PROCEEDINGS

NINETY-EIGHTH
ANNUAL MEETING

of the

UNITED STATES ANIMAL
HEALTH ASSOCIATION

THE AMWAY GRAND PLAZA HOTEL
GRAND RAPIDS, MICHIGAN

October 29-November 4, 1994
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UNITED STATES ANIMAL
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AMWAY GRAND PLAZA HOTEL
GRAND RAPIDS, MICHIGAN
October 29-November 4, 1994
This book is dedicated in memory to the members on USAHA who passed away in 1994

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Dr. E. Tom Thorne, Laramie, WY
Dr. J. K. Veatch, Manhattan, KS
Mr. Dave Whittlessey, Steamboat Spr., CO
Dr. E. S. Williams, Laramie, WY
Dr. Richard W. Winters, Brady, TX
Mr. Steve Wolcott, Paonia, CO
Dr. Jerry M. Woodall, Perkins, OK

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Dr. Charles O. Thoen, Ames, IA
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Dr. Daryl K. Thorpe, Pierre, SD
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Mr. Dave Whittlesey, Steamboat Spr., CO
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Mr. Sam Witham, Cushing, OK
Mr. Steve Wolcott, Paonia, CO
Dr. Jerry M. Woodall, Perkins, OK
Dr. Glen L. Zebarth, Alexandria, MN
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
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<tbody>
<tr>
<td>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>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>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>Oct. 2-3, 1900</td>
<td>Louisville, KY</td>
<td>*Mr. C. P. Johnston, Springfield, IL</td>
<td>*Dr. E. T. Eisenman, Louisville, KY</td>
</tr>
<tr>
<td>Oct. 9-8, 1901</td>
<td>Buffalo, NY</td>
<td>*Dr. E. P. Niles, VA</td>
<td>*Dr. E. T. Eisenman, Louisville, KY</td>
</tr>
<tr>
<td>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>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>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>Aug. 15-16, 1905</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>Aug. 15-16, 1906</td>
<td>Springfield, IL</td>
<td>*Mr. M. M. Hankins, Quanah, TX</td>
<td>*Dr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>Sept. 16-17, 1907</td>
<td>Richmond, VA</td>
<td>*Dr. D. F. Luckey, Columbia, MD</td>
<td>*Mr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>Sept. 14-16, 1908</td>
<td>Washington, DC</td>
<td>*Dr. Charles G. Lamb, CO</td>
<td>*Mr. C. E. Cotton, St. Paul, MN</td>
</tr>
<tr>
<td>Sept. 13-15, 1909</td>
<td>Chicago, IL</td>
<td>*Dr. W. H. Dalrymple, Baton Rouge, LA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>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>Dec. 5-6, 1911</td>
<td>Chicago, IL</td>
<td>*Dr. John F. Devine, Goshen, NY</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>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>Dec. 2-4, 1913</td>
<td>Chicago, IL</td>
<td>*Dr. Peter F. Bainsen, Atlanta, GA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>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>Dec. 2-3, 1915</td>
<td>Chicago, IL</td>
<td>*Dr. J. L. Gibson, Des Moines, IA</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>Dec. 5-7, 1916</td>
<td>Chicago, IL</td>
<td>*Dr. O. E. Dyson, Springfield, IL</td>
<td>*Mr. J. J. Ferguson, Chicago, IL</td>
</tr>
<tr>
<td>Dec. 3-5, 1917</td>
<td>Chicago, IL</td>
<td>*Dr. J. G. Wills, Albany, NY</td>
<td>*Mr. S. H. Ward, St. Paul, MN</td>
</tr>
<tr>
<td>Dec. 2-4, 1918</td>
<td>Chicago, IL</td>
<td>*Dr. M. Jacob, Knoxville, TN</td>
<td>*Mr. D. M. Campbell, Chicago, IL</td>
</tr>
<tr>
<td>Dec. 1-3, 1919</td>
<td>Chicago, IL</td>
<td>*Dr. G. W. Humphry, Lansing, MI</td>
<td>*Mr. Theo Burnett, Columbus, OH</td>
</tr>
<tr>
<td>Nov. 29-Dec. 1, 1920</td>
<td>Chicago, IL</td>
<td>*Dr. S. F. Musselman, Frankfort, KY</td>
<td>*Mr. Theo Burnett, Columbus, OH</td>
</tr>
<tr>
<td>Nov. 28-30, 1921</td>
<td>Chicago, IL</td>
<td>*Dr. W. F. Crewe, Bismarck, MD</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Dec. 6-8, 1922</td>
<td>Chicago, IL</td>
<td>*Dr. T. E. M. Munce, Harrisburg, PA</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Dec. 5-7, 1923</td>
<td>Chicago, IL</td>
<td>*Dr. W. J. Butler, Henena, MT</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Dec. 3-5, 1924</td>
<td>Chicago, IL</td>
<td>*Dr. J. G. Fenerhough, Richmond, VA</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Dec. 2-4, 1925</td>
<td>Chicago, IL</td>
<td>*Dr. J. H. McNeil, Trenton, NJ</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
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<tr>
<td>Dec. 1-3, 1926</td>
<td>Chicago, IL</td>
<td>*Dr. John R. Mohler, Washington, DC</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Nov. 30-Dec. 2, 1927</td>
<td>Chicago, IL</td>
<td>*Dr. L. Van Es, Lincoln, NE</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Dec. 5-7, 1928</td>
<td>Chicago, IL</td>
<td>*Dr. C. A. Cary, Auburn, AL</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
</tr>
<tr>
<td>Dec. 4-6, 1929</td>
<td>Chicago, IL</td>
<td>*Dr. Chas. O. Lamb, Denver, CO</td>
<td>*Dr. O. E. Dyson, Kansas City, MO</td>
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<tr>
<td>Date</td>
<td>City, State</td>
<td>Author</td>
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<tr>
<td>Nov. 30-Dec. 2, 1932</td>
<td>Chicago, IL</td>
<td>*Dr. Peter Malcolm, Des Moines, IA</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 6-8, 1933</td>
<td>Chicago, IL</td>
<td>*E. T. Faulder, Albany, NY</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 5-7, 1934</td>
<td>Chicago, IL</td>
<td>*Dr. T. E. Robinson, Providence, RI</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 4-6, 1935</td>
<td>Chicago, IL</td>
<td>*Dr. Edward Records, Reno, NV</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 2-4, 1936</td>
<td>Chicago, IL</td>
<td>*Dr. Walter Wisnicky, Madison, WI</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 1-3, 1937</td>
<td>Chicago, IL</td>
<td>*Dr. R. W. Smith, Concord, NH</td>
<td>Chicago, IL</td>
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<tr>
<td>Nov. 30-Dec. 2, 1938</td>
<td>Chicago, IL</td>
<td>*Dr. D. E. Westmoreland, Frankfort, KY</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 6, 1939</td>
<td>Chicago, IL</td>
<td>*Dr. J. L. Axby, Indianapolis, IN</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 4-6, 1940</td>
<td>Chicago, IL</td>
<td>*Dr. H. D. Port, Cheyenne, WY</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 3-5, 1941</td>
<td>Chicago, IL</td>
<td>*Dr. E. A. Crossman, Boston, MA</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 2-4, 1942</td>
<td>Chicago, IL</td>
<td>*Dr. I. S. McDady, Auburn, AL</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Dec. 1-3, 1943</td>
<td>Chicago, IL</td>
<td>Dr. W. H. Hendricks, Salt Lake City, UT</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 6-8, 1944</td>
<td>Chicago, IL</td>
<td>Dr. J. M. Sutton, Atlanta, GA</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 5-7, 1945</td>
<td>Chicago, IL</td>
<td>Dr. C. U. Duckwork, Sacramento, CA</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 4-6, 1946</td>
<td>Chicago, IL</td>
<td>*Dr. William Moore, Raleigh, NC</td>
<td>Chicago, IL</td>
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<tr>
<td>Dec. 3-5, 1947</td>
<td>Chicago, IL</td>
<td>*Dr. Will J. Miller, Topeka, KS</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 13-15, 1948</td>
<td>Denver, CO</td>
<td>*Dr. Jean V. Knapp, Tallahassee, FL</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 12-14, 1949</td>
<td>Columbus, OH</td>
<td>*Dr. T. O. Brandenburg, Bismarck, ND</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 1-3, 1950</td>
<td>Phoenix, AZ</td>
<td>*Dr. C. P. Bishop, Harrisburg, PA</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 14-16, 1951</td>
<td>Kansas City, KS</td>
<td>*Mr. F. E. Mollin, Denver, CO</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 29-31, 1952</td>
<td>Louisville, KY</td>
<td>Dr. Ralph L. West, St. Paul, MN</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Sept. 23-25, 1953</td>
<td>Atlantic City, NJ</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 10-12, 1954</td>
<td>Omaha, NE</td>
<td>*Dr. T. C. Green, Charleston, WV</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 16-18, 1955</td>
<td>New Orleans, LA</td>
<td>Dr. H. E. Wilkins, Helena, MT</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 28-30, 1956</td>
<td>Chicago, IL</td>
<td>Dr. A. L. Brueckner, Baltimore, MD</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 13-15, 1957</td>
<td>St. Louis, MO</td>
<td>Dr. G. H. Good, Cheyenne, WY</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 4-6, 1958</td>
<td>Miami Beach, FL</td>
<td>Dr. John G. Milligan, Montgomery, AL</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Nov. 15-18, 1959</td>
<td>San Francisco, CA</td>
<td>Mr. F. G. Buzzell, Augusta, ME</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 17-21, 1960</td>
<td>Charleston, WV</td>
<td>*Dr. J. R. Hay, Chicago, IL</td>
<td>Chicago, IL</td>
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<tr>
<td>Oct. 30-Nov. 3, 1961</td>
<td>Minneapolis, MN</td>
<td>Dr. A. P. Schneider, Boise, ID</td>
<td>Chicago, IL</td>
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<tr>
<td>Oct. 30-Nov. 2, 1962</td>
<td>Washington, DC</td>
<td>*Dr. W. L. Bendix, Richmond, VA</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 15-18, 1963</td>
<td>Albuquerque, NM</td>
<td>*Dr. T. J. Grennan, Jr. Providence, RI</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 19-23, 1964</td>
<td>Memphis, TN</td>
<td>*Dr. L. A. Roeder, Jefferson City, MO</td>
<td>Chicago, IL</td>
</tr>
<tr>
<td>Oct. 25-29, 1965</td>
<td>Lansing, MI</td>
<td>Dr. J. W. Safford, Helena, MT</td>
<td>Chicago, IL</td>
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<tr>
<td>Oct. 10-14, 1966</td>
<td>Buffalo, NY</td>
<td>Dr. C. L. Campbell, Tallahassee, FL</td>
<td>Chicago, IL</td>
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* indicates an address in the USA.
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<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<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>
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<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>
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<tr>
<td>73. Oct. 12-19, 1969</td>
<td>Milwaukee, WI</td>
<td>* Dr. John L. OHarra, Reno, NV</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>
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<tr>
<td>76. Nov. 5-10, 1972</td>
<td>Miami Beach, FL</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
<td>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>
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<tr>
<td>78. Oct. 13-18, 1974</td>
<td>Roanoke, VA</td>
<td>Mr. O. H. Timm, Dixon, GA</td>
<td>Dr. W. L. Bendix, Richmond, VA</td>
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<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>
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<tr>
<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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<td>82. Oct. 21-Nov. 3, 1978</td>
<td>Buffalo, NY</td>
<td>Dr. L. E. Bartell, Sacramento, CA</td>
<td>Dr. J. C. Shook, Hyattsville, MD</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>
</tr>
<tr>
<td>84. Nov. 2-7, 1980</td>
<td>Louisville, KY</td>
<td>* Mr. B. W. Hawkins, Ontario, OR</td>
<td>Dr. J. C. Shook, Hyattsville, M</td>
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<tr>
<td>85. Oct. 11-16, 1981</td>
<td>St. Louis, MO</td>
<td>* Dr. L. W. Hinchman, Indianapolis, IN</td>
<td>Dr. J. C. Shook, Hyattsville, MD</td>
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<td>86. Nov. 7-12, 1982</td>
<td>Nashville, TN</td>
<td>Dr. G. B. Rea, Salem, Or</td>
<td>Dr. J. C. Shook, Annapolis, MD</td>
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<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>
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<td>88. Oct. 21-26, 1984</td>
<td>Ft. Worth, TX</td>
<td>Mr. J. O. Pearce, Jr. Okiechobee, FL</td>
<td>Dr. J. C. Shook, Annapolis, MD</td>
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<tr>
<td>89. Oct. 27-Nov. 1, 1985</td>
<td>Milwaukee, WI</td>
<td>* Dr. David U. Walker, Montpelier, VT</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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<tr>
<td>90. Oct. 19-14, 1986</td>
<td>Louisville, KY</td>
<td>* Dr. N. W. Kruse, Lincoln, NE</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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<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>
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<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>
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<td>93. Oct. 28-Nov. 3, 1989</td>
<td>Las Vegas, NV</td>
<td>Dr. P. E. Bradshaw, Griggsville, IL</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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<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>
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<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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<td>96. Oct. 31-Nov. 6, 1992</td>
<td>Louisville, KY</td>
<td>Dr. J. Lee Alley, Montgomery, AL</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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<td>97. Oct. 23-29, 1993</td>
<td>Las Vegas, NV</td>
<td>Dr. T. J. Hagerly, St. Paul, MN</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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<td>98. Oct. 29-Nov. 4, 1994</td>
<td>Grand Rapids, MI</td>
<td>Mr. J. B. Finley, Jr., Encinal, TX</td>
<td>Dr. J. C. Shook, Mechanicsburg, PA</td>
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INVOCATION

Michael R. Marshall, D.V.M.
Salt Lake City, Utah

Our Father In Heaven:

This evening we are very grateful to be participating in this meeting which represents the animal kingdom and the food and fiber network of sustainable agriculture. We thank thee for our families at home who give us love and support, and we ask Thy blessings upon them.

We appreciate the opportunity to meet in this free land of America. We pray for Thy inspiration upon the leaders of the world, that they will guide us in the ways of peace and prosperity. Humbly, we ask for wisdom and discernment, that we might make proper decisions and give good counsel. Please help us to be a righteous and obedient people, so that we may glorify Thy name.

We ask for Thy forgiveness of our faults and errors, and pray that honesty, dignity, and charity will always be foremost in our minds. These things we humbly pray for, in the name of Jesus Christ.

Amen.

MEMORIAL SERVICE

Mr. President, members of USAHA, AAVLD, Ladies and Gentlemen, it our privilege at this time to pause for a moment of Silent Prayer and Personal Meditation to pay tribute and honor to our colleagues and friends who have passed away since our last meeting.

They are:

Dr. Raymond Bankowski - Life Member, USAHA - University of California - Davis, CA - November 3, 1993.
Mr. Clint Booth - Life Member, USAHA - Dallas, TX - March 20, 1994.
Dr. Grant S. Kaley - President, USAHA, 1967 - 1994
Dr. Everett S. Bryant - Past President, AAVLD, 1982 - Regional Representative, USAHA, Storrs, CT - June 12, 1994
Dr. Robert Rice - Life Member, USAHA - Punta Gorda, FL - August 29, 1994.
Dr. Richard Talbot - Former Dean of the Veterinary School at VPI, Blacksburg, VA - September 8, 1994.
WELCOME TO MICHIGAN

Dr. Gordon Guyer
Director, Michigan Department of Agriculture

I am pleased and proud to welcome the United States Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) to Michigan for your joint 1994 annual meeting.

When most people think of Michigan they probably think of us as the state that put America on Wheels. Our automotive industry continues to make manufacturing Michigan's largest economic sector. But agriculture is our state's second leading industry and the state's second largest employer. This year agriculture, food processing and related businesses will contribute $37.5 billion to the state's economy.

Michigan is one of the most diversified agriculture regions in the nation, second only to California in the variety of crops grown. We produce over 70 commercial crops and rank in the top ten nationally for the production of 66. Each year, Michigan exports agricultural commodities valued at nearly $2 billion. We send tart cherries to Japan, blueberries to Western Europe and Hong Kong, cranberry beans to Italy and black beans to Mexico.

Animal agriculture produces half of our agricultural income - almost 14 billion dollars in 1993 and more than 56,000 jobs. Cass County, a rural county just south of here, is one of the nation's leaders in the production of pork and pork products. A 64 million dollar animal livestock initiative at Michigan State University will provide the research and resources to help strengthen and expand Michigan's animal agriculture industry.

Speaking of Michigan State University, this outstanding state-supported institution and the first land-grant college in the nation, continues to provide cutting edge research and development in the areas of crop and soil science, and veterinary medicine.

We are proud you have chosen our state for this year's meeting, and for your selection of Grand Rapids as the convention site. Grand Rapids is one of Michigan's showcase cities, and the Amway Grand a true jewel among Michigan hotels and inns.

I hope you will extend your stay in our state to visit other areas of Michigan, including Northern Michigan where you will find some of the most breathtaking scenery in America.

Best wishes for a productive meeting. A warm Michigan welcome to all of you!
RESPONSE TO WELCOME

Jack N. Armstrong, D.V.M.
Reno, Nevada

On behalf of the United States Animal Health Association and the American Association of Veterinary Laboratory Diagnosticians, we wish to thank you, Director Guyer, for your warm and gracious welcome to Michigan, the "Wolverine State."

Dr. Guyer, we look forward to the enjoyment of your many beautiful lakes and streams, the ambrosia of your red and white wines, and delicacies such as apple cider, thimbleberry jam and slabs of cherry pie. Those gastronomical pleasures are meaningful to those of us who do food!

Let us borrow the latin word "Tuebor" from the Great Seal of the State of Michigan. Tuebor means "I will defend" - which describes the theme of our annual meetings. As a team we defend against diseases that are of concern to animal and human health. Our composition - industry, regulatory, diagnostic and research members - makes up a four horse team. It's a great team. Remember, a great team requires good care. They need food, rest and fluids. So let the team relax and enjoy the bounties of Michigan.

Our 1995 meeting site is John Ascuaga's Nugget in Reno/Sparks, Nevada. The Nugget offers an indoor Olympic-size pool; health club and spa; hair salon; eight award winning restaurants; two coffee shops and a deli, and 24 hour cabaret entertainment. Beautiful Lake Tahoe, legendary Virginia City and historic Carson City are all within 45 minutes driving time from the hotel.

From sights and activities to hotel amenities, northern Nevada will provide you a memory bank full of pleasant treasures.

I wish to welcome you to the "Silver State" next year. In Nevada - the latch string is out and the coffee pot is on - come by and stay a while.
A MESSAGE FROM THE SECRETARY'S OFFICE
THE HONORABLE PATRICIA JENSEN
ACTING ASSISTANT SECRETARY OF AGRICULTURE, USDA
Grand Rapids, Michigan
November 4, 1994

It seems almost deceptively simple, the succinct motto that describes the mission of one of our Marketing and Regulatory agencies, the Animal and Plant Health Inspection Service (APHIS). I'm sure many of you are familiar with it—"Protecting American Agriculture."

Although these words accurately sum up APHIS' overall purpose, they also can be used to represent the foremost concerns of this body. All of us attending this meeting—the veterinarians, laboratory diagnosticians, researchers, Federal and State regulatory officials, industry representatives, and countless others who make up our national animal health infrastructure, along with those who work with us on an international level—have a vital interest in preserving the health of U.S. livestock populations. I don't think it's stretching the point to say that we can all think of ourselves as stewards of a precious resource...a resource that must be guarded constantly against pest and disease threats from within and without.

As modern guardians of agriculture, we stand in good company. It's been over 400 years since the first organized U.S. animal disease eradication program to combat contagious bovine pleuropneumonia was established through the creation of APHIS' predecessor Agency, the Bureau of Animal Industry (BAI). Until 1883, livestock diseases received little Government attention. However, that year proved to be an important turning point. Bovine pleuropneumonia was reaching epidemic proportions in the United States; the disease had developed to such an extent that our cattle and sheep were denied entry into Great Britain. In addition, hog cholera was decimating our swine herds. As a result, U.S. pork was prohibited from most European markets. Scabies, tuberculosis, and brucellosis were also on the rise throughout much of the Nation. And innumerable cattle ranchers were being held tightly in the grip of Texas fever, which was sweeping through the country's midsection like an uncontrollable brushfire.

The outlook was certainly grim. Livestock groups urged direct governmental intervention to control and eradicate the devastating contagious bovine pleuropneumonia to protect their growing industries. It took 5 years of hard, relentless work by the fledgling BAI and its State and local cooperators and over $1.5 million—in pre-turn-of-the-century dollars—but by 1889, we were successful and contagious bovine pleuropneumonia was finally eradicated from the United States.

This experience taught our BAI predecessors a valuable lesson, one that they would put to good use later in ridding the country of most of the other destructive diseases I mentioned. The lesson was that effective con-
trol and eradication of contagious diseases depends on an all-out cooperative effort amongst all the parties involved. Only by working together at the community, State, national, and international levels can we be afforded any degree of security from such threats.

On that note, I'd like to take a moment to commemorate the completion of one of the most outstanding of our recent cooperative endeavors—the 40th anniversary of the final eradication of foot-and-mouth-disease (FMD) in Mexico. Without the efforts of the dedicated group of individuals who comprised the U.S.-Mexico FMD Commission, our livestock industries would undoubtedly look much different than they do today. The economic losses to both our countries from FMD—both in terms of lost export markets and eradication costs—would be staggering; some estimates run as high as $10 billion during the first year of an outbreak alone. Fortunately, because of the hard work and commitment of Commission workers, most of us in this room have never had to deal firsthand with the destruction of an FMD outbreak.

However, the efforts of the Commission were not without cost. A heavy toll was exacted upon the lives and health of its workers—as Dr. Frank Mulhern, a former APHIS administrator who was directly involved in this history-making endeavor, will attest in his talk later today. The work was difficult and dangerous, and the Commission workers often met with armed resistance from the very people they were trying to help. Nevertheless, they persisted and, by 1954, achieved their mission.

Persistence in the face of adversity and a commitment to working together for mutual benefit...these qualities will continue to serve us well as we work toward eradication of the pathogens that still threaten the health of both livestock and human beings.

Of all the infectious agents that attack humans and animals, tuberculosis (TB) is still one of the leading causes of misery and death. Here in the United States, outbreaks of the disease in animals occur only rarely, largely because of the strong preventive measures we already have in place. In humans, however, the incidence of the disease is rising at an alarming rate. It has been estimated that over one-third of the world's population is currently infected with TB or has been exposed to the disease. With more and more people traveling to the United States each year and the reduction of nontariff barriers to worldwide agricultural trade, we must continue to maintain a unified defense against possible sources of reinfection.

