEE ON TUBERCULOSIS

Chairman: Dr. V.P. LaBranche, Boston, MA
Vice Chairman: Dr. T.J. Hagerty, St. Paul, MN

R.D. Angus, IA; L.R. Barnes, IN; R.D. Buckmaster, IA; T.F. Conner, IN; M.A. Essey, MD; R. Frost, CA; G.H. Frye, MD; D.E. Hensel, CO; B.R. Hillman, ID; E.M. Himes, TX; S.B.S. Hurley, WI; J.L. Jarnagin, IA; C. L'Ecuver, Can; H. Lloyd, FL; C.W. McGinnis, NH; A.R. McLaughlin, WI; R.M. Meyer, CO; S. Musick, MT; M.E. Oetting, MO; W.J. Owen, IA; J.B. Payeur, IA; J.O. Pearce, Jr., FL; J.T. Prichard, NM; W.A. Rotenberger, ND; N. Stirling, SD; C.D. Stumper, KS; R.L. Tharp, MO; C.O. Thoen, IA; D.L. Thompson, CA; D.K. Thorpe, SD; R.D. Walker, KS; D.L. Whipple, IA; B. Widger, NY; S. Withiam, OK; R.E. Yoxheimer, PA

The Tuberculosis Committee of the United States Animal Health Association met at 1:30 p.m. on Wednesday, October 30, 1991, at the Town and Country Hotel in San Diego, California. Ninety-nine participants attended the committee meeting. Eighteen committee members were present. Dr. T. J. Hagerty, Vice Chairman, conducted the meeting in the absence of the Chairman, Dr. Victor LaBranche.

Fourteen presentations were made to the Committee.

Dr. Mitchell Essey described the status of the State–Federal Bovine Tuberculosis Eradication Program in FY 1991. He pointed out that no joint U. S./Mexico efforts against tuberculosis in Mexico are in progress, except for efforts to equip two laboratories. He reported that there were 16 tuberculous herds in the United States in FY 1991. Thirteen were infected and three exposed. Ten were newly diagnosed in FY 1991, and six were carried over from the previous year. Only four of the 16 were depopulated. These four were small herds which accounted for only 4% of the total tuberculous cattle. In the last ten years, 24 infected herds have not been depopulated, and infection was diagnosed two or more years after release from quarantine in 11 of those 24 herds.

There were 293 positive samples submitted from surveillance of carcasses. This is the highest number in a single year in the history of the program.

Two hundred forty-three investigations of positive samples were completed, and 77% were traced to Mexico. This percentage is significantly higher than average and reflects the record number of cattle being imported from Mexico.

Dr. Essey reported the first confirmation of bovine tuberculosis in llamas raised in agricultural conditions. He also summarized information about infected herds of cervidae in the U. S.

Dr. Dorothy Davidson–York reported about an infected California
REPORT OF THE COMMITTEE

herd of fighting bulls of Mexican origin. This herd was initially formed from
60 animals legally imported through El Paso, Texas', six years ago. This
herd is the second confirmed example of transmission from ex-Mexican
cattle to native U. S. cattle reported in the last two years. She also showed
some dramatic slides of other fighting bull herds at California dairies which
were pastured adjacent to Holstein heifers.

Dr. Dennis Thompson summarized past activities to address the
threat posed by Mexican cattle and discussed some current proposals to
address this threat.

Dr. Chris Boland reported that the presence of M. bovis in New
Zealand's "Australian Bushtail Possum" population has stopped progress of
their national eradication program. He reported that the single most
important factor determining the number of bovine reactors is the level of
possum control. Possum control is now only being done where it is cost
efficient, until better tools become available.

Dr. Essey reported about ten infected herds of cervidae in the U. S.
during FY 1991. There is also action pending on two herds where owners
will not permit further testing or necropsy due to a lack of indemnity. In late
1990, U. S. policies for testing cervidae were revised to establish the single
strength cervical test as the test of choice in herds of unknown status. Dr.
Essey indicated that c.c. testing at the time of reading or within seven days
of injection of the ssc antigen produced satisfactory results in cervids. Dr.
Essey also described a joint U. S./Canadian effort to complete test trials of
the BTB (LBA) test in more than 1,000 elk.

Dr. Mike Miller described an infected herd of elk where approximately
1/2 of the herd had tuberculous lesions and M. bovis was cultured from
approximately 1/3 of the depopulated herd. A complete case report of this
herd is contained in the report of the Committee on Wildlife Diseases.

Dr. Maria Koller, with Agriculture Canada, reported about infected
herds of cervidae, bison and llama in three provinces. Canada paid
$11,000,000 in indemnity on these animals. A number of these cases were
attributed to animals of U. S. origin, and some of the spread has been
attributed to infected cervidae from New Zealand and fallible testing of those
imports. Canada is considering the revision of their import policies.

Dr. Don Ferlicka reported to the committee on the occurrence of
bovine tuberculosis in ranched captive elk and fallow deer in Montana.
Montana's animal health laws and regulations were described. They provide
very broad authority to prevent and eliminate animal diseases that can affect
livestock.

In 1990, an outbreak of bovine tuberculosis in game farm elk in
Canada was traced to an elk exported from Montana two and onehalf years
earlier. The epidemiologic report linking this elk to Montana led to extensive
testing of ranched elk, which was done in consultation with USDA and Dr.
Charles Thoen. After testing a large number of ranched elk and fallow deer,
Dr. Ferlicka reached the following conclusions:

Single strength cervical and comparative cervical skin tests are valuable and cost effective for identifying infected and noninfected ranched elk and fallow deer herds. The comparative cervical test can be applied at the time of reading the single strength cervical test. Two negative double strength cervical tests 90 days apart are sufficient for quarantine release and should be followed with annual retests.

Dr. Essey reported that a field evaluation of the gammainterferon test kit (IDEXX) for the diagnosis of bovine tuberculosis is in progress. It will determine whether official test status is warranted for use in the national tuberculosis eradication program. The objectives are to determine its value as a supplemental test for freeing infected herds of tuberculosis and for high-risk herd evaluations. To date, the test has been applied to five herds having about 10,000 cattle. About 19 other herds in various categories are still needed to provide 8,000 cattle for further evaluation.

Dr. Charles Thoen described efforts for new techniques to test cervidae. He emphasized that collaborative efforts are needed that will include many groups.

Dr. Bob Bokma summarized efforts against bovine tuberculosis in Puerto Rico. They are making steady progress, had only three infected herds last year, and are being aided by the Southeastern Tick Eradication Program.

Dr. J. Hernandez reported about research of four antigens for use in an ELISA test. The predictive value of the tests may have value in areas with high prevalence.

A subcommittee reported that the gamma interferon test should be evaluated much more before being considered for approval as an official test in the national program.