The National State-Federal Cooperative Bovine Tuberculosis Eradication Program, which began in 1917, remains an integral part of this nationwide defense system. The program is now winding toward its conclusion, with few known infected cattle herds remaining in the United States. Forty-two States and the Virgin Islands are currently accredited free of the disease. Virginia's accredited-free status will soon revert to modified-accredited because two cattle herds there were recently found positive for tuberculosis. Nevertheless, we are still making good progress. In fact, just last year two additional States, New York and Louisiana, achieved accredited-free status.
Although the TB program has encountered significant challenges in the past few years, we believe we have a firm handle on the problem, and eradication is still set for 1998. However, we cannot forget that pathogenic organisms like tuberculosis are exceedingly opportunistic. Our hard-won experience has taught us that we cannot relax our vigilance. At this stage of the game, it is especially critical to maintain our momentum and to emphasize preventive measures.

As most of you are aware, during the past decade, more than half of all cattle with tuberculous lesions detected at slaughter in the United States have been traced back to Mexico. In recognition of the severity of the problem, the Mexican Government last year allocated $92 million for an ambitious National Tuberculosis Eradication Program. Modeled after the U.S. program, it requires, among other things, mandatory inspections of slaughter facilities and the implementation of uniform methods and rules for testing and eradication procedures. Prior to our action to ban importations of Holstein cattle from Mexico last year, Mexico had also placed a voluntary ban on exports of these animals. I’m pleased to support these efforts, and I’m proud that APHIS personnel were able to play an important role in the development of the Mexican program by providing their advice regarding training in post-mortem inspections, laboratory procedures, and field testing. In fact, some of the binational initiatives outlined in the Mexican eradicación program are already paying dividends. For example, since the prohibition on Holstein feeder cattle went into effect last year, we’ve seen almost a 50 percent reduction in the number of tuberculous lesions found at slaughter plants in the United States.

Mexico’s actions are of critical importance in addressing the long-term threat that TB holds for our country. However, earlier this year, we took additional measures to further safeguard our domestic herds. In this regard, we have proposed increasing our post-entry testing requirements for Mexican cattle and denying indemnity claims for Mexican cattle that are positive for TB or for other cattle that are exposed to reactor animals. This change, if enacted, would increase the financial stake importers have in importing healthy cattle. Cattle from herds that qualify for TB-free status under the Mexican Government’s TB eradication program would be exempted from the additional restrictions.

Moreover, in response to a USAHA resolution passed last year, as well as program needs, we have incorporated captive-farmed Cervidae under the umbrella of our general TB eradication program. This action provides uniform program standards and testing requirements for these animals which, because of their growing numbers and popularity, pose an increased risk of transmitting Mycobacterium bovis to domestic cattle herds, as well as to wild Cervidae. With the closure of these and other loopholes, we are confident that final eradication of this insidious disease, in all its forms, is attainable...an event many of our great grandparents never dreamed was possible.

We are also closing in fast on our goal of eradicating brucellosis, an
other longstanding disease threat. Back in the early 1930's when the coop-
erative State-Federal campaign began, few thought that full eradication
was attainable. However, today, 33 States, Puerto Rico, and the Virgin
Islands have been declared free of the disease. With the continued aid of
our cooperators, we know we can complete the task by 1998. In fact, we've
already seen nine other countries, including Canada, Australia, and Great
Britain, eradicate brucellosis using methodology that we developed.

An interim rule that went into effect last March providing APHIS with
the authority to depopulate all known brucellosis-infected swine herds and
to reimburse herdowners for the market value of their animals was an im-
portant step in the right direction. Most of the infected swine herds have
now been depopulated, largely due to the enactment of this measure. We
are continuing to monitor potential sources of reinfection and to depopulate
all newly discovered infected swine herds.

Still, despite the great progress we've made thus far in battling
brucellosis, holes remain in our defenses. For instance, we estimate that
perhaps as many as half of the bison and up to 10 percent of the free-
ranging elk in the Greater Yellowstone area may carry the disease. These
wild herds are outgrowing the parks and refuges designed to hold them.
And, more and more often, they are venturing out to surrounding areas or
States for food. This trend of higher infection rates, population increases,
and greater herd mobility make an outbreak in area livestock almost inevi-
table.

Again, this is an area where coordination of efforts and wholehearted
cooperation are going to be essential if we are to eliminate this disease
threat. Recently, other Department officials and I participated in a sympo-
sium to explore ways to address the problem with officials from the Depart-
ment of the Interior and the States of Montana, Wyoming, and Idaho. We
are confident that all the parties involved will soon reach a consensus on a
unified approach, or combination of approaches, that will be both effective
and have minimal impact on the wild herds.

We are also moving ever closer toward eliminating pseudorabies (PRV)
from the United States. At this time, the owners of nearly all of the infected
herds are participating in herd-cleanup plans. Thirteen States have achieved
free status, and Massachusetts recently advanced to Stage III. With Iowa-
-the State with the greatest concentration of PRV-infected swine herds-
enrolling all of its counties in the program, we are on target to eradicate the
disease by the year 2000.

These past and present achievements in disease control and eradica-
tion I've just discussed have certainly had a tremendous impact on the
production of livestock in this country, as well as on our economy and qual-
ity of life. However, today's livestock producers must cope with diseases
their 19th century counterparts could never have imagined. For example,
the emergence of a potentially fatal form of bovine viral diarrhea (BVD)
represents a serious threat to the health of cattle throughout the United
States. A study recently conducted by APHIS' Centers for Epidemiology
and Animal Health found that, among other things, BVD mortality rates can be severe because most U.S. cattle have not been adequately vaccinated against the disease. Our officials are monitoring the situation using data supplied by State veterinary diagnostic laboratories and rendering facilities across the country. We will continue to work closely with other Federal officials and State and industry representatives to get information to herdowners regarding effective BVD management techniques.

And there are still other problems that need to be faced. Problems that have far-reaching consequences, and, unfortunately, no easy solutions. One of the most critical is the contamination of our food supply.

As you know, adulteration of meat and poultry products remains a significant public health problem in the United States. A number of surveys indicate that as many as 10,000 cases of foodborne illness occur annually from ground beef contaminated with \textit{E. coli} 0157:H7. Similarly, \textit{Salmonella enteritidis} contamination of raw poultry contributes to hundreds of thousands of cases of sickness each year, through cross-contamination, incomplete cooking, or other means. Those most at risk are among the most vulnerable segments of our population—young children and the elderly.

As many of you are probably aware, Dr. Michael R. Taylor, the Administrator of USDA’s Food Safety and Inspection Service (FSIS), was recently named Acting Under Secretary for Food Safety under the Departmental reorganization. The creation of this position both elevates the food safety functions within the Department and separates them from the marketing functions, ensuring that the overriding public health concerns involved in this complex issue are addressed thoroughly and in a coordinated manner. Under Dr. Taylor’s leadership, the meat and poultry inspection functions of FSIS will continue to form the basis for the Nation’s food safety strategy. In addition, it will include the preharvest activities and \textit{Salmonella enteritidis} programs now being conducted by APHIS, as well as the Agricultural Marketing Services’ Egg Products Inspection Program. I look forward to working closely with Dr. Taylor as he and his colleagues further consolidate food safety functions within USDA and continue to build a science-based program that fully meets the public’s high expectations for safe, wholesome meat and poultry products. At the same time, I know that officials within the new USDA food safety organization will be soliciting input from you, our stakeholders in the scientific community, to ensure that their actions are feasible and effective in reducing or eliminating the bacteria that endanger our Nation’s food supplies.

The consolidation of food safety functions I’ve just touched upon is one of the first phases of the general reorganization of USDA. As most of you know, Congress approved the landmark legislation for the reorganization on October 5, providing USDA with the authority to totally restructure and streamline operations at both the headquarters and field levels. Over the next 4 years, we anticipate the USDA reorganization plan will save the taxpayers \$2.5 billion by closing 1,100 field offices, reducing the workforce by at least 7,500 positions, and eliminating 14 of the 43 USDA agencies.
As I've mentioned, some initial steps have already been taken toward this goal, including the reorganization of USDA's headquarters structure, which names six new Under Secretary positions. Moreover, as a result of recent buyout legislation, over 3,400 USDA employees have already separated from the Department, and it is likely that another 1,700 will retire or resign next year.

At this time, I want to stress that none of the savings that will be realized through the reorganization will be taken away from our agricultural programs, such as animal disease eradication. In fact, we believe that, by reducing needless duplication of efforts, consolidating field offices to provide "one-stop" shopping for USDA customers, and upgrading our communications systems, we will greatly improve our delivery of program services. I'm proud to be a part of this newly-reinvented USDA: an organization that is at the forefront of change and committed to making the best use of its resources to better serve its customers. And I'm looking forward to continuing our cooperative tradition with USAHA as we prepare to meet the challenges facing agriculture during the next century and beyond. Together, we have confronted a multitude of disease threats—contagious bovine pleuropneumonia, hog cholera, FMD, and others—threats that at times seemed insurmountable. And, together, we succeeded. I'm confident that the strengths that have served us so well in the past—perspective, professionalism, and an unparalleled sense of commitment—will enable us to move ever closer to eradicating the destructive diseases that remain.
It is indeed a privilege for me to address you this evening as president-elect of the United States Animal Health Association. This is our 98th annual meeting, and if our registration so far is any indication of success, this should be one of our best meetings ever. I believe the key to the longevity of our organization is our ability to keep many irons in the fire. By that, I mean we continue to address the obvious problems of controlling and/or eradicating serious livestock and poultry diseases. In addition, we have always seemed to possess the unique ability to identify emerging problems as they appear on the horizon. After identifying them, we then set about to address them, either through our in-place committee system or by enticing the recognized authorities in those areas to present the latest information on them in our general session. Aquaculture, Feed Safety and Animal Health Information Systems are examples of some relatively new areas of interest. I believe that it is our ability first to enlarge our areas of focus, and then be able to re-adjust our priorities as needed that keeps the U.S.A.H.A. in touch with today's changing face of agriculture. I also believe that these characteristics of our organization is what attracts new members and new allied organizations to our rolls.

I mentioned earlier that the organization was able to enlarge its focus to encompass new industries, issues and concepts. The term "enlarge" simply means to widen one's perspective. It certainly does not mean to lose sight of the original aims and goals of the organization's founding fathers - that of controlling and/or eradicating serious livestock and poultry diseases. This is still the cornerstone of our existence, and we will hear a little later in the program of just one of the organization's many success stories.

Remembering our goal is more important than ever now that we are in the countdown stages of conquering several major livestock diseases. The diseases to which I refer are cattle and swine brucellosis, as well as pseudorabies. It is of utmost importance that all the major players - industry, state and federal governments and universities - are functioning in the most cooperative, cost efficient and effective ways to accomplish the goal of eradication. The U.S.A.H.A. annual meeting provides the forum where the overall gameplan can be comprehensively reviewed and refined by all players, and the specific play for the following year can be formulated. This is what the U. S. Animal Health Association has been doing so well for nearly the last hundred years, and we hope, for the next hundred as well.

During the next year while I am president, I hope to be able to carry on the task of making our organization more vital and responsive on a day-to-day basis. I want us to continue the new format of our quarterly newsletter, which contains current items of interest to our membership. I have set a goal for myself to have the committee chairmen and committee members in place by Christmas so that a lack of committee appointments will not be a source of delay in getting out the published proceedings from this meet-
ing. We hope to continue the process of more efficient computerization of the proceedings, thus resulting in their being published at an earlier date and with considerable cost savings. As Public Relations Committee chairman, I have appointed a very able sub-committee to look into the possibility of some form of a more modern, up-to-date, easily transportable and accessible form of promotion for our organization. This new promotion vehicle would be available for use in veterinary schools, state veterinary medical associations, industry meetings, etc. Later this year, I plan to name a committee that will begin to plan our centennial meeting. Lastly, I have recently pledged the support of this organization to Assistant Secretary Jensen by offering her the organizational structure of U.S.A.H.A., as well as the expertise of a wide selection of our members in addressing the current issue of pre-harvest food safety. In light of her recent comments, I will have to ask my secretary to find that letter in the file and change and addressee to Mr. Taylor. Seriously, I hope to do whatever I can to assist in this difficult period of transition.

I know this is an aggressive agenda for a state veterinarian that is the only veterinarian employed by his state's government. That is why I ask now for your suggestions, your help and your prayers as I prepare to lead our great organization through the next year.
MESSAGE FROM THE AAVLD PRESIDENT
HERB SMITH, DVM, PH.D.

Distinguished guests, ladies and gentlemen. It is indeed an honor to address this group tonight. I have been forcibly aware, at this meeting, of the value of the partnership between USAHA and the AAVLD. Not only has diagnostic veterinary medicine come into its own but also that partnership.

Tonight I want to brag on North Dakota, not only because I want to but also because it may be some years before it can be done again. To start with I want to introduce some of those here who are from North Dakota. I have counted 14-15 at this meeting from North Dakota, which, on a per capita basis, I doubt can be matched by any other state.

I first would like to introduce my wife, Arlene. I forgot to introduce her at our state meeting back in August and I've been hearing about it ever since. We also have our state veterinarian, Bob Velure, and our deputy state veterinarian, Bill Rotenberger. These are two of the most dedicated veterinarians in North Dakota and we are mighty proud of them. Irving Huff, the North Dakota AVIC is also here. Leonard Lodoen, a seed stock producer from West Hope, and a recent president of The North Dakota Board of Animal Health is also present. Ken Throslon, our resident expert bison veterinarian, Dennis Joyce, an elk rancher and Jim and Jody Hague, North Dakota pork producers are also here. We in North Dakota pride ourselves in saying our aim is to make North Dakota the healthiest place in the world to raise livestock.

I would also like to echo the words of wisdom of Dr. Van Es, the first state veterinarian in North Dakota, appointed in 1903, who said that "The first element in animal disease control is a rapid and accurate diagnosis." His untiring work on equine infectious anemia, glanders, dourme, and avian and bovine tuberculosis is a model for disease control and eradication in this century. It has been rightly said that if we do not learn from the lessons of history we are bound to repeat them. I have here the documentation of some of Van Es' work in swine fever where he singlehandedly took the temperature of an infected horse (#636) daily for 14 years. You can imagine that he got much better acquainted with that horse's anus than he ever wanted to. Yet it is this dedication, this attention to detail that is part of the lesson I want to leave with you tonight. Dr. Van Es worked as a veterinarian until he was 80 years old. This is the second part of the lesson from history I want to leave with you. I see in this room many stalwart veterinarians who have given so unselfishly of their careers far beyond normal retirement age. I see Harold Chute from Maine who has kicked off the AAVLD Foundation with a generous contribution that ensures the viability of the newly established Foundation. I also see Dr. Ben Pomeroy, a recognized senior statesman in veterinary medicine - I need say no more.

The conclusion, and the lesson is, that the talent, experience, knowledge, and wisdom of these gentlemen, and many more like them, should be used to the full advantage of both of our organizations. They have so
much to offer and we should be so pleased that they are still willing to contribute.

We have two most excellent organizations and as we look to the future of that partnership we should dedicate ourselves once again to excellence, cooperation, and integrity. We have good days ahead and thank you once again for the honor of having a part in this meeting.
If the obstacles ahead seem insurmountable, we need to recall the experience of the past, or we may have to re-live it, as a scholar told us years ago.

Forty seven years ago, in 1947, when this organization was called the United States Livestock Sanitary Association, it sent a committee of four state veterinarians to Mexico when Foot and Mouth Disease was introduced into that country and quickly spread to 16 states. The members of that committee were Will J. Miller of Kansas, who was then president of USLSA and who served as chairman of the committee; H. F. Wilkins of Montana; Ivan G. Howe of New York, and C.E. Kord of Tennessee. Imagine the concern of the livestock industry in the U.S. at that time—no reliable vaccine was available anywhere in the world. The only solution was the slaughter of all infected and exposed herds. Quotes from the report submitted to USDA and the appropriations committees of the U.S. Congress seem appropriate tonight when USAHA is commemorating the successful eradication of Foot and Mouth Disease in Mexico, which occurred 40 years ago this December. When the committee arrived in Mexico City the members saw a build-up of equipment similar to the Gulf War or Haiti in miniature. The report states: 

"We were informed that over 1,400 pieces of automotive and heavy equipment are engaged in the campaign, with additional items arriving daily for field assignment. A random sampling of the major equipment items received from the U.S. and now in active field use, included 180 jeeps, 100 light trucks, 71 bulldozers, 59 scrapers, 10 power shovels, 35 heavy trucks, 122 trailers, 268 power sprays and 25 paymaster cars." Later, LCVP boats used to land our military on beaches in World War II were sent to move heavy equipment to the tropical areas where transportation was mainly by boat. The committee toured the entire infected area by automobile, jeep, airplane and boat. They traveled throughout the 16 infected states of the 28 in Mexico and along the U.S.-Mexican border. They described the travel "over unusually high, rugged mountain sections in which nothing but foot paths or burro paths or burro trails could be seen; large, open, intensely cultivated valleys and apparently almost impenetrable tropical areas along the coasts where rivers slowly and tortuously course their way through jungle to the sea. In these coastal areas most travel and movement of supplies is by boat or on foot. "Rugged mountains, up to at least 10,000 feet high, are inhabited and cultivated in spots to the very top. It must be remembered that such areas contain a large number of susceptible animals which can only be satisfactorily located and brought out by native inhabitants when needed to be sacrificed or inspected. "In other words, the infected area is almost as open as when discovered by the Spaniards and certainly much more densely populated. Every plot of tillable ground in this southern section of Mexico is cultivated and much of it by hand as the steep mountain sides rise at an angle up to approximately 45 degrees, where neither oxen
nor mules can be used. In the open valleys, oxen, mules and tractors are employed. Most of the people live in communal villages from which the cattle, sheep and goats move out to graze during the day and are brought in at night, when distribution is made to the numerous owners. While grazing, the herds from adjoining villages are frequently mixed, so the herders may visit, or if the herds are not mixed, the herders do congregate; hence when infection is present in one of the village herds, it is soon found in the surrounding ones. “The people, most of whom in these poorer, rural areas are Indian, move about by foot or on burro and can be seen at all times of the day and night going to market and elsewhere or returning home. At the present time, infection is not known to exist in these hostile areas, but without much better control of the human and livestock factors, it is reasonable to presume that all territory within the north and south lines will become infected, except perhaps for some marginal or isolated area. “The topography of the country involved varies from high, steep, rugged mountains, barren, rocky, arid regions to fertile valleys intensively tilled and heavily populated, and to wet, tropical, malaria-infected, mosquito-infested jungles and everglades. In the swampy coastal area transportation is, of necessity, by boat and on foot. “Although the spread of the disease has been slow, it has been relentless. Energetic eradication work has kept this spread to a minimum, but it has not been able to stop it without the same energetic handling of the quarantine operations.”

The committee observed:

1. The continued effort has contributed to the better understanding of the quarantine restrictions on the part of some of the military officers and soldier personnel.

2. Commission people are being placed with the military personnel at the most important places.

3. The new line approaches the natural dividing line between the communities toward the north of Mexico and those which depend upon Mexico City, and the other areas of central Mexico.

4. The character of the terrain in many places is such that it acts as a natural barrier to the spread of the disease.

5. These quarantine lines are approaching the areas where the local inhabitants are cognizant of the unbearable cost and loss associated with attempting to live with Foot and Mouth Disease and are, therefore, naturally actively desirous that the disease be kept from their territory. This has been evidence in some cases through the local decision of the people to employ guards to augment the quarantine force of the Army. This point is probably the most important.

“It is obvious that the success of the campaign will depend a great deal upon the unselfish and wholehearted cooperation of the people, as well as that of the Mexican government and the Mexican and American forces assigned to the task of eradicating this most devastating animal plague.”

The committee also listed some of the obstacles, including:

1. Continued disagreements over appraisals. Each such disagreement stops slaughter operations, with an attendant waste of man hours, until a solution can be found.
2. The lack of transport for Mexican Army troops. Work cannot go on without the presence of these troops to keep order and the lack of transport has often delayed the work until the troops arrived.

3. Resistance of the people to the work of the Commission, in many ways necessitating delays in the work until the resistance can be dissolved through the use of educational aids, persuasion or military force.

"The present extent of the disease is such that the control and eradication effort will have to be continued for some considerable time and the results will most assuredly cause a tremendous impact upon the economy of central Mexico. A realization of this is probably responsible for some of the opposition to the campaign, particularly on the part of political leaders in some of the infected areas. The government is making every effort to counteract this effect through the formation of educational committees throughout the infected areas and the establishment of an over-all committee for rehabilitation of the areas that have been deprived of their livestock. These efforts have had considerable effect, but the impact of the disease and the program of eradication are so tremendous that it cannot be said whether the efforts of the government are sufficient to accomplish the desired ends.

"One outstanding feature that we observed was the high morale of the Commission forces. Taking into consideration the many difficulties and discouraging aspects that the many difficulties and discouraging aspects that have presented themselves, this high morale very forcibly demonstrates the sincerity of purpose and ability of the campaign personnel. To attempt eradication of Foot and Mouth Disease from such a large infected area and in a foreign country requires courage, foresight and hope—a hope that every interested individual and department will give that cooperation and immediate favorable response so indispensable to immediate control and ultimate eradication." The underlines were not in the original report. I have added them for emphasis.

"Action, speedy and positive, is the essence of success and it is not readily obtained in a country not geared to that way of doing and to a people who do not fear or realize the far-reaching effects of the disease or fully understand the cost of delays, even of hours. The effort to hold the north and south lines—to operate from without inwardly—to attempt eradication form within, concurrently in the hope of saving thousands of animals—is commendable. This, however, has not been attainable with all of the deterring factors enumerated, including adverse and conflicting propaganda and sabotage.

"In view of the almost impossible terrain and the many obstacles that present themselves, your committee considers that the problem of eradication of Foot and Mouth Disease in Mexico is one of the most difficult ever presented to the veterinary profession."

The final recommendations of the committee were:

"1. The establishment and maintenance of a research laboratory on an isolated island or other acceptable safe area for the intensive study of Foot and Mouth Disease." As a result we have had the benefits of the Plum Island Research Laboratory, built in the 1950's.
"2. Further research to determine methods for the immediate and proper disposal of carcasses by chemicals or means other than burial.

3. The building of an adequate fence along the U.S.-Mexico international boundary line or adjacent to, as previously suggested by others."

The disease was having a devastating effect on the economy, due to controls on movement of livestock, and the closing of the U.S. border, and to livestock production and marketing in northern Mexico, not to mention the political unrest due to eradication procedures. In spite of these conditions, the joint program continued, but the method of eradication changed from killing all infected and exposed animals to vaccinating 17 million animals between the north and south quarantine lines.

The theory was to keep the entire population immune during a 12-month period (the longest time the virus was known to live outside of an infected animal's body). The next step was to establish an inspection system that would find and eliminate any of those vaccinated animals that showed evidence of the disease.

So we established what I call the "first Peace Corps." The infected area was divided into sectors, the size being determined by the time it took a U.S. inspector and his Mexican counterpart, traveling together, to inspect all susceptible animals every 30 days. Vigilante committees were set up to contact the inspectors if any animals got sick between their monthly visits. The inspectors were allowed out of their sectors one weekend a month for rehabilitation purposes.

I say they were the original peace corps because in most cases they were the only persons who could read or write in their sector. They spent money for lodging, food, horse and mule hire within their sector. If any animals came down with the disease and had to be killed, the owner was paid on the spot. Needless to say they became great friends with the inhabitants of their sector.

Initially we ran into trouble with vaccines imported from South America, due to outbreaks associated with their use. Importation of vaccines from Europe turned out to be too much of a hassle for the amount that was needed. So the Mexican and U.S. researchers on the Commission built a production laboratory and turned out a very effective vaccine against the strain we were combatting. As with the few effective vaccines available at the time, the length of immunity was only 4 months, so the entire population had to be revaccinated at 4-month intervals.

The inspectors lined up the animals for the vaccination brigades in the villages within their sectors. Here again, local people were hired to help with the work involved. Those inspectors found approximately 12,000 infected animals among the 17 million that were vaccinated.

Many of us learned to speak out of both sides of our mouth at the various meetings with the local population. When the program was slaughter of infected and exposed animals, we belittled the use of vaccine. When the program was changed, we became advocates of vaccine use.

Within a seven-year period, 2550 veterinarians and support personnel were recruited or transferred to Mexico. At the height of the program in 1949, 8000 American and Mexican personnel were working in it. Nearly a
million infected and exposed cattle, sheep, goats and hogs were killed and 17 million were vaccinated every four months for a year. Some personnel became sick and died of diseases—malaria, malaria, typhoid fever and dysentery. Six Mexican veterinarians were killed and one U.S. inspector was stoned to death riding into a village to inspect livestock. A U.S. information specialist was killed with a shotgun. There were three main causes of deaths—civil mutiny which took 20 lives, highway accidents which took 19 and plane crashes which killed 14. Seven persons were shot from ambush; two were killed when thrown from their horse, one was run over by a train and one was accidentally shot.

In 1954, the disease was declared eradicated by the governments of the U.S. and Mexico. The insurmountable objective had been achieved.

In retrospect, the report of the USLSA committee was extremely pessimistic; however, I want to emphasize the committee's recognition of the dedication and positive attitude of the U.S. and Mexican personnel conducting the program and the high morale observed by the committee members.

I sincerely believe this experience gave both the state and federal governments and the industries involved in the U.S. a "can do" attitude that played a major role in these programs:

* Eradication of Vesicular Exanthema of swine;
* Changing the brucellosis goal from control to eradication;
* Hog Cholera eradication;
* Sheep Scabies eradication;
* Contagious Equine Metritis;
* Virulent Velogenic Newcastle Disease of poultry;
* Venezuelan Equine Encephalomyelitis;
* African Swine Fever in the Dominican Republic and Haiti;
* Screwworm eradication in the U.S.

Equally with our Mexican neighbors, the program established the competence and dedication of researchers. It strengthened the recognition and importance of the veterinary profession in both countries. It created effective, workable relationship between the governments and the livestock industries of both countries. It was a major factor in developing a successful joint U.S.-Mexican screwworm eradication program.

What was learned:

1. Don't avoid what may appear to be insurmountable challenges; where there is a will, a way will be found.
2. Be absolutely sure that you have the support of the industry involved, or chaos may result.
3. Effective communication is essential, both within and outside the governments and industries involved.
4. No job is too big; don't let logistics overwhelm your outlook.
5. Now, when we are facing the challenges of eradicating piroplasmosis, food safety, free trade, environment and animal welfare, remember the real message of the 1947 USLSA committee report—Keep a positive attitude and high morale and, based on past accomplishments, you will ultimately get the job done.
NATIONAL ASSEMBLY AWARD
Dr. Jones W. Bryan

It now becomes my pleasure to present the annual award of the National Assembly of Chief Livestock Health Officials.

This award is presented to a person who has given many years of service and is still active in regulatory veterinary medicine.

As I asked for nominations for this award this year, one name was presented by most of the responders.

His service to our field is well recognized by all in the regulatory field. He has given 37+ years in our arena. His first regulatory position was that of Field Veterinarian in Pennsylvania in 1957.

In 1964, he became Director of the Department of Animal Industry and served until 1971.

From 1971-1979 he served as Laboratory Director in Maryland, becoming Assistant Chief of Animal Health in 1979-1982.

In 1982 he assumed the position of State Veterinarian where he served until his retirement in 1986.

He has been a member of USAHA since 1962, served as President in 1972, and currently serves as Secretary and Treasurer.

He has been married to Jean for 50 years and they have two sons, three grandchildren and one great-grandchild.

It is a genuine pleasure to present to my dear friend, Dr. John C. Shook, this award.

Dr. Jones W. Bryan, President of the National Assembly of Chief Livestock Health Officials, presented the sixth National Assembly Award to Dr. John C. Shook, Secretary/Treasurer of USAHA. The award is given to an active regulatory official or an industry representative for outstanding service in animal health regulatory programs.
Good evening. It is with the greatest pleasure that I introduce Mr. Neal Black, the recipient of this year's Administrator's Award.

In many ways, Neal has proven himself to be like the swine industry's version of Superman. Like Superman, Neal began life in a small town, and started his career working at the local newspaper. In fact, at one point Neal even held the same job that Clark Kent did -- city desk reporter. However, like Clark Kent, Neal had a secret alter ego -- champion of swine health. This alter ego first emerged in the 1950's, when the editor of the Waterloo, Iowa, Daily Courier newspaper, where Neal was working, started a farm section and put Neal in charge. Under the stewardship of Bernard Ebbing and the late Lou Thompson, both of the Rath Packing Company, Neal met his destiny. He learned about agriculture, and more importantly, he learned about hogs.

Neal's transformation from a "Clark Kent-type" journalist to the Superman of Swine Health began in 1957, when he accepted the position of managing editor at the magazine National Hog Farmer. In his 23 years at National Hog Farmer, he devoted his energies to the swine industry, relentlessly investigating and reporting industry developments, potential threats, and improved opportunities for producers. Where there was a threat to swine health, Neal was there, the man of steely words who used his typewriter to demand truth from bureaucrats involved in the legislation affecting the industry; justice for producers threatened by the loss of essential disease-controlling drugs or the spread of potentially devastating diseases; and the American Way for producers who he urged to organize at local, regional, and State levels.

This Superman of Swine Health bravely combatted hog cholera and pseudorabies, the arch enemies of all pork producers, by running countless stories, at least one every issue for 15 years, on the importance of hog cholera eradication, and voluntarily serving as Chairman of the National Pseudorabies Control Board, where he helped to review the standards of the program. But his long list of courageous acts does not end there. In the late 1970's, when nitrate was being linked to cancer and the cured meat industry was threatened by FDA and USDA regulations, Neal was faster than a locomotive in researching and reporting the facts and helping the industry to surmount the tall opposition it faced.

In 1979, Neal left the National Hog Farmer to head the Livestock Con-
servation Institute, where for 8 years, he continued to closely monitor af-
fairs in the swine industry, ensuring that pork producers remained safe from African Swine Fever and other threats to their livelihood.

Dr. Lonnie J. King, Acting Administrator, presents the APHIS Administrator's Award to Mr. Neal Black.
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."

ARTICLE II - PURPOSE

The mission of USAHA is to be a forum for communication and coordination among State and Federal governments, universities, industry, and other groups on issues of animal health and disease control, animal welfare, food safety and public health. It serves as a clearing house for new information and methods which may be incorporated into laws, regulations, policy, and programs. It acts to develop solutions to animal health-related issues based on science, new information and methods, public policy risk/benefit analysis, and the ability to develop a consensus for changing laws, regulations, policies, and programs.

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 of such other governmental agencies 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.
INDIVIDUAL MEMBERSHIP

Any person engaged in animal health work for Federal, provincial, state, county, or municipal governments, and any other person interested in animal health science or milk and meat hygiene, may be elected to individual membership.

Any individual members who have 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 in USAHA 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. Fully retired life members, not otherwise gainfully employed in the field of animal science or health, shall also be exempt from the payment of annual meeting registration fees. 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,
The Board of Directors shall consist of the officers, including the immediate Past President with the exception of the Executive Committee. It shall handle the financial, administrative, and internal affairs of the Association during such time as the Association and/or the Executive Committee is not in session. It shall handle all other duties and responsibilities as may be assigned to it by the Executive Committee or as may be provided in the Constitution. The Board of Directors shall meet immediately after the adjournment of each annual meeting of this Association and at the same place. The purpose of such meeting is to review plans for the administrative functions of the Secretary for the coming year, to give administrative guidance to the Secretary, and to approve the operations of the office of the Secretary including, upon consultation with him, the employment of an Executive Director and such other employees as may be required which are not otherwise in conflict with the Constitution and Bylaws. The Board of Directors may meet at such other times and places as it, by a majority vote, deems necessary. The Secretary shall keep minutes of all meetings of the Board of Directors, and after approval of such minutes by the President, they shall be presented to the Executive Committee at the next annual meeting of this Association.

**EXECUTIVE COMMITTEE**

The Executive Committee shall be composed of the executive officer representing the animal health departments of the various states, the principal animal health officer of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive animal health officer of Mexico, Puerto Rico, the Virgin Islands, and of such other governmental agencies 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. 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.

There shall be five districts. Said districts shall be known as (1) The Northeast: consisting of the states of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont; (2) The North Central: consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri,
Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; (3) The Southern: comprising the States of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico, and the Virgin Islands; (4) The Western district: consisting of the States of Alaska, Arizona, California, 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 Committee 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 present to the Chairman of the Executive Committee for election by that body the names of individual members eligible and applying for life membership. 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 monies 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.

ARTICLE VIII-AMENDMENTS

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 - COMMITTEE ON NOMINATIONS AND RESOLUTIONS

There shall be appointed annually a Committee on Nominations and Resolutions which shall be comprised of the Association's living immediate past presidents from each of the five districts, and the current president of the Northeast, North Central, Southern and Western Animal Health Associations. The immediate past president of the United States Animal Health Association shall serve as chairman of the committee. The purpose of the committee shall be to receive, consider and present to the general assembly nominations for officers and elected regional delegates, as well as resolutions, following such procedures as are established in Articles X and XI.

ARTICLE X - ELECTION OF OFFICERS AND ELECTED REGIONAL DELEGATES

The Committee on Nominations and Resolutions shall annually report to the Association membership at the first morning general session. Its recommendations for the offices of President, President-Elect, First Vice-President, Second Vice-President, Third Vice-President and Treasurer, as
well as Elected Regional Delegates shall constitute its report. Except for
the office of Treasurer, nominations shall not originate within this commit-
tee but shall be submitted by the appropriate region after caucus of its
official and affiliate representatives who are members of USAHA. From
such caucus, there must originate every fifth year a nominee for the office
of Third Vice-President from the district of that of the retiring President of
the Association. Annually, by caucus, two nominees for Elected Regional
Delegate will likewise be selected and offered in nomination by each of the
four regional associations.

The recommendations of the Committee shall be posted on the regis-
tration bulletin board immediately following their presentations at the first
morning general session. Any member of the Association, at the second
general session, may propose amendments to the slate presented by the
Committee. Such amendments shall be made at a time certain specified in the
program for "Report of Action of the Committee on Nominations and
Resolutions" during that session; provided that if a paper is being presented
at that specified time, its presentation will be completed, immediately after
which the 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 estab-
lished in the program for the "Report of the Action of the Committee on
Nominations and Resolutions". The Report of the Committee on Nomina-
tions and Resolutions, and proposed amendments to the report, shall be
presented to the Executive Committee for consideration. The acceptance
of the report or amendments shall constitute election.

ARTICLE XI - RESOLUTIONS

As the concluding committee report at the final session of the meeting,
the Committee on Nominations and Resolutions shall present for consider-
ation by the membership those resolutions which it has properly received
and reviewed for ambiguity and redundancy. Such resolutions must have
been submitted in proper format to the Committee by officially designated
committees of the Association, including the Executive Committee, or by
its Board of Directors. Resolutions, properly submitted, will not be altered
as to intent by the committee. Majority approval of resolutions or amend-
ments made thereto by the general membership present and voting, will
constitute acceptance.

BYLAWS

ARTICLE I-ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary.
Report of Treasurer.
President-Elect's Address.
Reading of Papers.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nominations and Election of Officers and eight members to
Executive Committee.
Adjournment.

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 sixty dollars ($60) 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 between September 15 and November 15.

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 that the majority of those in attendance in comprised of the executive officers representing the animal health departments of their respective states.

ARTICLE IV-DUES

The dues for individual membership in this Association shall be sixty dollars ($60) per annum, payable in advance (on or before January 1st of each year) to the Secretary of the Association.

The dues for nonvoting junior members shall be three dollars ($3) per annum, payable (on or before January 1st of each year) to the Secretary of this Association.

The dues for official and allied organization memberships shall be 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.

ARTICLE VI - ALTERATION OF BYLAWS

For the purpose of changing the order of business or to facilitate important business, Articles I and III of the Bylaws, or any portion thereof, may be suspended during any single meeting by unanimous consent of the Executive Committee.

Amended November 1994.
As we enter into an era of more open trade it becomes increasingly important to document the health and management of domestic livestock and poultry populations. The goals are to document the health of the National herd and flock in order to obtain access to markets and also to be vigilant to emerging problems whether of domestic or foreign origin. To this end the USDA:APHIS:VS has been building a National animal health monitoring and disease surveillance system. This National monitoring and surveillance system is built upon the firm foundations of the Brucellosis and Tuberculosis control programs. In addition the program makes use of existing data from other government and private sources. When new data are required the information gaps are filled through a variety of activities including National surveys, targeted studies, and on-going monitoring efforts. Current on-going monitoring efforts include the Veterinary Diagnostic Laboratory Reporting System, feedlot mortality, and swine slaughter checks. The on-farm component of the National monitoring and surveillance system is the National Animal Health Monitoring System (NAHMS). To date the NAHMS has gathered data through National surveys of the swine and dairy industries. The third National survey effort for the NAHMS was a study of the cow/calf industry.

Critical information gaps for the beef cow/calf industry were identified by contacts with industry representatives, veterinary organizations, animal health officials, extension personnel, and monitoring the lay and professional literature. The information needs were compared to known sources of information about the industry. A National survey was selected as the most appropriate way to meet the need. Consequently, the National survey was not an independent effort but rather capitalized on the talents of many professional groups. Existing data from the National Agricultural Statistics Service (NASS) and Economic Research Service (ERS) were used to evaluate sample requirements and optimum design. The resulting Cow/calf Health and Productivity Audit (CHAPA) was a 16 month study of the beef industry initiated in October of 1992. Survey activities depended heavily on the collaboration of the State and regional VS forces and in some cases the State veterinary personnel along with the National Veterinary Services Labo-
U.S. COW/CALF HEALTH AND PREVENTIVE MEDICINE PRACTICES

ratory, the Centers for Epidemiology and Animal Health staff, the NASS, and university cooperators. The responsibility of each group is outlined in table 1. Over 2500 cow/calf operations from across the Nation provided information on their herds to NASS enumerators in phase one of the study. In phase two of the study attention was focused on herds:
- from 18 states with large beef cow populations,
- that calved at least 50% of their herd in the January through June period, and
- that had at least 5 cows or replacement heifers.

Phase two included 799 or 540 producers depending on the extent of data collected. During the course of the study producers were contacted five times to collect information on the health of calves, management of the herd, and reproductive efficiency of the herd. Data collected from these operations were used to make estimates of health and management of cattle on cow/calf operations throughout the U.S.

Pre-weaning death loss among calves born on cow/calf operations in the U.S. in 1992 was estimated at 3.5%. Death loss varies with herd size, larger operations tend to experience higher pre-weaning death losses than smaller operations. For herds represented by the phase two operations (18 states, 50%+ calving in January through June, 5+ cows or heifers) a comparison showed that 4.4% of calves died prior to 500 pounds in 1992 compared to 6.5% death loss in 1993. Recall that in 1993 there was a great deal of concern about "Weak Calf Syndrome (WCS)". In fact, in the first half of 1993, 25.5% of producers felt that they experienced above normal death losses among calves. In addition, 6.4% of producers felt they had seen WCS in their herds. "Weak Calf Syndrome" was most frequently reported by producers from the west (20.8%) and least frequently reported by producers in the southeast (1.3%). The occurrence of WCS was also related to herd size, being more common in herds with 300 or more cows.

Comparing morbidity of calves in 1992 with 1993 shows a general increase in several illness categories for calves up to 21 days of age. Scours was estimated to affect 5.5% of calves up to 21 days of age in 1992 compared to 7.0% or calves in 1993. There was a four-fold increase (from 0.3% to 1.4%) in the percentage of calves affected with respiratory disease in the first 21 days of life. The percentage of calves from 22 days to 4 months of age affected with respiratory disease increased from 0.4% in 1992 to 1.1% in 1993.

Nationally, 5% of all the calves born to beef heifers in the U.S. are stillborn and an additional 2.5% die in the first 24 hours after birth. Another 1.3% of heifers calves that are born alive die in the period from 24 hours to 3 weeks after birth. These stillbirths and early neonatal deaths represent a significant loss to beef producers. To evaluate the factors that contribute to
these losses operations were categorized into those with "high" losses (>5%) at birth and in the first 24 hours and those with "low" losses (5%). The analysis focused on operations where some calving was assisted by the producers or a veterinarian. Factors increasing the likelihood of being a "high" loss producer were; 1) calving less than 50% of the herd in a lot, 2) an increased percentage of deliveries that were attended by a veterinarian, and 3) having no set calving season. Factors associated with a reduced likelihood of being a "high" loss producer were; 1) the percentage of unassisted deliveries and 2) the percentage of deliveries classified as "easy pulls". In addition, there was a suggestion that larger operations were more likely to be "high" loss producers as were those from the southeast.

In addition to risk factor analyses, the data collected in the CHAPA were used to characterize the frequency of various preventive medicine procedure use on operations. The most common antigen used to vaccinate cows on operations represented by the phase two sample was Leptospira (32.5% of producers). Fewer producers vaccinated cows for BVD (19.4%), Campylobacter (18.2%), IBR (17.6%), Hemophilus somnus (5.5%), PI3 (15.6%), and BRSV (12.8%). These percentages are similar to those for producers vaccinating calves for each of the same antigens. Nearly two-thirds of producers vaccinate calves for blackleg and malignant edema.

The information generated by this study and other activities of the National monitoring and surveillance system has helped address a variety of issues. Widespread concern about WCS from producers, the media, and our trading partners was addressed through the VDLRS and the CHAPA study. It was concluded that there was an increase in beef calf deaths meeting the case definition of WCS, however, this did not appear to be a new agent but rather a series of environmental and nutritional events that resulted in increases in the occurrence of expected calf disease problems. The beef industry also is concerned with beef and by-product quality. The CHAPA has helped to characterize the extent to which management practices that favor injection blemishes are used as well as the extent of hide branding which has a significant impact on hide values. Information on vaccine use by producers (beef and dairy) is helping to characterize the risk associated with a relatively new strain of BVD virus. Subsequent plans for the NAHMS call for the continuation of National surveys to help monitor longer term trends in management and health of livestock and poultry populations. The current plan calls for revisiting each of the commodities with on-farm studies approximately every 5 years. In addition, efforts are underway to enhance the on-going monitoring component of the monitoring and surveillance system to look at additional key areas of animal production and health. These on-going efforts will include analyzing existing data from a variety of sources, as well as collecting new data through practicing.
veterinarians and others that have contact with producers. In all, the system will allow the generation of descriptive information to portray the baseline of health and management of livestock and poultry as well as do risk factor analyses and identify and characterize emerging disease concerns. This combination of activities will result in a system that can deliver timely information on a variety of topics relative to animal health, animal production, product wholesomeness, animal welfare, and the environment.

Table 1. Roles of collaborators in the CHAPA.

<table>
<thead>
<tr>
<th>Role</th>
<th>Responsibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEAH staff</td>
<td>study design, training, data analysis</td>
</tr>
<tr>
<td>Field staff</td>
<td>data collection, data capture, data validation</td>
</tr>
<tr>
<td>NASS</td>
<td>sample selection, initial contact of producer</td>
</tr>
<tr>
<td>NVSL</td>
<td>laboratory analysis, blood - copper, selenium, BVD titer, feces - scours agents, giardia, cryptosporidium</td>
</tr>
<tr>
<td>University</td>
<td>forage - proximate analysis of forage samples, economic - standardized performance analysis (SPA)</td>
</tr>
</tbody>
</table>
Importance of exotic animal disease (EAD) surveillance

In any country one of the main objectives of veterinary authorities is to prevent the entry of foreign pathogens. This is generally accomplished by the restriction of importation of animals and animal products from countries affected with one or several EADs. In the past this led to a ban of virtually all animal imports from these countries. Today it is possible to recognize disease-free zones within a country or to establish adequate safeguards to reduce the risk of a particular importation to tolerable levels. This new approach to animal health management facilitates trade whilst protecting national agriculture.

Nevertheless, despite all preventive measures the risk of an outbreak is ever present and veterinary services must keep a watchful eye on the health status of the national herd. In Mexico this became particularly relevant after the foot-and-mouth disease (FMD) outbreak during the late forties and early fifties, which implied the destruction of one million animals and caused a severe economic, social and political crisis. In 1952 the Mexico-US Commission for the eradication of FMD was transformed into a commission for the prevention of FMD. In 1988 it was further modified to include the prevention of other exotic diseases. Since its creation, CPA as it is commonly known has played a key role in exotic animal disease surveillance in Mexico.

Exotic Animal Diseases In Mexico

Table 1 & 2 Mexico is free from most of the great epizootic diseases, although some important diseases still exist, they have been placed under national control and eradication campaigns and their incidence has been reduced. Table 1 and Table 2 list those diseases included in the Office International des Epizooties (OIE) list A and B that are exotic to Mexico.