Four resolutions were passed.
SUMMARY

Epizootiological and diagnostic investigations revealed extensive bovine tuberculosis infection in a herd of 50 captive elk (Cervus elaphus nelsoni) held on a game ranch near Powderhorn, Colorado. Eleven of 18 elk responded to a single-strength cervical (SSC) tuberculin skin test. We then classified ten of eleven responders as bovine tuberculosis reactors using a comparative cervical tuberculin skin test applied 4 days after reading the SSC test. Tuberculous lesions were observed in lungs and/or lymph nodes in seven of the ten reactors, and Mycobacterium bovis infections were confirmed by histopathology and culture. Based on these findings, the entire herd was ordered destroyed. We observed tuberculous lesions in 19 of 26 (73%) captive elk ≥ 2 yrs old examined at depopulation, including one animal that had tested SSC-negative; none of seven yearlings showed tuberculous lesions. Overall, at least 69% (25/36) of resident elk ≥ 2 yrs old examined during the course of investigation and control programs were infected with bovine tuberculosis based on histologic lesions and/or culture of M. bovis. Additional monitoring has been planned to assure that bovine tuberculosis has not infected free-ranging deer and/or elk populations in the Powderhorn vicinity. The apparent duration and severity of tuberculosis in this captive elk herd suggest game ranches throughout the United States represent potential reservoirs for bovine tuberculosis that may threaten the success of national eradication efforts.

12 Colorado Division of Wildlife, Wildlife Research Center, 317 W. Prospect Road, Ft. Collins, CO 80526.

13 Colorado Department of Agriculture, Animal Industry Division, 700 Kipling Street, Suite 1100, Lakewood, CO 80215–5894.


15 Colorado Division of Wildlife, Commercial Wildlife Parks Program, 50633 Highway 6 & 27, Glenwood Springs, CO 81601.
BOVINE TUBERCULOSIS IN CAPTIVE ELK

INTRODUCTION

Bovine tuberculosis represents a significant threat to human and livestock health worldwide. In the United States, the State–Federal Bovine Tuberculosis Eradication Program has virtually eliminated tuberculosis from cattle populations (Essey and Meyer 1990). Despite the overall success of this program, however, foci of infection apparently persist in nondomestic ruminant species exempt from federal regulation. An extensive tuberculosis outbreak in bison in 1983–84 prompted inclusion of this species in the federal program, but other native and exotic wildlife species propagated in captivity for commercial purposes remain unregulated. Lack of effective regulation of tuberculosis in captive wildlife, inadequate and sometimes misleading testing procedures in these species, and exponential growth of wildlife commercialization nationwide have collectively created a situation that threatens to compromise the efficacy of the entire eradication program unless prompt actions are taken. Here, we report an outbreak of bovine tuberculosis in captive elk (Cervus elaphus nelsoni) held on a game ranch in southwestern Colorado; we describe epizootiological and diagnostic observations, as well as management of the infected herd.

HISTORY

The captive elk used to stock a game ranch near Powderhorn, Colorado, were originally purchased from a commercial elk ranch in Corwin Springs, Montana, in March 1987. Of the 30 elk included in that transaction, 24 had been acquired from a ranch in Oconto, Nebraska 2 months earlier, and 6 others apparently originated in Montana; the elk from Nebraska had apparently tested negative for tuberculosis by caudal fold tuberculin tests prior to entry into Montana. These elk (including 12 bulls and 13 cows of varying ages, as well as 5 calves) were released into an approximately 210 ha pasture constructed of 2.5 m woven–wire fence enclosing native sagebrush/grassland and mixed stands of ponderosa and lodgepole pine, located about 4.5 km northwest of Powderhorn, Colorado.

No domestic ruminants cohabited this facility, but a few horses were grazed in the pasture periodically and dogs occasionally roamed the facility. In addition, an undetermined number of wild mule deer (Odocoileus hemionus) fenced in during construction of the enclosure shared range with captive elk. Wild elk and deer ranged outside the facility. Captive elk were maintained on native forage supplemented with grass hay and/or a commercial pelleted feed. Live elk were probably not sold from this operation, and additional elk were probably not acquired from outside commercial sources; however, apparently no herd records were kept.
In late February 1991, this herd was linked via the Corwin Springs operation to an ongoing bovine tuberculosis outbreak involving captive elk in the United States and Canada. The herd was quarantined 4 March 1991 pending completion of whole-herd tuberculosis testing, but lack of adequate animal handling facilities and adverse weather precluded skin testing until late May. No health problems had been noticed in this elk herd since 1987, although the ranch caretaker did report that 4 or 5 animals had died of "old age" during the winter of 1990-91.

Testing began on 28 May 1991. On 31 May 1991, we detected responses in 11 of 18 elk subjected to a single-strength cervical tuberculin skin test administered according to U.S. Department of Agriculture/Animal and Plant Health Inspection Service/Veterinary Services (USDA/APHIS/VS) guidelines for tuberculosis testing in cervids (King 1990). Four days later, the 11 responders were subjected to a comparative cervical skin test applied to the opposite side. On 7 June, we classified 10 of these as tuberculosis reactors using criteria recommended for interpreting tuberculin test results in cervids (King 1990) (Table 1). Among reactors, skin thickness responses to mammalian purified protein derivative (PPD) averaged 3.5 mm greater than avian PPD (range: 0.5 to 15 mm greater). Reactors were both male and female, ranging in age from 2 to 8+ years as estimated from dental replacement and wear. All reactors appeared in good to excellent condition, and bulls showed exceptional antler growth -- the only clinical abnormalities noted were 1) a large (20-25 cm), fluctuant swelling protruding caudoventrally from the right side of a 7-yr-old cow along the caudal border of her ribcage, and 2) a 15 cm lateral swelling at about the center of the left hindquarter of an 8+-yr-old cow.

All ten reacting elk and three dependent calves were killed on 7 June pursuant to Colorado's statutory requirements for disposition of tuberculosis reactors (Colorado Revised Statutes, 1984, §35-50-132). We subsequently necropsied each reactor, examining carcasses for lesions suggestive of tuberculosis and collecting diagnostic specimens. Representative paired samples of lymph nodes and other lesioned tissues, when present, were preserved in sodium borate solution and 10% buffered formalin and submitted to the National Veterinary Services Laboratories (NVSL)(Ames, Iowa) for mycobacterial culture and histopathological examination.

We observed gross lesions regarded as compatible with bovine tuberculosis infection in seven of the ten reacting elk (Table 1). In six of these first seven cases, we detected both pulmonary and lymphatic involvement; lesions in the seventh case were restricted to the retropharyngeal, submandibular, mediastinal, and hepatic lymph nodes. Tuberculous lesions varied widely among cases. These ranged from "granular" foci to caseous, suppurative or liquefactive abscesses to calcified,
BOVINE TUBERCULOSIS IN CAPTIVE ELK

sometimes encapsulated or multi-layered "onion-like" tubercles; lesion diameters ranged from <1 mm to >30 cm. Pulmonary involvement ranged in severity from 0.5–1.5 cm nodules distributed throughout the caudodorsal parenchyma of the caudal lung lobes to fulminating tuberculous pneumonia and pleuritis; in several cases, pedunculated 0.5–10 cm diameter tubercles extended from the costal and diaphragmatic pleura. In the most severe case, a single abscess containing liquified white exudate and mineralized flecks extended from the thoracic cavity through the diaphragm and into subcutaneous tissues overlying the ribcage.