One of the most important diseases in terms of EAD surveillance is vesicular stomatitis (VS). This is a list A disease, however it does not fulfill the definition for that category, the disease itself does not constitute a great threat to animal populations. Its importance lies in that it is clinically indistinguishable from FMD and every case that is reported must be confirmed by the laboratory.

For many years VS was practically the only concern for CPA, however
### Table 1. OIE List A Diseases Exotic to Mexico

<table>
<thead>
<tr>
<th>Disease Name</th>
<th>OCC.</th>
<th>Disease Name</th>
<th>OCC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot-and-Mouth Disease</td>
<td>1954</td>
<td>Rift Valley Fever</td>
<td>0000</td>
</tr>
<tr>
<td>Swine Vesicular Disease</td>
<td>0000</td>
<td>Bluetongue</td>
<td>+?</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>0000</td>
<td>Sheep Pox and Goat Pox</td>
<td>0000</td>
</tr>
<tr>
<td>Peste des Petits Ruminants</td>
<td>0000</td>
<td>African Horse Sickness</td>
<td>0000</td>
</tr>
<tr>
<td>Contagious Bovine Pleuropneumonia</td>
<td>0000</td>
<td>African Swine Fever</td>
<td>0000</td>
</tr>
<tr>
<td>Lumpy Skin Disease</td>
<td>0000</td>
<td>Fowl Plague</td>
<td>0000</td>
</tr>
</tbody>
</table>

### Table 2. OIE List B Diseases Exotic to Mexico

<table>
<thead>
<tr>
<th>Disease Name</th>
<th>OCC.</th>
<th>Disease Name</th>
<th>OCC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heartwater</td>
<td>0000</td>
<td>Q Fever</td>
<td>0000</td>
</tr>
<tr>
<td>Dermatophilosis</td>
<td>0000</td>
<td>Trypanosomiasis</td>
<td>0000</td>
</tr>
<tr>
<td>Haemorrhagic Septicaemia</td>
<td>0000</td>
<td>Bovine Spongiform Encephalopathy (BSE)</td>
<td>0000</td>
</tr>
<tr>
<td>Contagious Agalactia</td>
<td>0000</td>
<td>Contagious Caprine Pleuropneumonia</td>
<td>0000</td>
</tr>
<tr>
<td>Enzootic Abortion of Ewes</td>
<td>0000</td>
<td>Salmonellosis (S. Abortus Ov)</td>
<td>0000</td>
</tr>
<tr>
<td>Naarobi Sheep Disease</td>
<td>0000</td>
<td>Pulmonary Adenomatosis</td>
<td>0000</td>
</tr>
<tr>
<td>Srapie</td>
<td>0000</td>
<td>Maedi-Visna</td>
<td>0000</td>
</tr>
<tr>
<td>Contagious Equine Metritis</td>
<td>0000</td>
<td>Equine Encephalomyelitis</td>
<td>0000</td>
</tr>
<tr>
<td>Epizootic Lymphogtit</td>
<td>0000</td>
<td>Glanders</td>
<td>0000</td>
</tr>
<tr>
<td>Dourine</td>
<td>1973</td>
<td>Horse Pox</td>
<td>0000</td>
</tr>
<tr>
<td>Infectious Arteritis of Horses</td>
<td>0000</td>
<td>Salmonellosis (S. Abortus Equi)</td>
<td>0000</td>
</tr>
<tr>
<td>Japanese Encephalitis</td>
<td>0000</td>
<td>Surra</td>
<td>0000</td>
</tr>
<tr>
<td>Venezuelan Equine Encephalomyelitis</td>
<td>1995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterovirus Encephalomyelitis</td>
<td>0000</td>
<td>Porcine Reproductive and Respiratory Sindrome</td>
<td>+?</td>
</tr>
<tr>
<td>Duck Hepatitis</td>
<td>0000</td>
<td>Duck Virus Enteritis</td>
<td>0000</td>
</tr>
<tr>
<td>Psittacosis/Oriithosis</td>
<td>0000</td>
<td>Myxomatosis</td>
<td>1994</td>
</tr>
<tr>
<td>Tularemia</td>
<td>0000</td>
<td>Viral Haemorrhagic Disease of Rabbits</td>
<td>1991</td>
</tr>
</tbody>
</table>

Key: 0000 = Never reported; Year = Year of last occurrence; +? = Serology Only
since the addition of all exotic diseases to CPA's surveillance program a broad range of EAD's suspicions have been attended.

**Surveillance strategy and organization**

Although normally exotic animal disease surveillance tends to be passive, i.e. CPA investigates all reports coming from the field, during outbreaks active surveillance is carried out.

For practical purposes Mexico has been divided into 8 regions for which a coordinator is appointed (Figure 1). More recently in some regions a zonal assistant provides support to the region. Their tasks involve the attention of every suspicion of an exotic disease outbreak and to promote the awareness of private and official veterinarians on the importance of EAD surveillance and reporting. The latter is done by routine visits to state and federal authorities and by a series of short courses on exotic diseases and emergency programs.

The aim is to create, through training, an Animal Health Emergency Group in every state. These groups provide logistic and technical support during the first days of an emergency. To date 18 groups are already in place (Figure 2). This scheme has proven to be efficient particularly during the viral haemorrhagic disease of rabbits outbreak in late 1988, the new world screwworm re-infestation, the classical swine fever outbreak in Baja California Sur (a CSF-free zone) and finally the Venezuelan equine encephalomyelitis outbreak in Chiapas in 1993. It is important to mention that although CPA is a bilateral commission, the National Animal Health Emergency System (DINESA by its name in Spanish) is dependent solely from Mexican funds and personnel.

In every case DINESA has been able to control and eradicate these outbreaks. It is perhaps unfortunate but through real life animal health emergencies, the awareness on the importance and potential impact of exotic animal diseases has increased considerably during the last few years.

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* List A diseases. - Communicable diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic or public health consequence and which are of major importance in the international trade of livestock and livestock products. (FAO-WHO-OIE Animal Health Yearbook, 1992).
EXOTIC ANIMAL DISEASE SURVEILLANCE IN MEXICO

Figure 1 Exotic Animal Disease Surveillance
Regional Coordinations

Figure 2 State Animal Health Emergency Groups

18 GROUPS
475 MEMBERS
Information systems applied to exotic animal disease surveillance

Prior to describing the practical applications of several information systems in EAD surveillance, it is important to outline the general flow of information within the system.

When a producer suspects the presence of an exotic disease (basically a disease with high mortality or with unusual clinical signs) he is able to notify a variety of link persons or directly to CPA's regional coordinator or headquarters.

Once the notice is given the regional coordinator conducts an investigation of the case or an official veterinarian is appointed to carry it out. Samples are collected and sent to the high security laboratory in Mexico City. Diagnostic results flow in two directions simultaneously: to the investigator who in turn notifies the producer and to the Animal Health Director General. On occasions samples are sent directly to the laboratory by private veterinarians.

If laboratory results indicate the presence of an exotic disease the National Animal Health Emergency System (DINESA) is activated and is responsible of conducting and coordinating all activities for control and eradication.

General disease surveillance.- This type of surveillance is performed as described above. Data from EAD investigations are captured on an "universal" database called REGEE (REGistro de Enfermedades Exóticas). This database serves two broad purposes, on one hand it is a source of epidemiological information and on the other hand it is an evaluation tool for CPA's activities. The latter is done by measuring the rapidity of response from the time a report is received until a diagnosis is achieved and fed back to the initiating source, thus enabling to determine if improvements on timeliness of response are possible. A new database is initiated every year and information is cross-checked and validated regularly.

Emergency outbreak surveillance.- When an outbreak is detected, a specific database is designed to meet the information requirements for the emergency program. Normally the basis is REGEE that is adapted for the purpose. However, during the 1992-93 screwworm re-infestation, an entirely new database was designed to fit in more specific information related to an outbreak of this nature. A collateral benefit of creating a separate database is that excessively large and complicated files are not created, facilitating data analysis.

Specific disease(s) surveillance.- As mentioned above one of the main targets of surveillance, in terms of FMD prevention, is vesicular stomatitis. For this objective only a few relevant fields from REGEE are selected and fed to an exclusive VS database that expands for nearly 14 years. From it important information can be obtained such as seasonality, strain predominance over time and higher incidence locations or states. Figures 4 and 5 show part of this information over the 1981-1994 period.
EXOTIC ANIMAL DISEASE SURVEILLANCE IN MEXICO

An interesting facet of disease surveillance is to display data on maps, for this purpose EPI-MAP was developed. However the maps provided with the program (called boundary files) only include a general map of Mexico divided by state. Recently CPA produced for use in EPI-MAP all state maps (31 states and the Federal District) subdivided into rural development districts, which are the Ministry's of Agriculture (SARH) basic cell. This enables to represent graphically disease surveillance information with greater detail than before.

Perspectives and future developments

Information systems have changed the way we think about animal health and have improved the basis on which decisions are made. However these are simply tools and will not overcome any deficiencies in the quality of the data nor the flow of information within the system.

Recently CPA has linked to INTERNET, in the near future global disease information will flow by satellite communication through the Office International des Epizooties (OIE)* and it is not difficult to forecast subnational disease information being sent through these channels to communicate with central levels. A pilot program involving CPA's coordinators will be launched to test the system and assess benefits and drawbacks of communicating this way. To date only a few INTERNET nodes exist in the country, placing an important constraint for implementation. Gradually more nodes will develop, particularly at universities and other education institutions, and will constitute a true communications network in Mexico.

Geographical information systems (GIS) are becoming more accessible and provide a fascinating means to visualize and analyze epidemiological data6. An important drawback that hopefully will be addressed soon is that these systems are created for use in industrialized countries, therefore baseline information for other countries must be fed by the end user. This is a gigantic task that limits the practical application of these systems at present. Information needs are growing rapidly and will place pressure on manufacturers to include baseline information tailored for the country in question.

Conclusions

Computer technology and communications are evolving rapidly and with them a formidable challenge is developing: to keep the pace with technology while allowing improvements to permeate to lower levels of the system. In a country like Mexico this is not as simple as it may sound and often central and state levels improve, from a technological point of view, at different speeds. Careful planning is required before embarking in a technological revolution if the system is to be successful4.

One of the main constraints in the implementation of a computerized information system is computer literacy. This problem is being overcome
Figure 3 Information Flow through the Exotic Animal Disease Surveillance System
EXOTIC ANIMAL DISEASE SURVEILLANCE IN MEXICO

as people at all levels, but particularly administrators, realize the advantages of shifting from older information systems (though perfectly valid) to more modern information systems. Many short courses and demonstrations have been given by CPA to try to bridge the gap and facilitate adoption of the systems described.

In a globalized economy, trade barriers are being rapidly eliminated and sanitary barriers are emerging, more than ever, as the most important limitation to free trade of animals and animal products. Disease information systems in every country must improve to allow sound epidemiological information to be the basis to decide if sanitary restrictions are adequate or should be modified. In this sense risk assessment, regionalization and evaluation of veterinary services will play a fundamental role.

We are faced with a new reality that is demanding a different attitude on the way animal health is managed, this is placing great stress on veterinary authorities but at the same time is providing a great opportunity to strengthen veterinary services worldwide.

References

b EPI-INFO and EPI-MAP. Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA and World Health Organization, Geneva, Switzerland.
ZEPEDA, MATEOS

Figure 4 - Vesicular Stomatitis in Mexico 1981-1994

Figure 5 - Vesicular Stomatitis in Mexico 1981-1994

Cases

1 to 5
6 to 15
16 to 40
41 to 60
61 to 100
101 to 188

Note: Information until August 1994.
REPORT OF THE COMMITTEE ON ANIMAL DISEASE SURVEILLANCE AND ANIMAL HEALTH INFORMATION

Chairman: Dr. M.D. Salman, Fort Collins, CO
Vice Chairman: Dr. B.L. Akey, VA.

Dr. J. Lee Alley, AL; Dr. Lowell A. Anderson, IA; Dr. Charles W. Beard, GA; Dr. Douglas L. Berndt, DC; Dr. Jerry J. Bohlender, CO; Dr. Hector Campos, MEX; Dr. James T. Case, CA; Dr. Stanley L. Diesch, MN; Ms. Barbara R. Fox, MD; Dr. Thomas W. Freas, IN; Dr. Michael J. Gilsdorf, MD; Dr. Harvey S. Gosser, MO; Mr. Francis D. Gregerson, CO; Dr. Dale M. Grotelueschen, NE; Mr. Fred Gvillo, CA; Dr. Farouk Hamdy, FL; Dr. B. R. Heron, CA; Dr. David W. Hird, CA; Dr. John P. Honstead, MD; Dr. William D. Hueston, CO; Dr. M. E. Hugh-Jones, LA; Dr. Larry Hutchinson, PA; Dr. Tari P. Kindred, MD; Dr. Lonnie J. King, MD; Dr. Donald H. Lein, NY; Dr. Herbert Lloyd, FL; Mr. Larry D. Mark, VA; Dr. E. Hunt McCauley, MT; Dr. Thomas J. McGinn, III, NC; Dr. Robert W. Mead, WA; Dr. L. G. Morehouse, MO; Dr. John C. New, TN; Dr. Charles Palmer, CA; Dr. Archibald B. Park, MD; Dr. Phillip A. Pickerill, TX; Dr. John C. Prucha, MD; Dr. Frank Ross, IA; Dr. Leon H. Russell, Jr., TX; Dr. Vaughn A. Seaton, IA; Dr. Robert L. Tharp, MO; Dr. William L. Thomas, OH; Dr. William Utterback, CA.

The committee met for two days. Total attendance of the two day meeting was 63 members and guests. This year the committee had one theme for each day of the meeting. The two themes were:

1. Standardization of disease definitions and classification in veterinary medicine.
2. State/local disease reporting systems: A model for a national program.

Dr. J. Case, California Animal Diagnostic Laboratory System, was our first speaker on Monday. The title for his presentation was “Standardized Nomenclature in Veterinary Medicine: Potential of SNOVET International”. Dr. Case gave the history of the standardization of nomenclature in veterinary medicine. The first efforts began in 1938 through the AVMA with their first set of guidelines published in 1955. These guidelines were inappropriately used as the nomenclature itself which was unfortunate. The second effort was the Standardized Nomenclature of Veterinary Diseases and Operations (SNVDO) released in 1964, revised in 1966 and 1977 and still in use today. A third effort launched in 1983 resulted in the development of Standardized Nomenclature in Veterinary Medicine (SNOVET) in 1985. In 1990 the AVMA refined SNOVET and incorporated it into Standardized Nomenclature in Medicine (SNOMED), published in 1993. A nomenclature should not be confused with a classification, the two are independent and one does not dictate the other. Disease coding is inherently subjective.
however, individuality must be abandoned for standardization to succeed. Early systems lacked hierarchical relationships and contained no method for recording the relevance of findings in a case or the level of confidence in the findings. Benefits of a standardized nomenclature include: improved precision and recall of cases, allows for spatial and temporal trend analysis, provides the basis for quality control, creates accurate statistics useful confidence in the findings. Benefits of a standardized nomenclature include: improved precision and recall of cases, allows for spatial and temporal trend analysis, provides the basis for quality control, creates accurate statistics useful for administrative and other decision making processes. SNOMED International is constructed along 11 axes and contains 133,000 primary terms and synonyms including 7500 veterinary specific terms, is hierarchical in construction and allows the assignment of modifiers and descriptive terms to address relevance and confidence in the diagnosis. SNOMED does contain some inconsistencies in the primary terminology and the hierarchy, the ineffective use of subclass terms in some instances and there is a relative lack of veterinary synonyms. To begin to implement a standardized nomenclature begin with what you have already done, use it effectively by mapping to a standardized nomenclature, establish a consensus on terms and modify it as needed. A standardized nomenclature provides the foundation for accurate communication of medical data and is essential for the accurate exchange of information. It does not have to supersede an in-house nomenclature as long as that in-house nomenclature is “mapped” to the standard nomenclature.

The second speaker was Dr. J. Alexander from Pan American Veterinary Laboratories. The title for his presentation was “Sero-prevalence of Johne's Disease in Selected US goats”. He stated that testing has been conducted on Spanish and Boer goats from across the U.S. using an ELISA directed against a protoplasmic antigen. Results indicate about a 5% seropositive rate in Texas and about 3.2% for all submissions. Within the submissions in which positive samples were detected, the overall seroprevalence was 4.6%. Several Pygmy goat herds have been identified with large losses attributed to Johne's disease. This is an area in which further investigation is needed to determine if there is a breed related susceptibility to this disease. Conclusions cannot be made yet concerning the prevalence of this disease in goats due to the relatively small number of samples and submissions from states other than Texas.

The third speaker was Dr. J. Bender from the University of Minnesota. The title for his presentation was “How Representative Are Routine Diagnostic Laboratory Submissions of All Livestock Producers?”. He reviewed the sources of health information in the USA. The common sources of herd-based animal disease surveillance information currently available include state and federal disease eradication programs, the USDA's NAHMS system and the National Agriculture Statistics Service. Individual animal data include diagnostic laboratory data and field surveillance data collected for
REPORT OF THE COMMITTEE

various state programs. Diagnostic laboratory data have inherent shortcomings due to the skewed population being represented, the diagnostic bias of both the laboratory and the submitting veterinarian(s), limited access to the lab by producers, the number and quality of the specimens being variable and may not be representative of the problem, different diagnostic protocols and nomenclature and larger herds are usually over-represented. Data from The National Dairy Heifer Evaluation Project in Minnesota was used as a comparison to the data generated through the state diagnostic laboratory for the assessment of the representativeness of that data. Both prospective and retrospective studies were conducted. Production parameters were similar for both groups except average herd size was larger and calf mortality rates were higher in the diagnostic lab study herds. A self assessment questionnaire was administered to veterinarians to determine their submission patterns and found that those with the highest level of involvement in dairy practice, with larger herds in their practice and which had ongoing surveillance programs in their practices were over-represented. The conclusion was reached that, although generally representative of larger herds, the diagnostic laboratory data could be used as a basis for estimation of regional (state) disease levels.

Dr. M. Smith, from the USDA:APHIS:VS: Centers of Epidemiology and Animal Health in Ft. Collins, was the next speaker. Her title was "Issues Related to Case Definition and Standardization as Reported by Selected Veterinary Diagnostic Laboratories". The USDAs Center for Epidemiology and Animal Health (CEAH) attempts to take data reported for diagnostic laboratories and add value to that data to increase it's usefulness in decision making. The Veterinary Diagnostic Laboratory Reporting System (VDLRS) uses case definition criteria for each reportable disease that were developed by consensus of diagnostic specialists for each disease. This includes those tests that are considered the minimum required for reporting. This may include both presumptive and definitive tests and therefore the data should be separated on that basis. Another potential complication is introduced when some tests have long turn-around times thus necessitating adjustment of time frames when reporting. In situations where there is no one best test for a disease there needs to be a consensus reached as to which test or combination of tests should be used as the standard for reporting. Otherwise, reporting based on tests with different confidence levels and unknown compatibility with other tests cannot accurately address comparative disease prevalence between regions, species or different data sources. Standardization of reporting at least may in some cases be sufficient to allow for combining data from different sources.

Dr. L. King, Acting Administrator of APHIS, gave a summary report of the last meeting (October 17-21, 1994) of the Office International Des Epizooties (OIE) working group on Information and Epidemiology. He reviewed the functions and the mission of the OIE. The OIE working group on epidemiology and information met to continue discussions on the structure
of the disease reporting systems for the participating countries in OIE. The group recognized that there were 4 pillars upon which international disease surveillance and subsequent trade must be based: Regionalization, Risk Assessment, Standardization and Equivalency. They looked at Nations/States, Economic Communities and World Trade Organizations (GATT) as different models to be addressed as regards standards. Information systems planning is critical to the development of long-range strategies to implement these models. A standard for disease reporting was discussed using the computer software called "Handistatus" that will be adopted by all participating countries. A Geographic Information System module will be a natural addition to this system. Attention is being focused as well on developing surveillance systems that can be implemented in all countries regardless of level of development of the country. A sub-group on Risk Assessment will continue to develop that area as the logical basis for decision making in international trade and movement of animals. The quality and transparency of data must be improved. Training in epidemiology, surveillance, data collection and analysis will be an important component of future efforts as well. The group will also formulate a process for evaluating the significance of each disease to determine its appropriate inclusion in either List A or List B of the OIE reportable diseases. Evaluation of animal identification systems are also underway.

Dr. C. Rossiter, from the New York State Animal Diagnostic Laboratory, was the next speaker. The title of her presentation was "Johne's Monitoring and Surveillance Program in New York". The disease monitoring system began in the mid 1980's. Subsequent to producer demand for a control program for Johne's disease, a testing program and field management evaluation program was implemented. An attempt was made to monitor individual animals over time. The program is a cooperative effort between the state diagnostic laboratory and the New York Department of Agriculture and Markets. Some bias is introduced because of the fees charged for testing which in many cases dictates the type of test performed and how many samples are submitted. Although test results are reported for individual animals the system also tracks incidence based on premises for the purposes of spatial and temporal analysis. Another source of bias in the testing data is the uniqueness of the testing methods used at the New York laboratory. It is felt that this does not invalidate the reporting of that data or the combining of that data with data generated by different testing methodologies as long as all of those methodologies are validated. There is a need for surveillance systems based on data collection from the field in addition to laboratory data.

Dr. C. Zepeda Sein, from the Mexican Exotic Animal Disease Commission, was the last speaker for Monday's meeting. His presentation title was "Information Systems from the Exotic Animal Disease Surveillance in Mexico". This paper was selected to be presented in the general session (see the complete manuscript in the proceeding). He demonstrated the
use of a simple computer database in reporting selected disease occurrence in Mexico. The database has used in determining the trend of these diseases and in a simple geographical information system.

On Tuesday the first presentation, "U. S. Cow-Calf Health and Preventive Medicine Practices; Findings from the APHIS:VS National Animal Health Monitoring System (NAHMS)" given by Dr. W. Hueston of the Center for Epidemiology and Animal Health, USDA:APHIS in Ft. Collins, CO dealt with the uses of different data sources to provide information for monitoring and surveillance. Significant findings from the most recent cow-calf health monitoring system. This paper was selected to be presented in the general session (see the complete manuscript in the proceeding).