Histopathological examination of tissues submitted from reactor elk revealed mycobacteriosis-compatible microscopic lesions and acid-fast bacteria in all seven animals showing gross lesions, and suspicious lesions in one animal without gross lesions (Table 1). [Detailed descriptions of microscopic lesions will be described elsewhere (Rhyan et al. in prep).] *Mycobacterium bovis* was subsequently cultured from six of seven elk with gross lesions (Table 1).

Based on the foregoing field and laboratory findings, the quarantined elk herd under investigation was deemed extensively infected with bovine tuberculosis. Under joint regulatory authority (Colorado Wildlife Commission Regulations 1990, #008D–E), the Colorado Division of Wildlife (CDOW) and Colorado Department of Agriculture (CDA) consequently identified perpetuation of the diseased state of this captive elk herd as threatening to have "a significant detrimental effect on wildlife and domestic livestock of Colorado." The ranch owner was notified on 20 June 1991 of CDOW's intent to destroy the remaining elk held in the infected facility.
Table 1. Summary of cervical skin test reactions, postmortem and histologic diagnoses, and mycobacterial culture results from captive elk (n = 43) examined during investigation and depopulation of a bovine tuberculosis-infected game ranch herd.

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<td>nd</td>
<td>TB (P)</td>
<td>Mb-C</td>
</tr>
</tbody>
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1 "TB Reactor" tags used to identify carcasses and diagnostic samples during depopulation.
2 Age (yr) estimated from dental replacement wear.
3 Single-strength cervical (SSC) tuberculin test, read 3/31/91: POS = response; neg = no response.
4 Comparative cervical (CC) tuberculin test performed on all SSC-reacting elk, read 6/7/91: POS = mammalian response ≥ avian; neg = mammalian response < avian.
5 Postmortem lesions: TB = lesions suggestive of tuberculosis (L = lymphosis, P = parenchyma, M = mastitis); nsg = no significant gross lesions suggestive of tuberculosis observed (although other lesions may have been noted).
6 Histopathology lesions: Mb = mycobacteriosis (C = compatible, S = suggestive); GRAN = granulomas observed, but stiolology unknown; other = other nongranulomatous lesions noted; naf = no significant histologic findings.
7 Mycobacterial culture results (neg = no Mycobacterium spp. isolated).
8 Not done.
9 Results not available.
10 Animal released from holding pen prior to reading SSC test.
11 Tissues accidentally destroyed prior to testing.
BOVINE TUBERCULOSIS IN CAPTIVE ELK

DEPOPULATION

On June 26, 1991, a total of 32 CDOW, CDA, USDA/APHIS/VS, and other agency personnel participated in an Emergency Tuberculosis Control Program organized and conducted by CDOW and CDA to destroy tuberculosis-infected captive elk residing on the affected game ranch. The program met all planned objectives: 1) all tuberculosis-exposed captive elk and other wild ungulates were eliminated from the facility within 8 hrs; 2) no exposed captive elk or other wild ungulates escaped from the enclosure during the removal operation; 3) postmortem examinations and diagnostic sampling were performed on all adult and yearling ungulates collected in or near the infected premises; and 4) the aforementioned objectives were accomplished in a professional manner that emphasized and assured safety to all participants.

A total of 37 captive elk (26 adults, 7 yearlings, 4 calves) were shot within the enclosure between about 0800 and about 1500; postmortem examinations and sampling were performed on 33 of these ≥ 1 yr of age. In addition to the captive elk, an adult mule deer doe was shot within the enclosure and a yearling elk cow was shot just outside the fence — both were necropsied and sampled. The only clinical signs noted during depopulation were poor condition and exertional dyspnea in a 10+-yr-old cow; otherwise, all collected animals appeared clinically normal and in good to excellent body condition.

All postmortem examinations and sampling were performed by veterinarians from CDOW, CDA, USDA/APHIS/VS, NVSL, and/or the Wyoming State Veterinary Laboratory (WSVL) (Laramie, Wyoming). Upon collection, each carcass from inside the facility was identified with a metal Official USDA "TB Reactor" eartag. Gross observations from each necropsy were recorded on separate forms, along with all identification numbers (including numbers from "reactor", Official ID, and ranch eartags) and other pertinent data. Samples collected for bacteriology, histopathology, and serology were identified with "TB Reactor" numbers. All samples were submitted to NVSL for diagnostic evaluations; select samples were also submitted to WSVL for confirmation. In addition to collecting diagnostic samples, all significant postmortem findings were photographed and/or videotaped.

Upon completion of postmortem examinations, carcasses were hauled to an open earthen pit lined with wooden pallets. Carcasses were layered into the pit, with layers separated by pallets and rubber tires. After all carcasses were added, they were covered with additional pallets and tires. The resulting pyre was burned using napalm. Once all organic material had burned completely, the ashes were covered with lime and buried. All corrals and feeding areas, as well as vehicles, equipment, and the ground around the postmortem and burial sites, were disinfected with a mycobactericidal
disinfectant (One-Stroke Environ®) applied with a high-powered sprayer.

As suspected from initial testing and sampling, postmortem examinations of captive elk collected during depopulation confirmed that this elk herd was extensively infected with tuberculosis. We observed gross lesions regarded as tuberculous in 19 of 26 (73%) captive adult elk (≥ 2 yrs old) examined; one of the lesioned animals had previously been SSC-negative (Table 1). None of seven yearlings showed tuberculous lesions. Neither the wild mule deer nor the wild elk showed gross lesions of tuberculosis.

Postmortem findings during the depopulation resembled those encountered during examinations of reactor elk 3 weeks earlier. Ten of the 19 cases showed only lymphatic lesions, two showed only pulmonary lesions, and the remainder revealed both lymphatic and pneumonic tuberculosis; we observed one case of tuberculous mastitis. As with earlier cases, the severity of infections ranged from subtle lesions in one or more lymph nodes to fulminating tuberculous pneumonia and mastitis. Surprisingly, several older animals had no tuberculous lesions; one bull showed gross evidence of scarring in the caudal lung lobes suggesting successful recovery from some form of pneumonia, possibly tuberculous. However, the wide variety of lesions and severity of some cases we encountered suggested tuberculosis was an extensive and ongoing disease problem in this captive elk herd, thereby justifying measures taken under the Emergency Control Program.

In all, we examined 43 of 50 resident elk during the course of our investigation and control programs. At least 69% (25/36) of elk ≥ 2 yrs old and 58% (25/43) of elk ≥ 1 yr old were infected with bovine tuberculosis based on histologic lesions and/or culture of *M. bovis* (Table 1) (Fig. 1). Of 25 cases, 16 showed both gross and histologic lesions and yielded *M. bovis* on culture; seven others (including the SSC-negative cow) showed gross and histologic lesions but were culture-negative, and one showed only suggestive histologic changes. *Mycobacterium bovis* was cultured from pooled lymph nodes of one 2-yr-old bull in the absence of gross or histologic lesions. Two cases with suggestive gross lesions yielded *M. avium* complex or Runyon Group IV isolates from culture. Based on gross observations, tuberculosis in these elk appeared to originate in the lymphatic and/or respiratory systems, with localized and/or systemic spread subsequently occurring during a prolonged clinical course. We observed some form of gross lymph node involvement in all but 2 of 23 cases showing tuberculous lesions, and pneumonic tuberculosis in 15 of these cases (Fig. 1). Retropharyngeal lymph nodes showed lesions in 13 cases, and in 16 cases at least one cranial lymph node (parotid, mandibular, or retropharyngeal) was lesioned. The latter findings suggest examining cranial lymph nodes in eviscerated elk carcasses could be useful for tuberculosis surveillance in hunter-killed animals.
Figure 1. Gross and/or histologic lesions were observed in lymph nodes and/or lungs from 24 of 36 captive elk ≥ 2 years old examined during investigation and depopulation of a bovine tuberculosis–infected game ranch herd in Colorado. No lesions were observed in subadult individuals.

Although tuberculosis remained undetected for over 4 years, it undoubtedly affected survival and overall performance of these captive elk. Few deaths had been documented in this herd. However, we identified only nine of the 30 founder animals during our activities, and examined only 16 elk ≥ 5 yrs old. Ages of founder animals were unknown, but at depopulation older age classes appeared underrepresented (Fig. 1). Recruitment since 1987 also seemed lower than expected. Our postmortem findings revealed that most elk in this herd developed tuberculosis by 2 yrs of age (Fig. 1), suggesting a majority were probably infected with M. bovis within the first 2 yrs years of life. Judging from the extent and chronicity of several cases we examined, some elk can apparently survive longstanding disease and may shed organisms for several years, thereby perpetuating herd infections. Our observations may provide some insight into mechanisms whereby bovine tuberculosis can spread within and among captive elk herds. They also offer disturbing testament to the potential extent of the bovine tuberculosis reservoir in game ranches throughout the United States.

MANAGEMENT IMPLICATIONS

Colorado's livestock industry currently enjoys tuberculosis–free status under the State–Federal Eradication Program. Moreover, bovine tuberculosis has never become established in native wildlife populations in Colorado or
elsewhere in the United States. The Powderhorn area southwest of Gunnison, Colorado supports significant livestock and wildlife resources. Over 30,000 cattle are grazed on private and public rangelands in Gunnison county. In addition, over 12,000 mule deer and 3,000 elk are estimated to reside in the Powderhorn vicinity, often sharing range with livestock. Revenues from both agriculture and sport hunting contribute to local economies. It follows that maintaining healthy livestock and wildlife populations in the Powderhorn area are important and inseparable pursuits.

We do not believe that bovine tuberculosis has spread to wildlife or livestock herds in the Powderhorn area. However, in light of the apparent duration and extent of the tuberculosis problem in this captive elk herd, as well as the uncertain management history associated with that facility, we have recommended undertaking additional monitoring to assure that bovine tuberculosis has not infected free-ranging deer and/or elk populations in the Powderhorn vicinity. Components of this surveillance program will include 1) additional collections of wild deer and elk in the immediate vicinity of the affected facility, 2) submissions and examinations of road-killed deer and elk in the Powderhorn area, 3) check station and field examinations of harvested deer and elk in game management units surrounding the infected premises, and 4) collection of sera from hunter-killed animals for analysis by serologic assays currently under development. Our approach will incorporate CDOW, CDA, and USDA/APHIS/VS personnel in implementing a monitoring plan for the Powderhorn area over the next several years to assure that bovine tuberculosis has not spread from this infected game ranch to local wildlife and/or livestock populations.

ACKNOWLEDGMENTS

Extensive interagency cooperation was required in investigating and managing this tuberculosis outbreak. Many agencies and individuals contributed significantly to the overall success of these efforts. The Colorado Division of Wildlife and Colorado State Veterinarian's Office thank C. Coghill, R. Lowry, P. Mason, T. Malmsbury, T. Speeze, J. Young, and other CDOW Area 16 and Southwest Region personnel, D. Boyd and Drs. J. Bohlender and J. Malsby of CDA, Drs. G. Kane and P. R. Henry and other USDA/APHIS/VS staff, Drs. D. A. Saari and J. B. Payeur and other NVSL personnel, Dr. E. S. Williams of WSVL, and the U.S. Forest Service for their respective roles in, and the U.S. Marshall's and U.S. Attorney's Offices for full cooperation throughout, this operation. We also thank R.M. Bartmann, and Drs. D.L. Baker, D.L. Hunter, V.F. Nettles, and E.T. Throne for reviewing earlier drafts of this manuscript. Our work was supported in part by Federal Aid to Wildlife Restoration Project W-153-R.
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Rhyan, J. C., D. A. Saari, E. S. Williams, M. W. Miller, A. J. Davis, and A. J. Wilson. _____. Gross and microscopic lesions of naturally occurring tuberculosis in a captive herd of wapiti (Cervus elaphus nelsoni). In prep.
The Committee on Wildlife Diseases met at 1:30 p.m. on Monday, October 28, 1991. Sixty-five members and 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. Recommendations Formed by the Game Farming Workshop in Boise, Idaho.

Dr. David Hunter, Idaho Department of Fish and Game and Idaho Department of Agriculture, reported on a two day workshop held in Boise, Idaho in January, 1991. State wildlife and agriculture agencies, Federal regulatory agencies, Canadian wildlife and agriculture departments, Agriculture Canada and game farm industry representatives participated in discussions of disease, genetic, and competition issues associated with game farming and ranching that possibly could affect native free-ranging wildlife or domestic animals, especially in the western states and provinces. A final publication of suggested model regulations governing disease and genetic considerations for interstate movement of captive commercial wildlife was eventually published and distributed.

Recommended Action:

The Committee on Wildlife Diseases recommends that states developing permitting procedures and import regulations for captive commercial wildlife adopt stringent regulations, such as those suggested in the Boise Report, to prevent the introduction and occurrence of diseases that could threaten native free-ranging wildlife or domestic animals. State wildlife management agencies should work cooperatively with state veterinarians toward that goal.
2. **Bovine Tuberculosis in Captive Commercial Cervidae, an Industry Viewpoint.**

   Dr. Donald Davis, Texas A&M university, summarized the position of two of the United States' major captive commercial wildlife organizations, the Exotic Wildlife Association and the North American Deer Farmers Association, as follows:

   A. The Exotic Wildlife Association and the North American Deer Farmer's Association are concerned about the presence of tuberculosis and other animal diseases in any type of wildlife.

   B. The associations will cooperate in governmental disease eradication and control programs.

   C. The associations request recognition and consideration of the significant differences which exist between the management and husbandry of captive commercial wildlife in farmed and ranched situations and those of domestic livestock before animal disease testing programs are implemented.

   Comments were made from the floor by deer rancher Scott Petty, Jr., Vice President of the North American Deer Farmers, concerning the need for improved tuberculosis tests with greater sensitivity and more widespread applicability to game-ranched animals. The deer farming industry is concerned about handling losses that can occur with skin testing and encourages validation of serologic tests. Mr. Petty indicated that his industry is willing to help implement programs to eliminate tuberculosis from game-ranched animals.