Dr. Hueston also presented the work of Dr. J. Traub-Daragatz on "Opportunities for Equine Health Monitoring Systems". The first obstacle to establishing effective systems is identifying available data sources and their usefulness. Effective monitoring can identify disease trends, target scarce research dollars on those issues creating greatest economic loss and identify risk factors for disease. The horse industry is a multi-faceted industry which makes getting data that is representative of all equine activities difficult. Each data source has advantages and limitations. Equine census information provides a list frame for further monitoring but estimates vary widely, are not done annually and it does not reflect the economic impact of the horse industry. A lack of standardized definitions of farms has, in the past, led to these widely varying estimates. University veterinary hospital data is another potential source that is accessible, has wide geographic distribution and good diagnostic capabilities. Limitations of this data source include lack of a reference population and no standardization of diagnosis or reporting criteria. Diagnostic laboratory data benefits from expertise and has wide geographic coverage but again a lack of standardization of tests and recording, no reference population, lack of links with medical history and poor accessibility of data limit its usefulness. Private veterinary practices have reference population information and diagnostic expertise but again lack standardization of diagnoses, record keeping or reporting as well as a lack of willingness to report due to confidentiality and other issues. Rendering operations can provide enumeration data and identify changes by region but have no diagnostic capabilities and also suffer from a lack of availability of data. Slaughter plants have readily available records with uniform reporting of condemnation codes but many of these codes are for non-terminal diseases and represent symptoms or pathologic changes observed instead of diagnoses. Horse farms have a reference population but no standardization of recording, poor representation of cases (self-selection) and again a lack of willingness to report. Breed registries contain inventories by state and information for list frames but record no health information and the inventory is based on births only with deaths not reported. Race Tracks have a reference population with veterinary expertise on the premises but again no standardization of diagnosis or reporting.
ANIMAL DISEASE SURVEILLANCE AND ANIMAL HEALTH INFORMATION

State Veterinarian's offices have outbreak information on reportable diseases but little information for non-reportable diseases and often offer no incentive for reporting. Equine insurance companies have many advantages with mortality measures, denominator data, accurate diagnoses and wide geographic distribution but problems with accessibility due to confidentiality. Central reporting systems such as the OIE often lack the manpower and the adequate network for representative coverage. The ideal monitoring system will need incentives to gain cooperation and the standardization of diagnoses and reporting as the foundation.

Dr. B. Akey from the Virginia Department of Agriculture and Consumer Services spoke next on the topic of "VAHMS - The Virginia Animal Health Monitoring System". The VAHMS is based upon a regulation requiring all practicing veterinarians in the state to report any of a list of reportable diseases established by the State Veterinarian to that office and also on the requirement of the state-federal accreditation program for accredited veterinarians to comply with all state and federal regulations. Two lists of reportable diseases have been established, List A are essentially those considered to be foreign animal diseases as defined by the List A of the OIE while List B diseases are those additional diseases deemed of interest by the State Veterinarian. Occurrences of List A diseases are required to be reported within 24 hours of their observation while those from List B are reported monthly. Each large animal or mixed animal veterinary clinic as well as the state diagnostic laboratory system is considered a reporting entity and a monthly compilation of reporting serves to identify those entities that have not reported for follow-up telephone contact. Monthly reports may be made either by filling out a pre-paid mailer or by calling a toll-free hotline number. For each disease entities report the number of herds/flocks affected, the number of individual cases seen, whether these were clinical or laboratory-confirmed diagnoses and in which county they occurred. The submitted information is compiled in a simple database for correlation and generation of a variety of reports including entity reporting tracking, individual diseases by county, all diseases by county and disease occurrences over any given time period. This data can also be exported to a Geographic Information System for additional spatial and temporal analysis. Each month a summary report is prepared in the form of a VAHMS Newsletter containing descriptive information as well as tables and graphs of the month's data which is sent not only to all reporting entities but also to a wide variety of industry organizations and other interested parties. The VAHMS has the advantages of incorporating both diagnostic laboratory data and reporting from practicing veterinarians but as in many other systems there remain problems with standardization of diagnoses and reporting as well as a constant struggle to maintain compliance with reporting requirements. Nonetheless, the VAHMS may serve as a useful model for the development of a standardized state monitoring and surveillance system.

Dr. F. Elvinger of the Georgia Veterinary Diagnostic Laboratory System
presented a report on this year's AAVLD Sub-Committee on Animal Disease Reporting. The subcommittee voted to develop a mechanism for rapid collection and dissemination of diagnostic information to a restricted audience (Laboratory Directors and State Veterinarians) of an emerging disease, disease agent, or other condition with a significant impact (economic, public health, trade) in any state of the Union. The alert will be combined with a request for information on incidence of such condition in each state. The information is to be evaluated (with requests for more detailed information if needed) and analyzed prior to broader dissemination in the DxMonitor.

Development of Focus Articles. The subcommittee voted to publish summary articles based on veterinary diagnostic laboratory data pertaining to particular conditions (disease, agent, substance). The format of the reports will be variable and will depend on the type of condition. The condition should not warrant continuous monitoring and publication on a quarterly basis, but present valuable information generated by the laboratories for a given audience. Requests for inclusion of a condition should be made to and approved by the Editorial Review Group of the DxMonitor.

Function, Composition and Authority of the DxMonitor Editorial Review Group. The subcommittee voted to define and provide written guidelines to formalize function, composition and authority of the Editorial Review Group.

Feasibility of Inclusion of New Conditions. The subcommittee endorsed a request to conduct a feasibility study prior to including a new condition in the reporting system. When a new condition for inclusion in the reporting system is identified, a questionnaire is to be developed to determine the availability and quality of data available. The decision to include such a condition will be dependent on the results of this questionnaire. The following items were suggested for further consideration or action by the subcommittee: 1) contact chairpersons of AAVLD specialty committees to determine interest in and need for expanding reporting of particular conditions. The support of specialty committees is needed for the formulation of case definitions, determination of availability of data in diagnostic laboratories and the design of pertinent questionnaires, 2) contact the Chairman of the Publications Committee to determine if publication of data in a focus article/editorial or other form in *DxMonitor* prevents the author(s) from publishing the information in a peer-reviewed journal like the Journal of Veterinary Diagnostic Investigation. 3) contact the Editor of the Journal of Veterinary Diagnostic Investigation for information on the function and authority of their editorial board to help establish guidelines for the DxMonitor Editorial Review Group. 4) set up a LISTSERV on the Internet for discussion of issues among Committee members and invited guests.

Dr. K. Shank, USDA:APHIS:IS, reported on the "1993 Michigan Captive Cervidae Project". Information was gathered from 228 of 362 eligible participants for a response rate of 63%. Michigan is in the top three states for numbers of captive cervidae, with over 6000 animals. The two most common species are white-tailed deer and elk. The median herd size is
less than 10 animals, and there was a net increase of 7.9% of the population during the year preceding the study. Only 31% reported that cervidae were kept purely for profit. Nearly three-quarters of Cervidae owners use a veterinarian with 63% reporting at least one health problem in the preceding year and the most common causes of death were injuries and respiratory disease. Over 80% of herd additions came from within the state of Michigan, with on-farm breeding and private sales being the major routes. A little over 80% reported that contact of captive cervidae with wild deer was occurring. Fifty-five percent of owners felt regulations were necessary for the industry, with humane care of animals being the number one reason. Nearly two-thirds of respondents felt current regulations were inappropriate, with tuberculosis regulations being cited by 41%. The range of years of ownership of cervidae was 1-35 years with a median of 4 years.

"An Overview of Animal Health Disease Reporting Systems in Africa" was given by Dr. Bruckner, representative from the Republic of South Africa to the OIE Informatics Committee. Data on disease status of a country must, in the future, be transparent to other countries to ensure safe and efficient international trade. A survey of data resources available in the countries of southern Africa was conducted and resulted in a recommendation for the development by the OIE of an Ideal System for Gathering of Information (CIRAD). Individual owner data is not important in these countries and the emphasis of monitoring and surveillance will be on OIE List A diseases. The dipping-tank area will be the key field of reference in this system as this represents an efficient gathering point source where all livestock animals are seen on a regular basis. The epidemiology of disease/control measures is not related to individual owners because of the prevailing practice of communal grazing areas. The boundaries of these communal grazing systems determines the control measures/epidemiological framework of the monitoring system. Tracebacks of active surveillance are virtually impossible. In most of these countries the entire surveillance system is a one man job hampered by poor computer literacy and equipment shortages. Existing manual systems must be integrated with new electronic systems. Reports must be kept simple with an emphasis on visual interpretations. The OIE will likely launch a pilot project in one of these countries to develop a system appropriate to the level of development and the cultural milieu.

Dr. N. Frank then addressed the committee concerning "Michigan's Reportable Disease Program". The system is based in law and requires any person aware of a reportable disease to report it. The State Veterinarian determines which diseases are reportable and a procedure is in place to capture those reports manually. Currently there are problems with underreporting as there is no monthly reporting requirement and with getting information back to practitioners and the industry. Another facet of disease surveillance in Michigan is the Michigan Equine Monitoring System (MEMS). This system has been funded directly by the legislature with industry sup-
port and is based on on-farm visits similar to the NAHMS format.

Next year the committee will host a workshop on identification and consolidation of existing data sources and the standardization of disease definitions and reporting. Drs Elvinger, Hueston, Case, Hamady and Mateos will form a working group to begin addressing these issues leading up to the workshop. The committee also agreed to begin the development of a standardized state monitoring/surveillance system. An initial survey will be conducted this coming year by Dr. B. Akey to elucidate current systems in each state and the USDA:APHIS:CEAH agreed to develop a free, EPIINFO based data entry program for distribution to all states willing to join in a national reporting network similar to the DxMonitor Veterinary Diagnostic Laboratory Reporting System.
The meeting of the Committee on Animal Welfare was called to order by Chairperson John Lang on November 1, 1994 at 1:30 pm. The meeting was attended by 22 members and 27 guests and speakers. The chairperson’s opening remarks included the continuing journey of animal welfare issues with solutions which are a “win-win” for the individual animal and the industry.

Dr. Dale Schwindaman, USDA-APHIS-REAC, reported on the agency’s budget, inspection statistics, and issues including stolen dogs, captive marine mammals, human deaths by elephants, environmental enhancement for non-human primates and the external review of the Horse Protection Program. Future issues for REAC include budgetary challenges, amendments to Animal Welfare Act, the negotiated rulemaking process for marine mammals, farm animal regulations for research and exhibists, and the morbillivirus of east coast dolphins.

Dr. Norman Willis, assisted by Mr. Steve Sullivan, Agriculture Canada, discussed the influence of the media in animal welfare issues in Canada, the organized effort to draft a consensus on transportation of animals by facilitating all stakeholders in the issue, the regulations pertaining to the import of approximately 20,000 puppies/year, and the research into the acceptable practice for transporting horses by air.

Mr. Adam Roberts, Animal Welfare Institute, presented the organization’s concerns on the effects of GATT on laws and regulations in the U.S., the
REPORT OF THE COMMITTEE

upcoming CITES conference including the endangered species listing criteria, enforcement deficiencies, steel-jaw leg hold trap, and the use of Bovine Somatrophin. He supported USDA's effort to change from hot to freeze branding of Mexican cattle along with the mandatory utilization of anaesthesia in ovariectomy procedures in cows.

Dr. Mort Silberman summarized report "The External Review of the Horse Protection Program". The findings of the report included the definition of soring, a checklist to standardize DQP and VMO inspections, publicizing the violator's list, recommendations for compliance of non-sanctioned shows, and the quarterly meetings of the stakeholders.

Dr. Ken Olson, American Farm Bureau Federation, announced the completion of the national voluntary guidelines, "Caring for Dairy Animals", for dairy animal care by the release of two complementary publications, "Reference Guide" and "On-Farm Evaluation Guide". The program will be targeted at producer leaders, veterinarians, consultants, educators, and the media.

Dr. Alan Stern, Advocate for Animals, Inc., recommended several alternatives to improve current inspections including incorporating local enforcement officers and State Veterinarians, consistency of inspections, improved preparation of cruelty cases for prosecution, and focus on the condition of the animals rather than the facility during inspection.

The attached resolution was presented and unanimously approved by the committee.

The meeting was adjourned at 5:00 p.m.
MARYLAND AQUACULTURE

Dr. A.B. Park
Assistant Secretary, Animal Health
Maryland Department of Agriculture

This paper is a report card on the Aquaculture program in Maryland. More than 17 years ago Dr. Frank Hetrick, professor of Microbiology at the University of Maryland initiated a fish disease diagnostic service at the University. About 5 years later, he was joined by Dr. Ana Baya and together they established a registry of fish diseases in Maryland. This data base is extraordinarily useful in assessing the potential impact of any disease that might be imported into the state. Two years ago on the occasion of his retirement, Dr. Hetrick agreed to join State government and he and Ana moved their fish lab to the College Park Animal Health Diagnostic Lab where it exists today. Ana was and still is an employee of the Maryland Extension Service.

In 1986 immediately after his election, our current governor, William Donald Schaefer let it be known that aquaculture was going to be a major new initiative in his administration. Some years before, in 1981 in fact, there was an Ad Hoc Committee on Aquaculture. This committee produced a document published in 1982 called "Perspective on Aquaculture". It was to be 5 years before this plan was to be requested by the Governor but because of this early work a completed document was presented to the Governor within six months of his request for it. In fact in January of 1988, this plan was forwarded to the Maryland legislature and became the basis for the creation of the Aquaculture Office in the Maryland Department of Agriculture as well as the legal basis for our current Advisory Committee. Of historical note is the approval by the legislature of an Aquaculture Loan Fund. An applicant can be loaned up to $250,000.00 at 2 points below prime for 50% support of an approved project. In 1988 we had 6-10 producers with a value of over 10 million dollars. In 1994 we are over 200 producers at a value of over 20 million dollars. We are in the process of monitoring the progress of an enormous undertaking in shrimp production.

In a very large tank inside a very much larger building, the business venture will attempt to grow shrimp to a 2 gram size for delivery to growers who will then grow them to a 31 count size (approx. 15 grams). This will take about 75 days. At this point, they will be delivered to a single buyer for direct consumption in the restaurant trade.

This business venture, if successful, will yield 8-900 million dollars annually. An additional facet to the business is that they will be using a closed loop water system which will be cleaned and disinfected on a regular basis with the feces being diverted and collected in a large digester for methane production.
The shrimp selected for this venture has been found in Chesapeake Bay waters but is normally found in waters south of the Bay and all the around to and in the Gulf. It is a pink shrimp (Penaeus duorarum) and one of the fastest growing species. The methane will be used to generate electricity and to heat water for the tank. The shrimp will be grown in 92 degree salt water. There is no state money in this project but we are helping as we can in other ways. The building is owned by the state, having been deeded to the state by the Corps of Engineers. This is the site of the now closed "Bay Model", a large 12 to 1 scale model of the bay used for hydrological studies.

At a recent meeting of the Health Sub-Committee of the Advisory Committee it was decided that a Model Aquatic Animal Health Regulation be created. Since the author had been the scribe of record for the Mid Atlantic Poultry Health agreement, and since it was agreed that this too should be a regional document, the author agreed to once again do the writing. After a few paragraphs on why we need such a regulation there is a section on definitions. Then there is a section on reporting aquatic animal diseases, a section on the requirement to survey aquatic animals and their pathogens, and then a section on the quarantine of animals and premises. The regulation assumes that there will be dealers and that they will be as troublesome as they are with terrestrial food animals. We also assume that one day there will be trade in these animals at auction markets. As we do in poultry, we treat hatcheries with care and we are mindful of exhibitions. Then follows a section on the handling of animals and premises confirmed to be infected with one of the proscribed pathogens. Again there is a special section for hatcheries. Then there is a section on controlled slaughter as we do it in poultry; last day in the week, last lot in the day. This facilitates cleaning and disinfection with minimal disruption.

Special provision is made for breeding animals of high value. Then there is a section on monitoring and surveillance. It should be noted that monitoring is a passive function in which routine testing is done whereas surveillance is an active function where specific tests are run on selected premises looking for a specific pathogen. Then there are parts on indemnity, on vaccination policy, and finally a section on the movement of animals and products in to the state. It is important to note that we determined that because of a lack of federal law on the subject we decided to invoke the Lacey Act which requires the federal regulatory agencies to enforce state laws governing the importation of animals and products into our states from foreign countries. At the end, we wrote a special section on the water that is used to ship the aquatic animals.
The U.S.A.H.A. Aquaculture Committee met Thursday, November 3, 1994, from 1 to 6 p.m. Fifty-three persons were in attendance.

The aquaculture coordinator for Michigan, Mr. Robert Craig, and Mr. Robert Baldwin, President of the Michigan Fish Growers Association, presented an overview of aquaculture in Michigan and the past, present, and planned future development of aquaculture in Michigan, including legislative history, and the Michigan Aquaculture Development Act presently before the legislature updating and modernizing legislation for the aquaculture industry. Acts passed in the 1920s made all fish in Michigan the property of the state, and established riparian water rights for Michigan. The new act changes the ownership statute. A seven-year process organized Michigan fish farmers, legislators, health agencies, and academia to develop legislation favorable to development of aquaculture in Michigan, and clearly defining private ownership of fish and private water rights.

Dr. Robert Goetz, Keo Fish Farms, chairman of the committee, gave a legislative update. He discussed the Akaka bill, S-1288 (and its companion bill, HR 4744) which was introduced, had sixty co-sponsors, and proceeded through the hearing process, but was referred to the Senate Commerce Committee and died without passage. These bills will be reintroduced in the next Congress and are expected to pass. Dr. Goetz discussed international aquaculture from the industry perspective, and the increase in international trade in aquaculture.

Dr. Mark Dulin, USDA, APHIS, VS, Mission to the European Union, discussed the impending deadline next year of the EU in implementing its
aquaculture and seafood directives.

Dr. Althaea Langston, USDA, APHIS, vice-chair of the committee, discussed regulation of aquatic animal imports by state and federal governments, and the permit system proposed to Congress by the Intentional Introductions Policy Review Committee of the federal multi-agency Aquatic Nuisance Species Task Force.

Dr. Otis Miller, USDA, APHIS, discussed the export certificates issued by APHIS for non-mammalian aquatic species utilizing the accredited veterinarian and state veterinary diagnostic/animal health laboratory system used by APHIS in the certification of other species of animals for export. From May through September 1994, APHIS certified 6.4 million salmonid eggs for export to Chile, and more than $2.5 million worth of live fish exports, primarily ornamentals.

Dr. Maura Jansen, Troutlodge, Inc., gave an overview of the salmonid industry, then discussed the role of the veterinarian in food fish production, which she sees as identical to veterinary activities in other food animal industries (except for species).

Dr. Joe Gloyd, AVMA, reported on the membership and the first meeting of the AVMA Aquaculture and Seafood Advisory Committee.

Dr. Robert Goetz described the economics, distribution, culture and husbandry practices of the bait farming industry.

Dr. Jerry Heidel, Oregon State University Veterinary Diagnostic Laboratory, chair of the Aquaculture Committee, discussed laboratory assistance and availability, and the goals and accomplishments of the AAVLD Committee in its first year of existence. The AAVLD Committee surveyed laboratories involved in aquaculture diagnostic testing and production of aquaculture diagnostic reagents, and produced a list of laboratories which was given as a handout at the meeting.

Dr. Arch Park, Assistant Secretary of Agriculture for Animal Health and Consumer Services for the state of Maryland, discussed the history of aquaculture laboratory services in Maryland and their integration into the Maryland Animal Health Laboratory. Aquaculture was designated a major priority of the present Maryland governor's administration, with redistribution of existing funds required in order to accomplish the program. Maryland presented an aquaculture plan to its state legislature in 1988, and instituted state assistance to and promotion of aquaculture. In 1990 an aquaculture loan fund was established for producers in Maryland, who were and are unable to obtain bank financing for aquafarming. In 1988, there were 6-10 animal aquaculture producers in Maryland, with production value of $10 million. In 1994, there were 200 producers and a value of more than $20 million. Species cultured in Maryland include hybrid striped bass, tilapia, and ornamentals. In addition to the farmers and values above, Maryland has oysters and crawfish, and is a major producer of ornamental aquatic plants and microalgae. A major shrimp production venture is starting, sup-
ported with in kind assistance from the state. Proposed is a vertically integrated operation modelled on the poultry industry with broodstock, postlarvae production, and growout operations expected to gross $800 million annually.

Dr. Park then briefly discussed Maryland's draft "Model Aquatic Animal Health Regulation," modelled after an existing multistate poultry regulation. This regulation will apply to all cultured and wild aquatic animals, both public and private. Discussion of the regulation with other states in the Chesapeake Bay drainage area (Virginia, West Virginia, New York, New Jersey, Pennsylvania, and Delaware) will assure acceptability to other states. The jointly implemented regulation will then encompass a major watershed, in conformity with EU requirements. The regulation, jointly administered by the state secretaries of agriculture and natural resources, will mandate a survey of wild and aquacultured populations of aquatic animals and their pathogens in the state.

Provisions of the regulation were briefly discussed. Drafts, and eventually the final regulation, will be available from Dr. Park. Comments and suggestions for improvement are welcome.

Two resolutions were introduced, discussed, and unanimously passed by the committee.

The meeting was adjourned at 6:00 p.m.
The Biologics Committee met Wednesday, November 2, 1994. Seventeen members were present and 14 guests.

The purpose statement of the committee was reviewed and Dr. Raymond Loan was thanked for his many years of service as chairman of the committee.

Dr. David A. Espeseth, APHIS, BBEP Deputy Director, Veterinary Biologics, presented an update on current licensing activities and reviewed APHIS initiatives to address current program issues.

There were 118 product licenses issued in Fiscal Year 1994, 27 of which were for new products. Sixty-four product licenses were terminated to give a current total of 2,144 active product licenses; including 159 for diagnostic products, 230 for products for further manufacture, and 61 for biotechnology products. Approximately 58 billion doses of licensed products were produced in Fiscal year 1994.

Two new establishment licenses were issued in Fiscal year 1994 to give a total of 116 licensed establishments and permittees. During the year 98 field trials were authorized including 16 for biotechnology products. Import permits were issued for 72 products for research and evaluation and 11 products for transit shipment only.

New initiatives being addressed by the veterinary biologics program include: 1. efforts to develop regulations to implement good laboratory
practices to assure the validity of prelicensing data; 2. the voluntary initiation of QA/QC procedures in licensed establishments; 3. standard procedures for in-vitro testing of killed products using relative potency parallel line immunoassay procedures; 4. international harmonization with Canada and the European Union; 5. improved licensing procedures through use of coordinated review teams, summary information formats and risk analysis; 6. the development of a new updated definition of "veterinary biological product"; and 7. improved post licensing monitoring procedures.