3. **Bovine Tuberculosis and Captive Commercial Cervidae in Montana.**

   Dr. Don Ferlicka, Montana State Veterinarian, reported to the committee on the occurrence of bovine tuberculosis in ranched captive elk and fallow deer in Montana. Montana's animal health laws and regulations, which provide very broad authority to prevent and eliminate animal diseases that can affect livestock, were described. There are 84 licensed game farms with captive commercial elk and/or deer in Montana. Import health standards are imposed by the Montana Department of Livestock on these animals.

   In 1990, an outbreak of bovine tuberculosis in game farm elk in Canada was traced to an elk exported from Montana two and one half years earlier. The epidemiologic report linking this elk to Montana led to extensive testing of ranched elk, which was done in consultation with USDA-APHIS-Veterinary Service and Dr. C. Thoen, Iowa State University. In February
1991, a stop movement order was issued by the Montana Livestock Board until all privately owned deer and elk could be tested for bovine tuberculosis. After testing a large number of ranched elk and fallow deer, Dr. Ferlicka reached the following conclusions:

A. Single strength cervical and comparative cervical skin tests are valuable and cost effective for identifying infected and non-infected ranched elk and fallow deer herds.

B. The comparative cervical test can be applied (opposite side of neck) at the time of reading of the single strength cervical test.

C. The double strength cervical test provides better readability in evaluating problems.

D. Isolation of test positive responders can lead to creating negative herds.

E. Two negative double strength cervical tests 90 days apart are sufficient for quarantine release. This should be followed by annual assurance tests.

F. The only alternatives to the above assumptions are long term quarantine and research until better tools become available or whole herd buy out.

4. Bovine Tuberculosis in a Captive Elk Herd in Colorado

Dr. Michael Miller of the Colorado Division of Wildlife reported to the committee on the diagnosis and depopulation of a captive elk herd near Powderhorn, Colorado that was infected with Mycobacterium bovis. Initial testing of this herd was on May 28, 1991, after the elk were linked with an infected herd in Montana. Subsequent skin testing and necropsies confirmed infection and the entire herd of elk was destroyed and incinerated by June 26, 1991. Gross lesions were present in about 70% of the animals 2 years or older. Fortunately, limited surveillance to date has not revealed any infection in native elk or deer that were in association with the game farm animals. There was potential for infection of native animals through fence line contact, incursion of mule deer into the enclosure, and possibly by escape of elk that were unaccounted for at depopulation. Further details of Dr. Miller's experience are presented as a supplemental publication of this committee report.
Recommended Action:

The Committee on Wildlife Diseases views the occurrence of bovine tuberculosis in captive commercial cervids with a great deal of concern because of threats to native free-ranging wildlife, humans, domestic animals, and the National Bovine Tuberculosis Eradication Program. After much discussion, a consensus was reached by game farm and ranch representatives, wildlife veterinarians, and regulatory veterinarians that ranched cervidae, exotic wildlife and other ranched animals must be included by USDA-APHIS-Veterinary Services under separate sections of the Uniform Methods and Rules and Code of Federal Regulations governing the National Bovine Tuberculosis Eradication Program. A resolution encouraging Veterinary Services to work with the affected constituencies to develop regulations was adopted.

5. AVMA Animal Welfare Forum

Dr. Victor Nettles announced that the American Veterinary Medical Association (AVMA) will hold its Second Annual Animal Welfare Forum on November 7, 1991, in Chicago, Illinois. The theme this year will be "The Veterinarian's Role in the Welfare of Wildlife". The AVMA is becoming increasingly interested in wildlife, and there is little doubt that veterinary rescue activities during oil spills and other newsworthy events produce a good public image. Most AVMA members are practitioners and their perspective tends to be on individual care, rescue, and protectionism. Many veterinarians are poorly informed on population-based health management of wildlife.

Dr. Nettles indicated that it was important that wildlife veterinarians and livestock health officials work collectively to keep the concept of population health management in the minds of the veterinary community. There are numerous examples of diseases and parasites that are shared among wild and domestic species, and wildlife population control often is necessary to ensure livestock and poultry disease protection. The control methods often require hunting, trapping, or habitat manipulations that some veterinarians find objectionable, even though the maintenance of wildlife species at lower densities often reduces disease transmission and malnutrition. In some eradication programs for domestic animal diseases, and certainly in the event of a foreign animal disease introduction, extreme population reduction, or even depopulation, may be indicated to stop infection from becoming a problem in wildlife. Such programs will be difficult to conduct over the objections of a well-meaning but misinformed veterinary organization.
WILDLIFE DISEASES

Recommended Action:

The Wildlife Diseases Committee encourages the USAHA to maintain an awareness of how animal welfare and animal rights issues associated with wildlife could have important repercussions on domestic livestock and poultry health programs. The USAHA should stand prepared to support the wildlife management profession in the use of hunting and trapping as legitimate methods to manage wildlife populations.

6. Brucellosis in the Greater Yellowstone Area, Progress Report

Chairman Dr. Tom Thorne, Wyoming Game and Fish Department, with the assistance of Dr. Don Ferlicka, provided a progress report on activities concerned with brucellosis in the Greater Yellowstone Area of Wyoming, Montana, and Idaho. Known brucellosis infected wildlife populations in the GYA include three interacting bison herds in Yellowstone National Park; the small Jackson Bison Herd that summers in Grand Teton National Park and winters on the National Elk Refuge; elk using 23 winter feedgrounds, including the National Elk Refuge in Wyoming; and elk in and adjacent to Northern Yellowstone National Park, which may be infected at a low level. These populations include as many as 90,000 free-ranging elk and 4,000 free-ranging bison in the largest and most remote complex of national parks, wildlife refuges, national forests, wilderness areas, and private land in the contiguous states. These wildlife commingle with cattle, primarily during summer on grazing allotments on public land and, to a much lesser degree, on private land during winter.

Although the risk of transmission is much greater when brucellosis-infected wildlife and cattle commingle in winter and early spring, cross-transmission is unlikely and has never been confirmed in a free-ranging situation. Brucellosis in wildlife is a cattle disease, probably acquired from cattle early in this century, that is becoming the source of conflicts between wildlife and livestock in the GYA.

Anti-hunting protests over public hunting of brucellosis-infected bison, partially as a means of reducing bison contact with cattle, resulted in the Montana Legislature rescinding its bison hunt and a lawsuit stopping a planned bison hunt in Wyoming. Montana, the National Park Service, and the U.S. Forest Service are currently following the NEPA process in developing a cooperative bison management plan for the Northern Bison Herd of Yellowstone National Park, because this herd frequently wanders outside the Park. A management plan is also being developed for the Jackson Bison Herd in Wyoming. A planned lethal collection of 25 bison in Yellowstone National Park for scientific purposes in the spring of 1991 was delayed in court by an animal rights group and canceled.

The Wyoming Game and Fish Department, in cooperation with
REPORT OF THE COMMITTEE

USDA–APHIS, has initiated an elk vaccination program with an objective of vaccinating all feedground elk against brucellosis. A ballistic delivery system has been tested and demonstrated to be effective. This expensive program may have to be continued indefinitely in order to maintain a high level of immunity among these elk and reduce brucellosis–induced elk calf loss and reduce opportunity of transmission of brucellosis to cattle. The Wyoming Game and Fish Department also has established an internal Brucellosis–Feedground–Habitat Task Force to integrate the elk vaccination program with habitat improvements and alterations in feedground management to reduce the number of elk using feedgrounds and the time they spend on feedgrounds. This will reduce intraspecific spread of brucellosis. Field Implementation Teams are developing site specific Herd Unit Action Plans.

Two regional committees are addressing the problem of brucellosis in the GYA. One is the ad hoc Technical Committee on Brucellosis in the Greater Yellowstone Area. This informal, interagency committee has been meeting twice a year for about four years. It serves primarily as a source of information exchange.

Wyoming's Governor Mike Sullivan has established a Statewide Task Force on Brucellosis, which has adopted a goal to "Protect the integrity of Wyoming's free–ranging bison and elk populations and the livestock industry by eradicating wildlife brucellosis by the year 2010". Eradication of brucellosis from wildlife may not be an attainable objective, but eradication was felt to be the appropriate goal. The time frame was established in recognition that solving brucellosis problems in wildlife will be a long–term task. The Task Force is currently completing its final recommendation, which likely will call for creation of an administrative level interagency task force involving Wyoming, Montana, and Idaho and appropriate Federal agencies. The Task Force would have a goal of eradicating brucellosis while protecting the integrity of free–ranging wildlife and livestock in the Greater Yellowstone Area. It would have serving under it a Technical Committee and an Information and Education Subcommittee.

Recommended Action:

The Committee on Wildlife Diseases recognized brucellosis in the Greater Yellowstone Area as one of the nation's most important wildlife disease problems, and the Committee endorses a regional, interagency approach dedicated to solving the problem. In addition, the Committee recommends that USAHA support proposals whereby at least 25 bison be taken by lethal means from the Greater Yellowstone Area for study of Brucella abortus sero–reactivity in relation to culture positivity and infectivity.

7. Implications of the Importation of Wild Hogs into the Northern United States
European boar and wild swine are not native wild animals in Minnesota. When it recently became known that wild hogs were being imported and raised in Minnesota, an investigation was begun by the Minnesota Board of Animal Health to determine the extent of these activities. Dr. Walter Mackey, Minnesota Board of Animal Health, presented a summary of that study. A preliminary investigation revealed that approximately 300 head of wild hogs are being maintained on eight farms located throughout the entire state. Wild hogs are imported and raised in Minnesota for a variety of reasons; sale as meat, wild game hunting purposes, exhibition on exotic animal farms, and as a hobby species.

In order to assess the potential hazards associated with wild hogs, the Board of Animal Health did a survey of all the other states to learn of their experiences with wild hogs. State Veterinarians and Chief Wildlife Officials of each state were asked if they had free-ranging wild hogs in their woodlands, and if so, what was their impact on livestock, wildlife, crops, and environment. They also were asked if they considered wild hogs to be a liability or an asset to their state. The following are some of the things that were learned from this survey: 50 states responded to the survey; 23 states said they had free-ranging wild hogs. Through compilation of individual state estimates, there may be in excess of two million wild hogs in the U.S. Thirty five states allowed importation of wild hogs; 14 states banned the importation of wild hogs; 10 states said wild hogs had a negative impact on disease control; 14 states said wild hogs had a negative impact on wildlife; 14 states said wild hogs had a negative impact on the environment; and 45 states said they considered wild hogs to be a liability to their states. Dr. Mackey concluded there are a number of reasons why any state that does not have wild hogs should take action to keep them out.

Recommended Action:

The Wildlife Diseases Committee appreciates Dr. Mackey's efforts and recommends State Officials considering regulations regarding wild hogs consult with agencies with experience in managing wild hogs.

8. Report from USDA's Feral Swine Technical Group

Dr. Victor Nettles, Southeastern Cooperative Wildlife Disease Study, Athens, Ga., gave the Committee a brief report on short- and long-term strategies for resolving problems associated with pseudorabies and swine brucellosis in feral swine. This report was derived from a Feral Swine Technical Group assembled by the Swine Diseases Staff of Veterinary Services, APHIS, USDA. Because the findings of this group are of interest to the Subcommittee on Swine Brucellosis and the Pseudorabies Committee, as well as the Wildlife Diseases Committee, the final report of the Feral
REPORT OF THE COMMITTEE

Swine Technical Group, as submitted to Veterinary Services, will be published as a supplement to this Committee's report.

The most effective short-term strategies identified by the Technical Group were education and regulation. Dr. Nettles distributed a new brochure entitled "Wild Pigs... Hidden Danger for Farmers and Hunters". This brochure is available from the USDA and can be used to educate both swine producers and hunters of disease problems. In regard to regulations, the Technical Group was supportive of stronger restrictions on movement of feral swine and felt that the Feral Swine Subcommittee of the USAHA should take the lead in their development. Potential long-term strategies, such as feral swine population control, and vaccination or possibly depopulation were briefly discussed.

Recommended Action:

None required except that the Wildlife Diseases Committee was pleased that Veterinary Services has taken the initiative to produce the brochure and is placing more emphasis on education of the public about feral swine.

9. Psoroptic Scabies in Bighorn Sheep

Drs. David Jessup and Walter Boyce, California, reported on ongoing research on psoroptic scabies in bighorn sheep and other native free-ranging wildlife in the West. Scabies has been present in bighorn sheep in California for over 80 years. Recent attempts to control scabies with drugs have not been successful. In recent years, psoroptic scabies has also been identified in elk, white-tailed deer, mule deer, and ranched llamas and alpacas in scattered locations throughout the West. Ongoing research is designed to elucidate the relationships between mites from these different species and develop effective treatment procedures.

Recommended Action:

None required.
Short- and Long-term Strategies for Resolving Problems of Pseudorabies and Swine Brucellosis in Feral Swine

by
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At the invitation of the Swine Diseases Staff, Veterinary Services, APHIS, USDA, a Feral Swine Technical Group was assembled on April 15–16, 1991, in Hyattsville, MD, to provide consultation on short- and long-term strategies for resolving problems associated with pseudorabies and swine brucellosis in feral swine. The Technical Group consisted of the following persons: Dr. Victor Nettles (Chairman), The University of Georgia; Dr. George Beran, Iowa State University; Dr. Don Davis, Texas A&M University; and Dr. Paul Gibbs, University of Florida. Also participating in the meeting with the Technical Group were Dr. Joe Annelli, Mr. Bill Clay, Mr. Joseph Corn, Dr. Richard Fite, Dr. Cyril Gay, Dr. Delorias Lenard, Mr. Samuel Linhart, Dr. Don Luchsinger, Dr. Frank Mulhern, Dr. Robert Ormiston, Dr. David Stallknecht, Dr. William Stewart, and Dr. Martin Van Der Leek.

Dr. Don Luchsinger, Veterinary Services, APHIS, USDA, welcomed the Technical Group and requested that the discussion examine the depth and spectrum of the problems with both pseudorabies and swine brucellosis in feral swine. He encouraged the Technical Group to utilize all forms of old and new technology to address the issues.

Dr. Victor Nettles, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, presented an outline listing the objectives for the Feral Swine Technical Group and gave a few remarks pertaining to each item. Those objectives were to be revisited at the end of the meeting and serve as a basis for forming recommendations.