The status of several regulations under development was also reviewed.

Dr. Don Randall, USDA, APHIS, BBEP, Veterinary Biologics Field Operations (VBFO), Ames, Iowa, reported on biologics field activities for the last year. VBFO performs check testing and marketing control on product batches, inspection of facilities, and post-license product monitoring of performance under field conditions. Dr. Randall reported that they reviewed 21,712 serials of product--21,022 were released, 744 were destroyed. They performed 103 inspections including in-depth, follow-up, and special with 14 inspectors.

Dr. Randall talked about the move toward Good Manufacturing Practices for biologics or "process inspection." He also discussed several new program initiatives to improve the inspection process and to handle serial release more efficiently. During the last year his group received 108 consumer complaints which is consistent with previous years. Also 31 investigations of alleged violations were done.

Dr. Mark Wood of the Animal Health Institute (AHI) presented the following issues and interests of the biologic manufacturers:

1. International harmonization efforts are continuing between AHI and Fedesa. Their initial focus will be on product quality assurance standards for sterility testing. Other quality assurance testing criteria will follow. The emphasis will be on establishing similarity in test results rather than similarity in test design. Collaboration will require evaluation of data from Europe and the U.S. Evaluation will use risk assessment models under accepted sensitivity and specificity criteria to demonstrate similarity and eventual mutual acceptance of product quality validation criteria from country to country.

2. The anticipation of APHIS/REAC guidelines for farm animal housing and care was discussed. Industry is concerned about the possible impact on the manufacturers of animal health care products. AHI will monitor this issue and has offered to help with comments regarding these guidelines.

3. AHI commends APHIS on the publication of the final rule prohibiting the repackaging of veterinary biological products. This should help to maintain the integrity of these product by preventing mislabeling and misrepresentation.
4. Industry is concerned about the institution of annual state licensing fees for the sale and distribution of veterinary vaccines within certain states. AH1 will monitor these programs with the intent of possible negotiations to help prevent losses in product availability within these states.

5. A report was given of recent concerns by the California Veterinary Medical Association that animal revaccinations are unnecessary. Industry maintained that without the appropriate duration of immunity data it would be impossible to validate.

Dr. Rick Hill, USDA, APHIS, BBEP, VBFO, reported on the post-licensing monitoring program. They are presently sharing data on consumer complaints with some manufacturers, participating in the NAHMS Project, doing a survey on adverse reactions to feline products, and a survey on Type 2 BVD.

Dr. Hill gave a presentation entitled "Epinephrine ... Don't Leave The Clinic Without It." This talk is normally targeted to veterinary practitioners concerning the reality in the use of biologics that reactions do occur. Dr. Hill summarized that there are four types of reactions which may be anticipated or unanticipated. These are: (1) Immune reaction: hypersensitivity, immunological interference or suppression; (2) Local: swelling, pain, lumps, abscesses; (3) Systemic: fever, malaise, abortion, death, endotoxic; (4) Failure to Protect: strain variations, overwhelming exposure, too many antigens, poor responding animals. Dr. Hill said it is important to understand that reactions will occur, and no product performs 100 percent.

Mr. Vaughn Kubiak, Director of Regulatory Affairs at SyntroVet described his company's efforts to obtain the first U.S. license for a viral vector biologic—"Newcastle-Fowlpox Vaccine Live Fowlpox Vector." Mr. Kubiak showed the technology involved in developing a viral-vector vaccine, and went through the standard licensing components of safety, efficacy, purity, and potency and the additional components in each category required for a viral-vector vaccine. The product provides a meaningful advantage not provided by conventional products, has been licensed in accordance with established guidelines, and has been subjected to academic and producer performance assessment.

The Committee discussed the potential that preservatives used in biologics might cause drug residue violations. Comments were made that there is no evidence showing this to be the case, and that current levels used in biologics are approved by the FDA as well as USDA.

There was also discussion within the group concerning the need for Rabies Vaccine to be recommended for goats and the difficulties involved
BOVINE LENTIVIRUS (BIV):
DIAGNOSIS, PREVALENCE AND PATHOGENESIS

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Introduction

The bovine immunodeficiency-like virus (BIV) was first isolated over 25 years ago, but BIV remains largely an unknown virus. Large gaps still exist in our understanding of BIV’s role in disease and other aspects of its natural history. As a result of overzealous comparisons with other lentiviruses, unsupported statements about BIV have commonly been referred to in scientific papers. This paper will try to put in perspective what published scientific research has determined about BIV and those things which remain in question about the virus.

BIV is a lentivirus that was isolated in the United States in 1969 from an eight-year-old dairy cow, R29, with lymphoproliferative lesions.²⁹ BIV was initially called bovine visna-like virus, because of its similarities to sheep visna virus, specifically in virus morphology, syncytial formation in cell culture, and the prolonged lymphocytic response in experimentally infected cattle.²⁹ After a long hiatus from study, the HIV epidemic prompted another look at this bovine retrovirus. Further analysis showed that the virus was indeed a unique member of the lentivirus family with some similarities to HIV and other lentiviruses.¹¹ In addition to the usual structural proteins, gag, pol, and env, BIV has several nonstructural regulatory proteins, specifically tat and rev.¹⁰,¹⁷,²¹ The tat protein has been shown to be able to upregulate BIV’s LTR expression.¹⁷ Cis-acting sequences with a stem loop structure have been shown to be of importance in this transactivation process.⁶ The rev regulatory protein may also be important in the regulation of virus production, and has been shown to localize in the nucleus similar to HIV.²¹

In 1993, two new isolates of BIV, FL112 and FL491, were isolated from a dairy herd in Florida and characterized.²⁶ Because of the availability of only a single isolate, until recently, all of the literature on BIV has been developed using the original R29 isolate. The view of BIV has a very narrow perspective because of the reliance on R29, and this must be realized in trying to objectively evaluate this virus, especially concerning its role in disease.
Diagnosis

Virus isolation - Isolation of BIV from field samples has been very difficult at best. Numerous attempts to culture the virus have resulted in failure. Currently, only two published reports have documented successful culturing of BIV, allowing three different isolates to be available for study. These isolations were made from cocultivation of primary fetal bovine spleen or lung cell cultures with blood buffy coat cells from infected cattle. However, this same technique has proven to be unsuccessful in other attempts at isolation from known naturally and experimentally infected animals. The prominent cytopathic effect (CPE) that has been described for BIV in cell culture is syncytial formation after multiple blind passes. However, it has taken up to 8 blind passages in cell culture before CPE is observed, which results in a 3- to 6-week lag time before infection with BIV becomes detectable in clinical samples. Contamination of cell cultures with more aggressive viruses, like bovine herpesviruses and bovine syncytial virus, is common. Experimental testing with R29-derived isolates has demonstrated that BIV does not grow well on established cell culture lines and it has best been propagated and isolated on primary fetal bovine cell cultures. The optimum conditions for culturing wild-type virus, however, may still remain to be identified. There have been reports that BIV has been grown on heterologous species cell cultures such as canine cells. These cell cultures have not been compared to the fetal bovine primary cell cultures either in sensitivity for virus recovery or in the production and processing of viral proteins. Currently available techniques in virus isolation are still too unreliable to be of routine diagnostic value.

Serology - The methods that have been reported for the serodiagnosis of BIV include immunofluorescence, Western immunoblot, and enzyme linked immunosorbent assay (ELISA) with whole virus antigens or recombinant antigens. Immunofluorescence, usually performed as an indirect fluorescent antibody assay (IFA), is a sensitive and specific test. However, there are multiple limitations to using this assay. The test has to be carefully controlled to assure that all of the reagents used, including cell cultures, reference sera and conjugates, are free of bovine viral diarrhea virus (BVDV). The pattern of cytoplasmic fluorescence produced with both BIV and BVDV are indistinguishable. Since BVDV is a common virus among cattle, the presence of contaminates could account for a high false positive rate of diagnosis. Sera from field cases, especially from older animals that have been exposed to multiple vaccinations and bacterial and viral antigens, tend to have a high, nonspecific background that interferes with the ability to interpret the test. In order to avoid this, the sera must be diluted, often times so much so that specific antibodies can no longer be detected, accounting for false negatives or a no test. Lastly, reading IFA accurately
demands highly skilled personnel and expensive, specialized equipment. The BIV Western immunoblot, is a highly specific and sensitive test that can detect the presence of viral polypeptide-specific antibodies. Since antibody reactivity is directed to specific viral polypeptides of known molecular size, results can be visualized with a high degree of confidence. Although the Western blot is a very good assay, it is too cumbersome to be used for processing large numbers of samples. A second drawback to the test is that it is difficult to prepare antigen that has sufficient amounts of env glycoprotein to give a reliably readable test. Different cell cultures express viral glycoproteins at varying levels, but even under optimal conditions only a small percentage of total viral protein is env glycoprotein. This is compounded by the fact that the glycoprotein is fragile and easily lost during preparation if care is not taken to minimize that loss. The two major polypeptides that are diagnostic for BIV are the major core p26 gag and the gp110 env. If viral antigen preparations are not optimized for glycoprotein, then Western blot reactivities are limited to p26. This could be somewhat averted by using recombinantly prepared gag and env proteins. We know from experimentally inoculated cattle, that reactivity to p26 commonly diminishes or falls below detectable levels. Even recombinant antigens cannot help if antibody levels in the animal have fallen below detectable levels.

A third type of serological test is the ELISA. Antigens for the ELISA can be prepared from either whole virus or recombinantly-derived material. Although this test is easily used with large numbers of samples, the specificity is usually confirmed by a Western blot assay. ELISAs are often highly sensitive tests, but because of nonspecific reactions from bovine serum samples due to extraneous proteins in the antigen preparation, false positives are common. The use of recombinant gag antigens have been reported. However, this test has never been described in detail and validation methods have not been published. Complete correlation between ELISA and Western blot assays is probably not possible because there are basic differences in the two tests, but the Western blot test is considered more specific because antibody to specific viral proteins can be compared.

With all of these assays it must be remembered that each type of test detects antibodies to different types of antigens. Comparisons of the different serodiagnostic tests have not been made. With experience borrowed from HIV, the ELISA test can be a very sensitive test for lentiviruses, but a confirmatory test with high specificity in the form of a Western immunoblot is required to prevent false positives. The IFA, because of the difficulties in interpretation, has become less prevalent as a routine diagnostic test for this virus. Our lab primarily uses the Western blot assay for final determination as a serodiagnostic test. As with HIV, it is advisable to use more than one type of test to confirm the serological status of a sample.
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**PCR** - A hot start single reaction\(^2\) and a nested PCR\(^2\) have both been described for the detection of BIV-infected cattle. Both of these tests are used to detect proviral DNA. Both techniques rely upon the use of multiple primer sets to amplify different areas of the BIV genome to overcome the potential of the primers missing amplification of variant virus strains. The nested PCR test has been shown to be more sensitive in other retroviral systems because of the greater number of cycles involved. This is an important feature since lentivirus-infected animals are thought to usually have a very low number of infected cells in peripheral blood. Both techniques use a Southern blot hybridization assay for confirmation of PCR product, but only the single reaction PCR test requires the Southern blot test for maximal sensitivity of the test. Direct comparisons between these two tests have not been made, and it is not possible to tell if one has a clear advantage over the other. Nested PCR can detect BIV both early after infection before virus specific antibodies appear and before virus can be isolated, and later in infection when antibodies fall below detectable levels and virus is not easily recovered.\(^2\) Neither PCR test has been used to examine large numbers of naturally infected animals. These tests have a great potential, but currently they are still untested in the field. Even a successful PCR test, however, does not lend itself to processing large numbers of samples and quality control assurances must be high to prevent false positives.

**Prevalence**

The prevalence of BIV infection in United States cattle herds is unknown and with existing diagnostic technologies, we can only estimate the true prevalence. Various serologic studies have been reported that show that BIV is widely distributed, being present in the United States, Canada, and other foreign countries.\(^1,2,7,9,12,13,15,19,25\) Two larger studies, generally using random samples from repository serum samples, showed detectable antibody levels of 4.0% and 5.5% of 1,997 and 928 animals tested.\(^2,19\) McNab's (1994) study of Canadian cattle also reported a herd infection rate of 18.1%.\(^19\) Other studies of individual herds have shown a higher incidence of infection using a recombinant ELISA system.\(^7,25\)

With the various studies that have been completed, a statement of seroprevalence of BIV-infected animals cannot be made. From the available data, the number of infected cattle in an individual herd may be very high, but the overall prevalence in the United States is probably much lower. It has been hypothesized that the rate of infection may be much higher in Southern states for a variety of potential reasons, but it is not possible to conclusively make this claim with the available data. The largest survey of cattle, by Black (1989), was almost exclusively of cattle from Southern states, and had a lower overall incidence of antibody detection as compared to McNab's (1994) Canadian study, which appears to contradict the
hypothesis of a higher seroprevalence in the South. The studies did use different detection methods (IFA and Western blot) which makes direct comparison of the two studies difficult. The incidence of BIV infection in dairy cattle has also been thought to be higher than in beef cattle, but again little published data is available to support this idea. The available data does support the hypothesis that BIV is very widely distributed, and suggests that it is probably present in every country with a large cattle population.

Pathogenesis

In examining the known pathogenesis of BIV it is important to understand the experimental models that were used to gain this information. Four distinct categories of BIV inoculum have been used in experimental studies, with each inoculum giving unique results. Three of these inocula were derived from the original isolate of BIV and can be divided into low passage R29, BVDV contaminated high passage R29, and BVDV-free high passage R29. The fourth category of inoculum is the recently described Florida isolates.

R29 was originally isolated from an eight-year-old dairy cow that had a persistent lymphocytosis, progressive weakness, and emaciation. Necropsy data showed a generalized hyperplasia of the lymph nodes and a mild perivascular cuffing in the brain. Virus was isolated on fetal bovine spleen cells and this inoculum was given to a group of colostrum-deprived calves. The principal observations from this initial inoculation of a low passage isolate was a transient mononuclear cell increase early in infection (10-20 days post-inoculation [p.i.]), a second mononuclear cell increase later in infection (60-150 days p.i.), and clinical evidence of enlargement of subcutaneous lymphatic nodules.

The R29 isolates of today have undergone significant changes in the intervening twenty years. The current R29-derived isolate, that we use and currently distribute, has been passed through fetal bovine spleen cells, canine thymocyte cell line, MDBK cells, and is currently being maintained in fetal bovine lung cells, and must be considered highly tissue culture adapted. The R29 isolate was also contaminated with a noncytopathic strain of BVDV during its time in vitro, and although BVDV-free isolates are available today, numerous animal studies were conducted with BVDV contaminated isolates. Evidence of attenuation of the currently available R29 isolate is the loss of the transient mononuclear cell increase early and late after infection. R29 does still appear to generate a lymphoproliferative response, but it is not as great as that originally described. Two infectious molecular clones of R29, R29-127 and R29-106, have been made and sequenced. R29-127 and R29-106 are very similar except for an 87 nucleotide base pair deletion in the 5' end of the surface envelope (SU) gene of R29-106. All of the R29-derived isolates that our lab has sequenced or examined by PCR have a R29-106 phenotype, with the 87 b.p. deletion.
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The R29-127 type genome does not appear to remain in our current viral stocks. This size difference in the envelope gene is further confounded by the observation that the Florida isolates and all other natural field isolates studied have a larger SU gene size than R29-127. It appears that R29 SU gene no longer represents the SU gene observed in BIV isolates that are out in the field. While R29 and its derivatives have been very valuable in the study of BIV, caution must be used when trying to interpret what a natural infection of BIV might cause in the way of clinical signs or possible disease as compared with what has been reported using R29. A further caution needs to be made for the BVDV contaminated isolates that were used in some studies, since BVDV is a known immunosuppressive agent early after infection, causing fever and lymphopenia.

The recently described Florida isolates, when examined in experimental inoculations, have some of the same observed clinical signs as the original BIV isolate, including a transient mononuclear cell increase early in infection and a lymphoproliferative response, composed of a mild follicular hyperplasia. The late mononuclear cell increase was not observed in the very limited number of animals followed for long periods of time. Further experimental studies with the FL112 isolate have revealed that the early mononuclear cell increase observed in experimental inoculations is predominantly B cells. No significant changes were observed in other cell populations during short-term infection with this isolate.

BIV causes a persistent infection in cattle with a detectable antibody response occurring typically two to four weeks p.i. Virus can be detected in peripheral blood by PCR as early as 3 days p.i. and it can continue to be detected for up to 3 years p.i. Virus can be isolated very early after experimental inoculation, from 3 to 7 days p.i. Antibody response peaks early in infection, and in many experimentally infected animals the antibody levels to p26 and gp110 declines, sometimes to undetectable levels.

Several studies have examined the potential role of BIV as an immunosuppressive agent. BIV has not been shown or implicated to cause a clinically apparent immunosuppression (i.e., greater susceptibility to infections with atypical pathogens) in experimentally inoculated cattle. Several reports have described specific changes in particular functions of immune cells, but no clear trend has developed between different research groups. For example, Martin (1991) described a decreased lymphocyte blastogenesis response after 6 months p.i. Flaming (1993) using a larger sample size, demonstrated a slight increase in lymphocyte blastogenesis response at 4-5 months p.i. and between 19 and 27 months p.i. Again the use of only R29-derived isolates, some contaminated with BVDV virus, have confounded some of these reports. To date, no convincing evidence for immunosuppression by BIV has been demonstrated, and therefore the use of the name bovine immunodeficiency virus is probably misleading.
BIV has also been implicated as causing infections of the brain, specifically causing perivascular cuffing in the brain. This histopathologic lesion is not pathognomonic of BIV infection and it has been associated with several other disease agents. The most commonly cited reason for believing BIV causes encephalitis is the data from the necropsy report of cow R29. Other unsupported observations for brain lesions have been cited in review papers. Anecdotal and other unpublished data suggests a potential role of BIV in causing perivascular cuffing in the brain, but no data at this time support an active BIV infection in the brain or evidence of clinically detectable encephalitis of infected cattle.

BIV has often been cited as causing a lymphadenopathy. Experimental inoculations do not support this clinical sign as being a common aspect of BIV infections. BIV has been reported to have a lymphoproliferative capacity in its ability to cause a transient mononuclear cell (primarily B cell) increase after infection and by the common observation of mild follicular hyperplasia in animals experimentally infected with BIV. In the initial experimental inoculation study by Van Der Maaten (1972), infected cattle were described as having palpably enlarged subcutaneous lymph nodes. However, a generalized peripheral lymphadenopathy was not described in this study or any other study of experimentally infected animals, and it is very much in doubt if lymphadenopathy is a clinically salient feature of BIV infection.

The role of BIV as a disease agent is still debatable. One serological study has suggested a role of decreased milk production in BIV-infected animals. Other than in experimental studies, no other studies have been reported that looked at objective parameters when comparing cattle infected with BIV. Serological studies must be carefully interpreted since the BIV infection may only be associated with another agent that is responsible for decreased milk production or some other clinical parameter. Results obtained using the attenuated R29 isolate may be masking the real disease role of BIV in natural infections. The question of whether BIV causes or does not cause disease is no closer to being answered today than it was 25 years ago. We know that BIV elicits a characteristic response in cattle and we assume it causes a lifelong infection. Because most other lentiviruses cause disease, it is tempting to want to believe that BIV also causes disease. More research is needed to objectively examine the potential role of BIV in disease.

References


BOVINE LENTIVIRUS (BIV)


STRATEGIES FOR IMPROVED BLUETONGUE DIAGNOSTICS

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Introduction

Bluetongue (BLU) viruses are arthropod-borne viruses that infect sheep, cattle, and wild ruminants. These viruses are transmitted by biting midges in the genus Culicoides. Bluetongue virus infection of sheep often results in severe clinical manifestations that can result in death of the animal. Infection of cattle with BLU viruses is usually asymptomatic; however, infection of pregnant cows can result in abortion. Bluetongue viruses are included in the List A viruses of the Office of International Epizootics. This inclusion has resulted in the imposition of regulatory restrictions requiring certification that animals are BLU virus and antibody free before allowing import/export of these animals or their germplasm.

Diagnostic procedures for BLU rely on virus isolation and antibody detection. Isolation of BLU virus has traditionally relied on inoculation of cell cultures, embryonated chicken eggs, or sheep with blood from infected animals or with homogenates of insects collected in endemic areas. This is followed by observation of cytopathic effects (CPE) or death, and isolation and serological confirmation of the virus. Although sensitive, these procedures can be labor-intensive, time-consuming and expensive. Various immunohistochemical staining techniques have been used to detect and identify BLU viruses in infected tissues and cell cultures. These techniques include immunofluorescent staining, immunoperoxidase staining, and immunogold labeling. These procedures are sensitive and specific; but they are often subjective, not easily quantifiable, and may require expensive equipment.

Detection of antibody to BLU virus from infected animals has relied on traditional serological techniques that include neutralization, complement fixation, and agar immunodiffusion (AGID) procedures. The AGID test has been the most widely used USDA approved assay in recent years for testing the sera of animals for the presence of antibody to BLU virus. However, false negative results, due to limited sensitivity, and false positive results, due to antigen cross-reactivity have cast doubts on the test’s reliability.

Advances in understanding the molecular biology of BLU viruses have led to the development of improved diagnostic procedures. Enzyme-linked immunosorbent assay (ELISA) procedures have been developed for the detection of either viral antigen or antibody; and polymerase chain reaction

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(PCR) procedures have been developed for the detection of viral nucleic acids. These new procedures offer improved sensitivity and specificity and considerable cost and time savings over traditional BLU diagnostic procedures.

**General Considerations**

BLU viruses are members of the orbivirus group of viruses in the family Reoviridae. The genome of BLU virus is composed of 10 segments of double-stranded RNA, which code for seven structural and three non-structural proteins. At least 24 serotypes of BLU virus have been identified worldwide and five have been isolated in the United States. The different BLU serotypes have common and unique antigenic determinants on their proteins. This phenotypic profile is also reflected in the genotypic profile of this group of viruses. The development of improved diagnostics must take into account phenotypic and genotypic conservation and variability, the large number of virus serotypes, and the propensity of this group of viruses to undergo reassortment of their segmented genome to form new genotypes. Group-specific diagnostic procedures, designed to detect all members of the group, are based on those genome segments and viral proteins that share a high degree of sequence homology and antigenic similarity among the serotypes. Serotype or strain-specific diagnostic procedures are based on characteristics unique to a particular serotype or strain of BLU virus. Understanding both the antigenic and genotypic characteristics of BLU viruses is essential to the development of improved diagnostic procedures.

**ELISA Based BLU Diagnostics**

ELISA based diagnostics can be configured for the detection of either viral antigen or antibody to the virus. An antigen capture ELISA has been developed for the direct detection of viral antigen in *Culicoides* infected with BLU viruses. Results of the antigen-capture ELISA correlated with the number of plaque forming units of virus. The procedure uses a monoclonal antibody that reacts with a group-specific epitope on VP7, which is a structural protein that is highly conserved among the BLU viruses. This antigen capture ELISA has also been adapted to the detection of BLU viruses in the blood of infected vertebrates; however, because of the relatively low titers of virus in the blood in these animals at the early or late stages of viremia, an amplification step in cell culture is required before detection by this test. In the antigen capture ELISA, virus or viral antigen is captured by a polyclonal antibody to BLU virus (rabbit or some species other than mouse). A mouse monoclonal antibody to BLU virus is then allowed to react with the captured virus or viral antigen. Reaction with biotinylated goat anti-mouse antibody and streptavidin conjugated to peroxidase followed by reaction with a suitable substrate, such as orthophenyldiamine, produces color that can be detected spectrophotometrically.
The degree of color production is correlated with the amount of virus or viral antigen that is captured in the assay and provides a quantitative measure of the amount of virus present as measured by traditional assays.

A number of ELISA procedures have been developed for the detection of antibody to BLU virus. Two different strategies have been used for these procedures. One design, termed indirect ELISA, detects the amount of virus-specific antibody in the serum of an infected animal that has bound to virus or viral antigen adsorbed to the wells of microtiter plates. This antibody is detected by addition of an anti-species antibody conjugated to horse-radish peroxidase followed by reaction with the appropriate substrate and measurement of color development. The second design, termed competitive or blocking ELISA (c-ELISA), measures the amount of competition between antibody in the test serum and a heterologous antibody (usually a monoclonal antibody produced in mice) for binding to the virus or viral antigen in the microtiter plates. The terms competitive and blocking refer to the order of addition of the test serum and the monoclonal antibody. The degree of competition between the antibodies is measured spectrophotometrically following addition of an anti-mouse antibody conjugated to horse-radish peroxidase and an appropriate substrate for color development. An alternative protocol uses biotinylated anti-mouse antibody and streptavidin-conjugated peroxidase in place of antibody conjugated to horseradish peroxidase. The degree of competition is indicated by a decrease in color development and is a measure of the amount of virus-specific antibody present in the test serum. The c-ELISA has become the procedure of choice because of its increased sensitivity and specificity. A number of such tests have been developed that are group-specific and capable of detecting antibodies in animals that have been exposed to any of the currently recognized serotypes of BLU virus without detecting antibodies to viruses of other antigenically related serogroups of orbiviruses, such as epizootic hemorrhagic disease (EHD) virus. The c-ELISA has been shown to be as sensitive or more sensitive than the indirect ELISA, the AGID test, the complement fixation test, and the plaque neutralization test. In addition, it has been shown to be more specific than the AGID test in not demonstrating reactivity with antibody to EHD viruses. The c-ELISA has been endorsed as an acceptable international test for serodiagnosis of bluetongue and there are currently several commercially available c-ELISA tests.

The different c-ELISA tests vary primarily in the monoclonal antibody and antigen preparation used. The majority use monoclonal antibodies that react with the antigenically conserved VP7 structural protein. In addition to being a conserved protein, VP7 is produced in large quantities during viral replication and induces a good serum antibody response in infected animals. These characteristics make it a good choice for group-specific ELISA tests. Other conserved viral proteins may also be useful in c-ELISA procedures. For example, a c-ELISA has been configured around
VP3, which is also a highly conserved structural protein. A c-ELISA using a monoclonal antibody against the largest non-structural BLU virus protein, NS1, has been also been described. Since non-structural proteins are produced only during virus replication, a c-ELISA based on one of these proteins may be useful in differentiating between infected animals and animals vaccinated with either an inactivated virus or non-infectious genetically engineered virus. The majority of c-ELISA tests described use cell culture derived viral antigen. However, a yeast expressed VP7 has been used in a c-ELISA with results that compare favorably with those based on cell culture derived virus.

Serotype-specific ELISA procedures would be useful alternatives to time consuming neutralization assays. However, no such tests have yet been described. Serotype-specific antigenic determinants are located on one of the structural proteins, VP2, and appear to be conformational in nature. Denaturation of these determinants during one of the ELISA steps may alter antigenicity and the ability to react with antibody in the test. Delineation of linear epitopes on VP2 and production of corresponding monoclonal antibodies may help overcome this problem. The observation that VP2 is produced in lower quantities during virus replication may also contribute to the difficulties of developing a serotype-specific ELISA. Cloning and expression of VP2 may be useful for obtaining adequate amounts of VP2 for coating test wells in a c-ELISA.

**PCR Based BLU Diagnostics**

The polymerase chain reaction (PCR) is based on *in vitro* amplification of a defined nucleic acid sequence. Application of this procedure to BLU viruses involves the isolation of viral RNA and synthesis of a complementary double-stranded cDNA molecule using specific oligonucleotide primers and reverse transcriptase. A thermostable DNA polymerase permits amplification of the cDNA in a three phase cyclic process. The first phase involves heat denaturation of the cDNA target. The temperature is then lowered to allow specific oligonucleotides to anneal to opposite strands of the target cDNA in opposite orientation. The temperature is then raised to the optimal temperature for DNA synthesis between the two primers by the thermostable DNA polymerase. These three steps are repeated for 25-35 cycles using automated thermocyclers. This process results in several million fold amplification of a DNA fragment of a size determined by the distance between the oligonucleotide primers that hybridize to the target cDNA.

Sequence analysis and comparison of the genome segments of the different serotypes of BLU virus has provided the information necessary for the development of either group-specific or serotypic-specific PCR diagnostics. In a typical BLU diagnostic PCR, RNA is isolated from the clinical sample and a piece of viral RNA is reverse transcribed and amplified using oligonucleotide primers of the desired specificity. Detection of a PCR prod-
uct of the correct size by agarose electrophoresis is taken as positive evidence of BLU virus RNA in the sample. This can be confirmed by hybridization, restriction digestion, or sequence analysis of the PCR products.

Polymerase chain reaction tests for BLU viruses have been developed based on the structural proteins, VP2, VP3, and VP7; and the non-structural proteins, NS1, NS2, and NS3. Tests based on the genome segments coding for the highly conserved structural protein, VP7, and two conserved non-structural proteins, NS3 and NS1, have been applied to the detection of BLU virus RNA in infected sheep, cattle, and calves. A PCR test has also been used to detect BLU virus RNA in ram semen samples to which known BLU virus was added.

Two PCR tests have been developed for the detection of BLU virus RNA in C. variipennis. One test, the nested PCR, is based on two amplification steps to produce two amplified nucleic acid sequences of the genome segment coding for a non-structural protein, NS1, that are conserved among the five U.S. serotypes of BLU virus, and is a group-specific test for all five serotypes. The other test, a multiplex PCR, is serotype-specific and is based on the gene coding for the variable outer capsid protein, VP2, of the five U.S. serotypes. In this test, five different primer pairs, corresponding to a unique region of the VP2 gene for each of the serotypes, were used to generate specific PCR products that were differentiated by size.

As few as 5000 infectious units of virus can be detected by PCR amplification of a single viral RNA target and detection by staining with ethidium bromide in agarose gels. The sensitivity can be increased 100 fold (50 infectious units of virus) by hybridization detection. Nested PCR procedures, based on amplification of two viral RNA targets, are capable of detecting <1 infectious virus particle. The sensitivity of nested PCR procedures has been further increased by including an enzyme-linked oligonucleotide sorbent assay (PCR-ELOSA). In this procedure, biotinylated and fluoresceinated probes are annealed to the PCR amplified BLU virus nucleic acid segments, and the complexes captured on streptavidin-coated microtiter plate wells. The complexes are then detected using a horseradish peroxidase-labeled antifluorescein antibody conjugate. This procedure has been shown to detect as little as 0.01 infectious units of BLU virus. A similar colorimetric test has been developed for detection of viral RNA using a commercially available kit. This test incorporates the Lac operon sequence into the second primer. The captured PCR product is incubated with a Lac-galactosidase fusion protein and the substrate to produce a color reaction that can be detected spectrophotometrically. There was good correlation between detection of BLU virus RNA with this system and detection of infectious virus from infected animals. Increasing the sensitivity of these PCR based tests can raise questions about the biological significance of the results. For example, BLU virus RNA was detected to 100 days in sheep and to 20 weeks in calves after infection using the PCR-ELOSA;
however, infectious virus was detected only to 38 days and 8 weeks in the respective species.\textsuperscript{16,18}

**Concluding Remarks**

Advances in understanding the molecular biology of BLU viruses has led to the development of improved diagnostic tests. These include the development of antigen-capture ELISA tests for the detection of virus and/or viral antigen, c-ELISA tests for the detection of antibody in infected animals, and PCR procedures for the detection of viral RNA. These new procedures offer improvements over conventional diagnostic procedures in terms of sensitivity, specificity, and time/cost savings. However, since each of these procedures measures a different parameter of infection, no single test should be considered a general "ideal" diagnostic test. Detection of specific antibody is an indication of past infection, but not necessarily of current infection. The detection of viral antigen and/or viral RNA does not necessarily correlate with the presence of infectious virus.\textsuperscript{16,18,21} These tests should be viewed as complementary to give a more complete picture of BLU infection. Continued surveillance and increased knowledge of the molecular biology of this group of viruses will permit us to update and improve diagnostic procedures. Improved diagnostic procedures will help protect domestic livestock and ensure safe international animal trade.

**References**


IMPROVED BLUETONGUE DIAGNOSTICS STRATEGIES


A Natural Outbreak of Bovine Immunodeficiency Virus

Infection in Holstein Dairy Cows

Dr. Ron, Speaker of the College of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana reported a collaborative study between Louisiana State University, Baton Rouge, Louisiana and the National Cancer Institute, Laboratory Institute at St. Louis, Missouri, conducted a collaborative study between Louisiana State University, Baton Rouge, Louisiana and the National Cancer Institute, Laboratory Institute at St. Louis, Missouri.

Chairman: Dr. Lie, M. Miller. Victoria, MD.

Chairman: Dr. James, O. Mechan, Laramie, WY.
fewer numbers of plasma calls and macrophages occurred. In the neuropil, lymphocytic infiltration and gemistocytic astrocytes were found. Lesions were present chiefly in medulla oblongata, cerebellar white matter, rostral brain stem, and cerebral white matter. Lymph node and splenic changes were predominately characterized by a lack of lymphoid proliferation in the presence of chronic inflammation in the field of lymph drainage. Follicular development was almost non-existent and there was a general lack of lymphocyte density in regions normally rich in lymphocytes.

Obliteration of normal lymph node and splenic architecture by neoplastic lymphocytes occurred in cases of lymphosarcoma. Hemal nodes were not appreciably enlarged. Bacterial isolations included *E. coli* and *Klebsiella* sp. often from cows with mastitis. ELISA tests for antibodies to bovine immunodeficiency (BIV) and bovine leukemia (BLV) viruses showed high infection rates by both agents. In 1987, 62 of 125 cows were seropositive for BIV and BLV. In 1990, 67 of 141 were positive. In the spring of 1991, 103 of 134 were positive and in the fall of 1991, after substantial restocking of the herd, 45 of 120 cows were seropositive for BIV and BLV. Of the 26 cows obtained for necropsy, all 26 were positive for BIV by ELISA and western immunoblotting tests, and 24 were seropositive for BLV. Dr. Snider compared the findings to those reported for human immunodeficiency virus infection and stated that they believe BIV or BIV in combination with BLV can cause a chronic, sub-clinical infection that predisposes cattle to a variety of other disease problems.

Questions about the role of other latent or persistent virus infections such as bovine herpesvirus and bovine virus diarrhea virus were discussed. Tests for chlamydia and viruses on various tissues from the affected cattle have usually been negative. Work is in progress to further characterize the role of BIV infection in this dairy herd.

**Studies on Cattle Naturally and Experimentally Infected With Bovine Lentivirus**

Dr. Martin Van Der Maaten, USDA, Agricultural Research Service, National Animal Disease Center, Ames, Iowa, reported on his work at the NADC. Bovine lentivirus was isolated and characterized in 1969 during attempts to identify the viral etiology of enzootic bovine leukosis. It was, however, immediately realized that the structural characteristics of the virus, the seroepidemiological evidence, and the limited lymphoproliferation in the animals under study made it unlikely that the isolate was a candidate for serious consideration in relation to the neoplastic disease. Many years later, with the advent of the human AIDS epidemic and the identification of a related lentiviral agent, there was a renewed interest, not only in all lentiviral agents, but in immunodeficiencies in relation to many human and animal health problems.

In response to questions regarding the pathogenicity of bovine lentivirus,
more than 75 cattle have been inoculated by various routes and with various inocula, including both low- and high-passage materials, and significant clinical disease problems have not been identified in the inoculated animals. Several farm herds that have reported problems thought to be related to immunodeficiencies have been investigated, but there has been little evidence to support a role for bovine lentivirus infections in these conditions. Sheep have been inoculated with bovine leukemia virus and subsequently superinfected with bovine lentivirus to try to identify a potential of oncogenic activity as a result of the coinfections. To date, none has been found. Thus, we conclude that bovine lentivirus, although it may have value in comparative medical studies, particularly because it seems to represent a lentivirus that has become very well adapted to its host, does not pose a serious health threat to the cattle industry. Similarly, we know of no evidence that the virus infects man or is in any way related to human health issues, including persistent indeterminant responses of some individuals to the standard HIV serological tests. One must, however, keep in mind the well-known genomic plasticity of the entire Lentivirus family and give serious consideration to divergent views regarding these matters. Furthermore, the repeated reports of cattle herds with problems that might be related to a mild immunodeficiency leads one to wonder whether there is, in fact, some type of mild disease condition present in our cattle population.

Bovine Lentivirus (BIV): Diagnosis, Prevalence, and Pathogenesis

Dr. Cecilia Whetstone, USDA, Agricultural Research Services, National Animal Disease Center, Ames, Iowa, contrasted Jembrana disease with the results of experimental infection of Banteng cattle with BIV virus. Jembrana disease affects cattle in southeast Asia and has been reproduced using tissue homogenates; however, a specific etiologic agent has not been identified. Typically, infected cattle develop clinical signs in 5 to 19 days resulting in death of some cattle and complete recovery in others. Inoculation of Banteng cattle with a U.S. isolate (FL112) of BIV did not induce clinical disease and also did not cause transient or persistent lymphocytosis as has been seen in U.S. cattle experimentally infected with BIV. Although a lentivirus has been isolated from cattle with Jembrana disease, it is either much different from U.S. BIV isolates or it is not the principal cause of Jembrana disease. Dr. Whetstone also gave an overview of the paper to be presented by Dr. David Suarez which will be printed in the proceedings.

Discussion

The response of USDA,APHIS to the committee's 1993 proposal entitled, "Standards for Certification of Cattle Herds as Bovine Leukosis Virus Free," was discussed. In a letter from Dr. Gary Colgrove, USDA,APHIS,Veterinary Services, he stated the desire of APHIS to modify the standards to give States singular responsibility for certifying herds in-
stead of having joint responsibility by state and federal health officials. The proposed modifications were accepted by the committee. The revised standards read as follows:

Standards for Certification of Cattle Herds as Bovine Leukosis Virus Free

I. Introduction

Owners of cattle participating in the voluntary certification program are required to obtain the services of accredited veterinarians and to submit samples to the National Veterinary Services Laboratories or other laboratories approved by the National Veterinary Services Laboratories to conduct tests for bovine leukosis. The serologic test(s) to be used must be approved by USDA, APHIS.

II. Definitions

A. Herd:

1. All cattle under common ownership or supervision that are grouped on one or more parts of a single premises (lot, farm, or ranch). More than one herd may be maintained on a single premises if they are separated to preclude any physical contact between herds and have separate feed, water and drainage systems.

2. All cattle under common ownership or supervision on two or more premises that are geographically separated, but on which cattle have been interchanged or where there has been contact among cattle on different premises. Contact between cattle on the different premises will be assumed unless the owner establishes otherwise.

3. All cattle on common premises, such as community pastures or grazing association units, but owned by different persons. Other groups of cattle owned by the persons involved that are located on other premises are considered to be part of a herd unless the epidemiologic investigation establishes that cattle from an affected herd have not had the opportunity for direct or indirect contacts with cattle from that specific premises.

B. Identification. All cattle in BLV certified free herds will be identified with a mark which identifies the animal to the herd and identifies the individual animal in the herd. This mark may be permanent or semipermanent. Permanent indelible marks may include tattoos, brands, electronic non-removable implants or registration certificates issued by a recognized breed registry organization that uniquely identifies each animal. Additionally, animals should have a semipermanent visible identification device. Alternatively, two semipermanent visible devices can be used. Semipermanent vis-
ible devices may include eartags or other tags which are surgically attached to the animal. Neck chains or other externally attached numbers are not considered semipermanent devices.

C. Representatives of the State Department of Agriculture shall approve applications for BLV herd certification and recertification.

III. Initial Certification
A. To qualify a herd for certification, all cattle must have two negative tests not less than 6 months nor more than 12 months apart.
B. Send application for Bovine Leukosis Free Herd Certification to the State Animal Health Official along with copies of the last two negative herd test reports. Application must be signed by the herd owner and the accredited veterinarian who did the herd testing.
C. On acceptance by the State Animal Health Official, certification will be approved for one year from the date of the second negative herd test. The Month and Day of the second negative herd test will become the anniversary date for subsequent recertification.
D. Herds certified by State Animal Health Officials will be recognized by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), as free of BLV. Accordingly, APHIS will certify the BLV-free status of such herds for purposes of export to countries requiring that cattle and bovine germplasm originate in herds that are free of BLV.

IV. Recertification
A. A complete test of all cattle in the herd must be completed within 60 days prior to the anniversary date to maintain certification of the herd. Certification terminates on the anniversary date if the herd test is not completed prior to the anniversary date. Certification may be reinstated with one complete negative test of all cattle in the herd conducted within 60 days after the anniversary date. Reinstatement of certification will not change the anniversary date.
B. Application for recertification must be submitted to the State Animal Health Official along with a copy of their herd recertification test. Test results of all herd additions during the previous certification period must be included. A complete accounting for all cattle in the herd including all herd additions or deletions is required.

V. Herd Additions
A. Cattle originating from a Certified BLV-free herd. Cattle from another certified herd must have one negative test 30 days prior to or 30 days after entry into the certified herd. Included are cattle returning to the herd from shows or sales or in transit situations where contact with cattle of unknown status could have occurred. Addi-
tions should be segregated from the herd until they have a negative test.

B. Cattle originating from a BLV negative herd. Cattle originating from a herd with a complete negative herd test within 1 year prior to addition may enter the herd with a negative test 30 to 60 days prior to addition to the herd and a second test 30 to 60 days after entry onto the premises of the certified herd. Additions must be segregated from the certified herd until after the second negative test.

C. Cattle originating from infected herds or herds of unknown status. Cattle from such herds must have 3 negative tests conducted at not less than 60 day nor more than 120 day intervals. During that time they must be segregated from untested and BLV seropositive cattle and not commingled with cattle in the certified herd. If cattle can be segregated at the place of origin, the first or first and second qualifying tests may be completed there; however, the third qualifying test must be conducted after entry onto the premises of the certified herd. If any cattle in the group are found to be positive none of the group may be added to the certified herd until the positive cattle are removed and the testing process started again from the beginning.

One resolution was presented and approved by a 12 to 0 vote. The resolution dealt with implementing a voluntary program for certifying cattle herds as bovine leukemia virus free.

Bluetongue (BT)

Dr. James Mecham, Vice-Chair of the Committee introduced Dr. James Pearson of the National Veterinary Services Laboratory, APHIS/USDA, Ames, IA for a report on bluetongue (BT) and epizootic hemorrhagic disease (EHD).

Bluetongue Epizootic Hemorrhagic Disease Isolation

In calendar year 1993, the National Veterinary Services Laboratories (NVSL) made BT virus isolations from New Mexico, California, and Florida and EHD virus isolations from Arizona, Colorado, and Idaho (Table 1). Domestic animals (cattle) were the source of two EHD type 2 isolates and one BT type 13 isolate. All other isolates were from wildlife.

In fiscal year (FY) 1994, there were 126 BT/EHD virus isolation submissions (including 16 import/export submissions), and there were 112 submissions of imported fetal bovine serum for BT safety testing requiring 181 sheep. During the calendar year 1993 portion of FY 1994 there have been six BT virus or EHD virus isolations. Completed virus identification studies on these isolates are still in process. Epizootic hemorrhagic disease virus was isolated from a white tail deer in North Dakota, BT virus was isolated from a bovine in Georgia, and BT virus was isolated from a red brocket
deer in a Florida zoo.

Bluetongue Survey

The 1993/94 BT survey of 19 northeastern and north central states plus Alaska and Hawaii was conducted from October 11 through December 21, 1993 (Table 2). It utilized the competitive enzyme-linked immunosorbent assay (C-ELISA) test rather than the traditional immunodiffusion (ID) procedure. A total of 8,646 slaughter samples were tested, of which 87 (1.0 percent) were C-ELISA positive. Two of the 14 geographic areas sampled had 2.0 percent or greater C-ELISA-positive samples; Virginia and Indiana had 5.5 percent and 2.3 percent C-ELISA-positive samples, respectively. Thirteen C-ELISA-positive samples were negative for neutralizing antibody against BT and EHD. The other samples had neutralizing antibody against BT 10, 11, 13, and 17 (39 samples), BT and EHD (33 samples), and EHD-2 only (2 samples).

Testing for Serum for Export to Canada

A decision by Agriculture Canada as of August 1, 1993, and to be implemented November 1, 1993, making the BT C-ELISA the only official test for BT for the importation of live ruminants, semen, and embryos into Canada has been changed. Either the agar gel immunodiffusion (AGID) or the C-ELISA test are currently being accepted.

BT Proficiency Test

The BT proficiency test was divided into enzyme-linked immunosorbent assay (ELISA) and AGID with 48 laboratories doing the ELISA test and 10 the AGID for a total of 58 USDA approved BT laboratories. The average number of BT ELISA samples missed was 1.77, and the results of 22 laboratories agreed with the NVSL on all 30 samples. The average number of BT AGID samples missed was 0.7, and 3 laboratories agreed with the NVSL on all 30 samples.