Dr. Frank Mulhern of the National Pork Producers Council (NPPC) gave an overview of the feral swine disease situation from the perspective of pork producers. Dr. Mulhern stated that the swine industry is working toward increasing sales through a program emphasizing pork as the "Meat of Choice" and stressing leanness, white meat texture, and quality assurance. He also indicated that swine producers were looking toward increasing the U.S. export sales for pork products, and in doing so, they must compete with several European countries with advanced swine-raising technology. At present, Dr. Mulhern indicted that PRV costs the U.S. pork industry $30,000,000 per year and that the elimination of PRV and other disease problems would allow U.S. producers to produce a consistent quality product. He also expressed interest on the behalf of pork producers that
RESOLVING PROBLEMS OF PSEUDORABIES AND BRUCELLOSIS

Swine brucellosis should be eradicated.

According to Dr. Mulhem, there is concern that infections of PRV and swine brucellosis in feral swine will become a hindrance to eradication programs if these disease problems become viewed as reasons not to proceed with the programs.

Dr. Nettles gave the Feral Swine Technical Group a brief overview of some data on PRV and swine brucellosis in feral swine. PRV antibodies have been identified in feral swine in 11 states, and the overall seroprevalence is approximately 19% nationwide. Two isolations of PRV have been made from feral swine and several others have been obtained indirectly from hunting dogs. Swine brucellosis has been diagnosed serologically and/or B. suis cultured from feral swine in 10 states, and the overall prevalence of seroreaction was 11%. Dr. Nettles indicated that he felt it was reasonable to consider most wild swine populations infected with these diseases until testing shows otherwise.

Information on feral swine entering domestic swine markets was provided by Dr. Martin Van Der Leek, University of Florida. Studies in Florida livestock markets revealed that feral swine or cross-bred feral x domestic swine were sold occasionally in feeder pig sales. In addition, private hog dealers that specialize in selling wild swine to hunting clubs and game preserves also move wild swine in an uncontrolled fashion. Dr. Van Der Leek gave several examples where wild swine were being removed from large acreages in Florida and most likely moved to unknown destinations.

Dr. Robert Ormiston, Swine Diseases Staff, APHIS, USDA, gave the Group some recent epidemiologic information related to PRV infections in domestic swine attributable to feral swine. In the first 3 months of 1991, 5 newly infected swine herds in Oklahoma were considered infected by exposure to feral swine. In Arkansas, 4 of 5 positive first point tests involved feral swine that were being trapped and sold through markets. Dr. Delorias Lenard, also of the Swine Diseases Staff, gave a review of several recent shipments of feral swine that were found in interstate commerce. Laboratory tests revealed that high percentages of the animals involved were infected with pseudorabies and/or swine brucellosis.

Dr. Lenard also presented a draft brochure that is being prepared by APHIS. The Group was asked to review the brochure and provide their input. Dr. Nettles remarked that he believed the brochure would be an extremely valuable educational tool. Education of swine producers and sportsmen was discussed, and the group considered various target audiences and media applications. Brochures, news releases, posters, brief videotapes, and magazine articles were suggested. It was generally recognized that educational efforts would yield the highest cost/benefit dividends as a short-term strategy.

Federal and state regulations regarding intra- and inter-state transportation of feral swine were discussed. Dr. Ormiston suggested that the
eventual resolution to reducing domestic swine exposure to feral swine was for the pork industry to institute highly rigid quality control requirements that were linked to market eligibility. By this method, only "Certified Domestic Hogs" could be sold and such animals had to be raised in total absence of feral swine exposure.

Dr. George Beran, College of Veterinary Medicine, Iowa State University, presented a few new guidelines that he proposed for addition to program standards for pseudorabies eradication. He also presented current language in the PRV program standards and swine brucellosis uniform methods and rules pertaining to feral swine. The Technical Group discussed these proposed additions at length and generally favored their implementation. The Group encouraged Dr. Beran to work through his Feral Swine Subcommittee of the United States Animal Health Association to develop these guidelines.

Mr. Bill Clay of Animal Damage Control (ADC), APHIS, USDA, provided information on ADC activities in regard to feral swine control. The agency does feral swine control in multiple areas in response to local problems. Mr. Clay indicated that funds were not available to conduct large-scale swine control efforts. Cost information on per head removed basis were not given but possibly could be derived.

A brief summary of the use of mathematical models to study the epidemiology of PRV or swine brucellosis was given by Dr. Van Der Leek. General factors in the model included: number of susceptible pigs; number of latent carriers; number of active shedders; population density; rate of culling (hunting, trapping, natural mortality); seasonal influences; and habitat influences. Dr. Van Der Leek indicated that modeling could predict threshold swine population densities required to maintain disease. Dr. Van Der Leek reviewed serologic data for Florida feral swine and gave overall prevalence rates of 34.7% and 43.1% for PRV and swine brucellosis, respectively.

Dr. Nettles reported on a pilot oral bait trial on Ossabaw Island, Georgia, that provided encouraging results in regard to the delivery of oral vaccines. Baits similar to the one designed to deliver oral rabies vaccine were placed on a 1,000-acre study area on the Island. A survey of feral swine on the area for 2 biomarkers revealed that 95% of the animals sampled had eaten at least 1 bait. Dr. Nettles said that additional research is needed in oral bait development and delivery techniques to increase cost effectiveness and reduce consumption by non-target species.

Dr. Paul Gibbs, College of Veterinary Medicine, University of Florida, gave the Group a brief report on bioengineered vaccines. He indicated that the poxvirus group has a strong potential as vector viruses because a poxvirus can accommodate multiple genetic insertions and is heat stable. Poxviruses often have a wide host range and will produce humoral immunity which can be useful in monitoring a target population. Researchers at the University of Florida currently are working on a swine pox-PRV recombinant
vaccine, and Dr. Gibbs indicated that they will soon test this product in swine experimentally.

Dr. Beran summarized the status of currently available modified live virus PRV vaccines and gave the Group some preliminary information on his studies with oral administration of a concentrated form of the Syntrovet vaccine. There appears to be a capability for the Syntrovet vaccine to work orally if concentrated to $10^6$; however, the pilot trial was flawed by the inadvertent introduction of street virus and must be redone.

A similar review on existing oral vaccine candidates for swine brucellosis was presented by Dr. Don Davis, College of Veterinary Medicine, Texas A&M University. Dr. Davis indicated that the Chinese oral vaccine, *B. suis* biotype 1, strain 2, was available for study and that he intended to test it in swine. Furthermore, he indicated that *B. neotomae* may have potential as an oral vaccine. *B. neotomae* is considered a rodent-adapted mutant that has been recovered only from wood rats in Utah. *B. neotomae* appears to be an excellent immunogen while being apathogenic. Dr. Davis will be experimenting with this organism in experimental swine in the future.

Dr. David Stallknecht, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, reviewed the status of PRV in swine on Ossabaw Island, Georgia. In-depth serologic study has revealed that juvenile swine during the spring/summer have a low seroprevalence of $<2\%$ but adult swine that have lived through at least 1 winter are infected at a rate of 28%. Seroconversions are occurring on an annual basis but vary in rate from year to year. Dr. Stallknecht outlined a research plan that would test the feasibility of PRV vaccination of wild swine as an eradication or control strategy. Intensive trapping and vaccinating of wild swine on Ossabaw Island would be done in conjunction with other studies to reduce costs. An injectable differential PRV vaccine would be used on all available swine on the study area to see if vaccination would be useful in reducing the prevalence of infection during a 2 year period.

The Feral Swine Technical Group was adjourned at 5:00 P.M. on Monday and reconvened at 8:00 A.M. on Tuesday. During the Tuesday morning session, the Group's list of objectives was reexamined and brief statements were prepared to be used by Veterinary Services, APHIS, USDA, in policy decisions regarding swine disease control programs. These recommendations are presented below:

1. The Group's position on the significance of these diseases in feral swine to certain groups is as follows:
   a. Domestic Swine/Pork Producers
      The eradication of pseudorabies and swine brucellosis is possible in domestic swine by the year 2000 despite an infected feral swine population.*
      The domestic swine producers of the U.S. have endorsed programs to eradicate pseudorabies and
swine brucellosis. Producers want to stress that efforts to minimize the prevalence of these diseases in feral swine and their transmission to domestic swine be better defined and accelerated to meet the needs of the current eradication programs. Unless this is accomplished, feral swine problems may impede the 10–year pseudorabies eradication goal.

*Chairman's Note: Although not incorporated into this statement, it was automatically assumed that measures would be taken to segregate wild from domestic swine.

b. Other Domestic Animal Species
Several diseases and parasites can potentially be transmitted from wild swine to domestic animals. Although clinical disease is rare in wild swine, subclinical infections may interfere with other disease eradication programs, notably bovine brucellosis. Livestock owners should protect their investment by minimizing contact between livestock and wild swine.

c. Free-ranging Wildlife
Diseased feral swine potentially present a threat to susceptible predatory species such as the endangered Florida panther, ocelot, jaguarundi, and red wolf. Pseudorabies is of particular concern because felids are known to develop a fatal infection.

d. Sport Hunters
Pseudorabies is not infectious to humans but occasionally can cause fatal infections in hunting dogs. Swine brucellosis is contagious to humans and can be a serious disease. Therefore, proper sanitary precautions should be taken in handling potentially infected feral swine and swine carcasses. To eliminate spread of these diseases, only proven uninfected feral swine can be legally relocated.

e. General Public
Feral swine are infected with several zoonotic agents. Of particular concern are swine brucellosis and trichinosis. These non-indigenous swine cause agricultural and economic losses, and they destroy native ecosystems. Population control measures may be necessary.

2. Educational strategies to be used to address feral swine
RESOLVING PROBLEMS OF PSEUDORABIES AND BRUCELLOSIS

disease problems:

a. Target audiences for educational efforts were determined to be wildlife agencies, hunters, producers, cattle ranchers, and livestock market operators.

b. Media tools to be used should include a video, a brochure, magazine articles, and posters.

c. The lead agency in the education effort should be APHIS's Swine Diseases Staff. An educational committee should be formed that would include a member of the Swine Diseases Staff; a representative from NPPC; Chairman, Feral Swine Technical Group; and a representative from Legislative and Public Affairs, APHIS, USDA.

3. The Feral Swine Technical Group recommended that movement of feral swine needs to be restricted to prevent spread of diseases. Implementation of rules and regulations should be initiated through deliberations of the United States Animal Health Association.

4. In regard to the feasibility of feral swine depopulation, the Feral Swine Technical Group recognized that current techniques have limited value in many wild swine populations and that such efforts are extremely expensive and possibly futile. Furthermore, removal of feral swine may provoke strong public resistance in many areas.

5. Research needs and information gaps exist in the following areas:

a. Population dynamics of feral swine as they relate to disease elimination or reduction

b. Swine population control methods

c. Delivery techniques for oral medications such as vaccines, sterilants, and toxicants

d. Feasibility of oral vaccination for PRV and swine brucellosis

e. Feasibility of elimination of diseases from distinct, isolated feral swine populations

6. The problem of lack of funding for the necessary research should be addressed since it is a major constraint to program success.
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 Association 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 member 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.
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 members 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.
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.

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, 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

*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.
district: consisting of the States of Alaska, Arizona, California, Colorado, 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; Southern; Region-at-Large; North Central. In the event that an elected officer is unable to complete an elected term, the District that originally 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 membership. 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 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 or 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.

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, occupation, and address of each applicant for individual membership for the approval of the Executive Committee. He shall prepare forms for applicants for allied organization membership and shall notify each of the elected officers upon receipt of such completed application. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, ex-officio secretary of the Board of Directors, and an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars ($10,000).

7. Treasurer: The Treasurer shall keep an accurate account of all Association moneys received and disbursed. He shall receive from the Secretary all moneys of the Association paid directly to the Secretary along with proper identification of such moneys. By and with the approval of the Board of Directors, he shall deposit the funds of this Association in such types of accounts as may be approved by the Board of Directors, and he shall invest the funds of the Association or liquidate Association investments in such manner as may be approved by the Executive Committee upon recommendation of the Board of Directors. He shall honor warrants for the proper expenditure of Association funds furnished him by the Secretary over his signature and that of the Executive Director. He shall honor warrants from the Secretary on the Secretary's own authority for incidental expenses of the Secretary's office in sums not to exceed one hundred dollars ($100) for any given expenditure over the sole signature on warrants signed by the Executive Director. He shall be given guidance and general administrative supervision by the Board of Directors, and he shall furnish the Executive Committee with a financial statement of the Association's funds annually. He shall be bonded for not less than ten thousand dollars ($10,000), and he shall receive such salary as the Executive Committee may from time to time determine.
The Constitution and Bylaws of this Association may be amended by a two-thirds vote of the members of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting, printed in the annual proceedings, and further provided that the amendment has received the approval of a majority of the Executive Committee members present and voting.

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.

ARTICLE IX - ELECTION OF OFFICERS AND ELECTED REGIONAL DELEGATES

The Nominating Committee shall annually report to the membership of this Association at the first morning general session. Their recommendations for the offices of President, President-Elect, First Vice-President, Second Vice-President, Third Vice-President, and Treasurer, 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 of the Nominating Committee, and proposed amendments of the report shall be presented to the Executive Committee for consideration. The acceptance of the report or amendment shall constitute election.

**The phrase "at the second morning general session" shall be deemed to mean at a time certain specified in the program, "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 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 Executive 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.

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 forty-five dollars ($45) 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 repre-
senting the animal health departments of their respective states.

ARTICLE V-DUES

The dues for individual membership in this Association shall be forty-
five dollars ($45) per annum, payable in advance (on or before January 1st
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 three
hundred dollars ($300) each per annum, payable in advance (on or before
January 1st each year) to the Secretary of this Association.

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.

NOTE: 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.
96th ANNUAL MEETING
October 31 – November 6, 1992
GALT HOUSE HOTEL
Louisville, Kentucky

97th ANNUAL MEETING
October 23 – 29, 1993
RIVIERA HOTEL
Las Vegas, Nevada