Table 1. Bluetongue (BT) and epizootic hemorrhagic disease (EHD) isolates for calendar year 1993.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Location</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHD-2</td>
<td>Arizona</td>
<td>White tail deer</td>
</tr>
<tr>
<td>EHD-2</td>
<td>Colorado</td>
<td>Cattle</td>
</tr>
<tr>
<td>EHD-2</td>
<td>Idaho</td>
<td>Cattle</td>
</tr>
<tr>
<td>BT-11</td>
<td>New Mexico</td>
<td>Bighorn sheep</td>
</tr>
<tr>
<td>BT-13</td>
<td>California</td>
<td>Cattle</td>
</tr>
<tr>
<td>BT-13</td>
<td>Florida</td>
<td>Bison</td>
</tr>
</tbody>
</table>
Table 2. 1993/94 BT Survey C-ELISA test results for the 14 geographic areas from slaughtered animals.

<table>
<thead>
<tr>
<th>State</th>
<th>Samples</th>
<th>Positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>566</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Hawaii</td>
<td>613</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Indiana</td>
<td>603</td>
<td>14</td>
<td>2.3</td>
</tr>
<tr>
<td>Maryland-Delaware</td>
<td>605</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Michigan</td>
<td>609</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Minnesota</td>
<td>610</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>New England</td>
<td>713</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>New York</td>
<td>682</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>North Dakota</td>
<td>601</td>
<td>4</td>
<td>0.7</td>
</tr>
<tr>
<td>Ohio</td>
<td>602</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Pennsylvania-New Jersey</td>
<td>609</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Virginia</td>
<td>604</td>
<td>33</td>
<td>5.5</td>
</tr>
<tr>
<td>W. Virginia</td>
<td>622</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>607</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8,646</td>
<td>87</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Infection of Carnivores with BT Virus

Dr. Bennie Osburn from the University of California at Davis presented a summary of results from samples obtained from dogs that had been vaccinated with a canine multivalent vaccine contaminated with BT virus and submitted to UC-Davis for testing. There were 153/691 (22%) samples that were positive for BT-specific antibody by the C-ELISA and 2/78 (2.6%) that were positive by a BT-PCR. Attempts to detect BT-specific antibodies in U.S. domestic dogs, coyotes and zoo held African wild dogs were negative. However, BT-specific antibody was detected in 1/4 mountain lions tested. This may be significant since deer make up a large part of a mountain lion's diet.

The study was extended to examine the seroprevalence of BT-specific antibodies in both domestic and non-domestic carnivores in Africa. Seroprevalence was ascertained for domestic dogs and cats in Botswana, Ethiopia, and Kenya and ranged from 0 percent to 39 percent positives. The seroprevalence for non-domestic carnivores in these three countries, plus Namibia, South Africa, Tanzania, and Zimbabwe was also studied. The species sampled included the wild dog, jackal, Simen wolf, cheetah, lion, spotted hyena, W. mongoose, M. mongoose, and spotted genet. The number of positives ranged from 0 to 96 percent. There was a wide distribution of serotypes of BT, that included serotype 2, 3, 4, 6, 8, 9, 10, 13, 14,
BLUETONGUE AND BOVINE RETROVIRUS

17, 18, and 19, to which the African carnivores had been exposed. The seroprevalence of BT specific antibodies among African ruminants suggests that ingestion of infected tissues might play a role in the infection of African carnivores with BT virus.

EHD and BT in Deer

Dr. David Stallknecht from the University of Georgia reported on the incidence of BT and EHD in southeastern white-tailed deer populations. Antibody prevalence studies during 1992 and 1993 suggested a decrease in herd immunity which appears to have changed in 1994. In 1994, isolates have been made in a number of states. All isolates were EHD serotype 2 except for a single isolation of BT serotype 10 from Georgia. Hemorrhagic disease, without confirmatory virus isolation, has also been reported. Most of the virus isolations were from penned animals were mortality can be very high.

Research Issues at ABADRL

Dr. Walter Tabachnick from the USDA, ARS, Arthropod-borne Animal Diseases Research Laboratory (ABADRL) discussed the major research issues for bluetongue disease at ABADRL. Current research is focused on understanding mechanisms controlling the interactions of the virus with the vertebrate host and the insect vector. Studies to identify genes controlling the vector's ability to transmit bluetongue, to identify virus genes controlling virulence in the host animal and interactions with the vector, and to identify factors influencing host pathogenesis offer new strategies for disease control and provide a basis to predict risk factors to U.S. livestock.

Strategies for Improved BT Diagnostics

Dr. James Mecham from USDA, ARS, ABADRL reviewed strategies for improved diagnostic procedures for bluetongue. These include ELISA tests for detection of both viral antigen and antibody to the virus, and PCR procedures for the detection of virus RNA in test samples.

Proposed Protocol for Testing Modified Live BT Virus Vaccine

Dr. Cindy Wolfe from the University of Minnesota requested that committee members review and provide comments on a draft proposal outlining guidelines for testing modified live virus vaccines. The proposal addresses the need to formulate guidelines for consideration by Veterinary Biologics/APHIS in future evaluation of BT vaccines. The proposal outlined four principle criteria to be assessed and included:

1. clinical signs
2. biochemical indicators
3. body temperature
4. level of viremia
USES OF THE LIVESTOCK GENETIC LINKAGE MAPS

Steven Kappes  
USDA-ARS  
U.S. Meat Animal Research Center  
Clay Center, NE

Prediction of genetic differences between animals in current selection practices is based upon phenotypic observations, a product of genetic and environmental factors. For qualitative traits, as coat color or horn development, selection has been quite successful since the phenotypes are easily classified and the variation associated with them is due to a few number of genes with large effects. However, most economically important traits, as growth and carcass traits, are quantitative in nature and the continuous variation underlying them is mainly due to segregation of many Mendelian loci with small effects plus a major influence of the environment. Present estimates of an animal's genetic merit for a quantitative trait is based upon the phenotypic performance of their progeny (expected progeny difference -EPDs). Therefore it is difficult, expensive and time consuming to identify genetically superior animals. Selection at the DNA level (marker assisted selection -MAS) would be more accurate, since it is based upon the genetic material causing the genetic variation, and it would allow selection at a very young age, reducing the time and expense for genetic improvement.

Genetic markers can be used to identify a single quantitative trait locus (QTL) even if the locus is only responsible for a small proportion of the total phenotypic variance (reviewed by Soller). Genetic linkage maps are currently being developed for many livestock species and they provide the framework required for identifying QTLs. Prerequisites for successful implementation of MAS are: 1) development of a high resolution genetic linkage map, and 2) identification of the regions of the genome responsible for the genetic variation (quantitative trait loci -QTLs).

Although genetic linkage maps contain 200 to 400 polymorphic markers for cattle, swine, sheep and poultry, they do not currently represent the entire genome and do contain large intervals between markers that decrease the probability of detecting QTLs, especially those with a small effect. The primary marker type used for building genetic linkage maps are called microsatellites. Microsatellites are a region of the genome that contain repetitive pieces of DNA (i.e., (CA)\(_n\)) with unique flanking sequences found randomly spaced across the genomes of many species. Microsatellites have a high mutation rate with additions or deletions of the repeat unit (CA) occurring. With the use of PCR technology, the length of the microsatellite can be determined. In spite of the polymorphic nature of microsatellites, they will not be informative in some families. A parent has to be heterozygous for a marker (have two different size alleles) before the segregation of the two alleles can be followed in their progeny. Heterozy-
Heterozygosity of a marker appears to be dependant upon the genetic diversity found within an animal. For example in cattle, straightbred Bos taurus animals only average 45% heterozygosity. Therefore a marker will only be informative in 45% of the straightbred parents. Heterozygosity levels for F1 Bos taurus/Bos taurus and F1 Bos indicus/Bos taurus animals are 60 and 72%, respectively. In swine, a 83% heterozygosity level is found in a F1 four breed white composite/Meishan while a 66 and 54% are found in F1 white composite/Duroc and white composite, respectively. Therefore, if a 10 cM map is desired for identifying QTLs, 300 polymorphic relative evenly spaced markers will be required (10cM/3000cM - estimate of bovine genome) to cover the genome. If a F1 Bos taurus/Bos taurus population is being used to identify QTLs, approximately twice the number of markers will be required for making a 10cM map for each parent because of the marker heterozygosity level. The reduction in marker heterozygosity in western breeds has set our group's goal to place 1000 polymorphic markers on the cattle and swine maps. Unfortunately, with 1000 polymorphic markers there will still be small regions of the genome that are not represented. A directed approach will be required such as using chromosome specific libraries and mapping information from the human and mouse maps to generate markers for these specific regions. Comparative studies have found that groups of genes are in the same relative order across species and this information from the human and mouse maps can be used in a directed approach for livestock species.

With high resolution linkage maps, a whole genome search for QTLs will be possible. Obviously, the great QTL hunt has been initiated prior to the completion of high resolution genetic linkage maps with some limited success reported. These efforts have not always been undertaken with the realization that some QTLs will be missed because polymorphic markers are not available from certain regions of the genome and markers may be spaced to far apart to detect QTLs with small effects. Specific populations will need to be designed for specific traits since the parents need to be heterozygous for the QTLs of each trait(s) being evaluated. And since a particular parent is unlikely to be heterozygous for all the loci controlling the genetic variation of a trait, several families will be required to identify all or most of the loci involved. The QTLs must be heterozygous to detect linkage with the markers in the same manner that two markers need to be heterozygous to detect linkage.

An accurate measurement of the phenotype is critical to identifying QTLs. If inaccuracies occur in phenotypic measurements and the environmental effects are large, it will be impossible to identify loci affecting the trait. With some traits as disease resistance little is known about the genetic component and additional work may have to be performed to reduce the environmental influences and obtain a more accurate phenotypic measurement. With disease resistance traits, an animal's susceptibility varies with their environment and the incidence of the disease is also
USES OF THE LIVESTOCK GENETIC LINKAGE MAPS
dependant upon many factors that are not well understood. Therefore these
traits will have to be evaluated under a strictly controlled environment to
allow accurate measurement of the phenotype.

When QTLs have been identified for a trait, then MAS selection can be
implemented. The value of a marker for a QTL is dependant upon the
distance between the two. If the marker is 10 cM from the QTL then 10% of
the time a recombination event will occur between the marker and the QTL.
With the 10% that recombine, MAS will select animals with the less
valuable allele. Incorrect selection of this kind can be eliminated by using
a marker from both sides of the QTL. Whenever a recombination event is
detected between the two markers then the markers cannot be used for
selection because it is not known on which side of the QTL that the recom-
bination event occurred. And since markers are not informative in every
animal, several markers located close to both sides of the QTL are going to
be needed.

Unfortunately MAS can only be practiced in families where QTL alleles
have been characterized. A particular marker allele is not always associ-
ated with the same QTL allele in different populations. Therefore, the QTL
and marker alleles have to be characterized in each family by progeny
testing. Once this has been done for a population then MAS can be used
within that population. The problem of associating a specific marker allele
to a QTL allele within each family can be overcome if the QTL is sequenced
and the mutation is identified that causes the genetic variation. Then the
mutation can be used as a marker for that QTL allele across all families.
However, any new alleles (different mutations) from the same loci present
in other populations will also need to be evaluated by progeny testing. The
task of identifying the specific gene mutation causing the genetic variation
is quite large. If a marker is found to be within 1 cM of a QTL this still
constitutes approximately 1 million bases and there are likely to be many
genes within this region that are not responsible for the QTL.

Application of MAS will have the largest impact on traits that require
progeny testing (carcass traits), traits that cannot be measured in field con-
ditions (disease resistance and production efficiency) or traits that have a
very long generation interval (maternal traits). Selection on quantitative
traits will improve with MAS because it will increase the accuracy and allow
selection to done at a very young age. Qualitative trait selection will not
benefit as much since selection is already quite accurate, but MAS can be
used to fix desirable recessive alleles in a population. Genetic selection
studies will also provide valuable insight to the molecular events controlling
the expression of certain traits which may allow future technology to be
developed.

Marker assisted selection will augment the traditional selection prac-
tices and enable the livestock producer to produce a high quality product in
a profitable manner. Selection for disease resistant animals as the K88 E.
coli resistant pigs will reduce vaccination and treatment costs and improve production efficiency. Identification of malignant hyperthermia susceptible pigs, which tend to produce pale, soft exudate pork, will allow selection against this mutation. But it was suggested that an association may exist between the mutation and lean body mass, so the mutation may be beneficial in market animals in the heterozygous state. A similar case exists with the weaver disease and milk production in cattle. Therefore, MAS should benefit the livestock producer and ultimately the consumer.

References
USES OF THE LIVESTOCK GENETIC LINKAGE MAPS


APPLICATIONS OF RECOMBINANT PROTEIN
EXPRESSION TECHNOLOGY AND GENE AMPLIFICATION
TECHNOLOGY IN VETERINARY DIAGNOSTIC VIROLOGY
AND SEROLOGY

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In the waning years of the 19th century, Von Behring, Ehrlich, and others groped towards what we now know as the antigen-antibody theory of microbial invasion and specific host resistance. Although greatly refined, modified, and supplemented, their concept of specific biomolecular interactions between invaders and defenders has guided much of the research and diagnostic development of medical microbiology in the 20th century. Much of modern diagnostics still relies upon identifying unknown disease agents using specific reference antisera and upon identifying unknown antibody responses using specific reference antigens.

Enormous technical advances have been made in the production of quality reference diagnostic antigens and antisera. The production of these reagents still involves the use of animals and mammalian/avian cell culture systems. Issues of biosafety, cost, and extraneous agent contamination still plague reagent production, especially those intended for foreign animal disease diagnosis. Undesirable nonspecific ("background") reagent reactivity is still a common problem, as is the need for complex reagent purification procedures in many instances. Nucleic acids technologies are now beginning to profoundly alter the ways in which we design diagnostic reagents and test methods. This technology is often successful in overcoming some of the problems alluded to above.

Practical modern nucleic acids diagnostic technologies can be classified as either observational or manipulational. Observational techniques are simpler and basically involve finding evidence of microbial presence using probes and/or primers. These techniques are also useful in providing sequence information about the microbial genes which encode protein antigens of diagnostic significance.

Manipulational technologies are more complex and involve the construction and placement of microbial genes into an environment in which diagnostically significant gene products can be produced in vitro, safely, abundantly, economically, and with minimal need for extensive purification procedures. It is imperative to understand that the value of a recombinant diagnostic antigen is only as good as the basic and applied research previously demonstrating that the antigen is a reliable, conserved, yet agent-specific marker of infection by the virus concerned. It is also usually neces-
RECOMBINANT PROTEIN
EXPRESSION TECHNOLOGY AND GENE AMPLIFICATION

sary that the virus genes encoding such antigens be known and that those
genes have been sequenced.

Most viral protein antigens range from 10 KDa to 100 KDa in size. This
implies that they may contain roughly 70 to 700 amino acids which would
therefore be encoded by virus genes from .2 Kb to 2 Kb in length. Virus
genes of this length are generally monocistronic and easily amplified by the
polymerase chain reaction (PCR) in order to produce large amounts of the
desired genetic material for expression cloning purposes. Much has been
made of the potential for copy errors introduced during the PCR process,
especially by "nonproofreading" DNA polymerase enzymes such as Taq
polymerase. While undeniable, many studies show that even the least
accurate enzymes make nucleotide copy errors at a rate of 1:400 to 1:4,000.
By taking a few simple precautions and performing amplifications at less
than "maximum power," the error rate drops to about 1:5,000 to 1:20,000.
Therefore in theory and in our experience, the fidelity of expression cloning
using PCR-amplified genes has not been a problem. Few, if any, errors
would thus be expected in genes of 200 to 2,000 nucleotides in length and
many such errors would be antigenically "silent" if they did occur.

There are currently five general methods of plasmid construction utiliz-
ing PCR-generated genes to be expressed. All of these methods will work,
and choosing between them is to a large extent personal preference and
experience. In some cases gene-dependent peculiarities may, however,
favor one approach over another. We have successfully employed two
methods: the restriction enzyme site incorporation approach and the high
efficiency blunt end ("universal") cloning strategy. The latter approach is
virtually failure-proof, although 50 percent of the clones will be "forward" (5'
3') orientation and 50 percent will be "backward" (3' 5') and be of no value.
Simple restriction analysis as well as DNA sequencing enable rapid and
clearcut selection of the clones inserted in the correct orientation.

A final consideration to be made involves expression of proteins either
intact and unmodified ("native") as regions of defined antibody specificity
(epitopic subunits) or as proteins fused covalently to other protein or pep-
tide tags. These chimeric fusion protein reagents may be useful in that
multiple, serologically important epitopes may be expressed in a single
construct, even those from different natural protein precursors. Fusion tags
may also allow the one step affinity purification of recombinant proteins.
The two affinity purification fusion protein tags in most common use today
are the glutathione-S-transferase (GST) and polyhistidine peptide systems.

*Escherichia coli* (*E. coli*), yeast, baculovirus/insect cell culture, and
mammalian cell culture systems have all been widely used to express re-
combinant proteins, the genes for which have been inserted into expres-
sion plasmids appropriate for each system. *E. coli* systems are inexpen-
sive, easy to construct, and productive but suffer from the practical disad-
vantage that contaminating *E. coli* proteins may produce unacceptable back-
ground when used in diagnostic tests involving serum from animals ex-
posed to everyday gram negative bacterial flora. In our experience with recombinant p72 antigen of African swine fever virus produced in *E. coli*, even high performance liquid chromatography (HPLC) failed to produce a completely acceptable reagent free of *E. coli* contaminant proteins. Yeast and mammalian expression systems may suffer a similar disadvantage in dealing with antibodies from "real world" animals. Mammalian systems suffer additional drawbacks in their relatively low level of recombinant protein production and potential for extraneous agent contamination. We have been generally pleased with the high productivity, authenticity, low background crossreactivity, low cost, and simplicity of the baculovirus/insect cell culture system. The increasingly widespread use of this system has also enabled us to acquire from other laboratories baculovirus vectors expressing proteins important in the diagnosis of such important diseases as avian influenza, African swine fever, and vesicular stomatitis (New Jersey serotype). In those cases where we have not been able to "clone by phone," we have made our own baculoviruses for expression of such diagnostically important antigens as the prion protein (PrP) of the spongiform encephalopathies (scrapie, bovine spongiform encephalopathy), an additional avian influenza conserved protein antigen (matrix protein), and the conserved capsid protein of vesicular stomatitis virus (Indiana serotype). A variety of enzyme-linked immunosorbent assay (ELISA), western blot, and immunoperoxidase monolayer assay (IPMA) tests have worked well for us using even crude homogenates of insect cell cultures and homogenates of insect larvae infected with these recombinant baculoviruses. The inherent animal biosafety of the baculovirus system is enabling the National Veterinary Services Laboratories scientists to currently construct plasmids for the expression of isolated protein antigens of foreign animal disease agents including foot-and-mouth disease and African horsesickness viruses. Our success in this endeavor will hopefully reduce some of the costs and risks involved with the serologic testing for the presence of diseases exotic to the United States.

Finally, the heterologous antigenic nature of insect and vertebrate cells culture systems and the high level of recombinant protein expression by recombinant baculoviruses has enabled the extremely easy production and screening for monoclonal antibody reagents directed against highly defined and diagnostically significant antigens expressed in rDNA form. The ability to generate paired recombinant protein - monoclonal antibody reagent systems has enabled us to construct a highly specific, species- indifferent competitive ELISA (C-ELISA) for vesicular stomatitis serology. We anticipate that in the coming years an increasing number of recombinant antigen-monoclonal antibody reagent systems will be produced for effective, specific, safe, and economical application to veterinary diagnostic virology and serology in ways never dreamed possible by Paul Ehrlich and his contemporaries 100 years ago.
REPORT OF THE COMMITTEE ON BIOTECHNOLOGY

Chairman: Dr. Joan M. Arnoldi, Ames, IA
Vice Chairman: Dr. Robert A. Crandell, College Station, TX

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The Biotechnology Committee met from 1:30 p.m. until 4:50 p.m. on Wednesday, November 2, 1994. There were 8 members and 16 guests in attendance.

Chairperson Dr. Joan Arnoldi called the meeting to order and welcomed all those in attendance. She introduced Vice-Chairperson Dr. Bob Crandell. Speakers and summaries of the scientific reports presented are given.

Dr. Steven Kappes, "Uses of Livestock Genetic Linkage Maps." Genetic linkage maps will provide the framework of information needed to use Marker Assisted Selection (MAS). MAS is a new selection tool that will improve the accuracy and time of selection of current selection practices. The prerequisites to MAS are 1) development of a high resolution genetic linkage map; 2) identification of the loci (region of the genome) responsible for the genetic control of the trait; and 3) implementation of MAS by characterization of the effect of loci upon the trait and then rising markers to select the genetically superior animals. MAS will be most beneficial for traits that are currently difficult to select for such as reproduction, disease resistance, production efficiency, and carcass traits.

Dr. Brad Bosworth, "Genetic Resistance to Porcine Post-Weaning Diarrhea Caused by Enterotoxigenic Escherichia coli That Express 2134 P Pili."

K88+ and F107+/2134P+ E. coli causes enterotoxic disease in weaned pigs. Genetic resistance to both has been documented and the gene that control the resistance to K88+ E. Coli is on chromosome 13 and may encode for a mucin-like receptor. The gene controlling resistance to F107+/2134 P+ E. coli is on chromosome 6 and is closely linked to the gene controlling susceptibility to porcine stress syndrome. Selection for the correct genotype should help in increasing disease resistance to enterotoxigenic diarrhea in swine.
Dr. Jon Katz, "Application of Recombinant Protein Expression Technology and Gene Amplification Technology in Veterinary Diagnostic Virology and Serology."

Nucleic acid cloning and amplification techniques have permitted the expression of important protein antigens for diagnostic use. Systems currently being developed at NVSL include African swine fever viral proteins, vesicular stomatitis viral capsid antigen, and prion protein antigen for diagnosis of scrapie and BSE. These reagents offer the potential for safer, more specific, and more economical veterinary virus diagnostic tests.

Dr. Fred Tatum, "Characterization of a Restriction Endonuclease from Pasteurella haemolytica serotype 1 and construction of a gene replacement aroA mutant.

1. Pasteurella haemolytic serotype 1 was found to contain a type II restriction endonuclease and modification system, Phal.
2. Protection of DNA against the restriction-modification system is a critical step prior to introduction of exogenous DNA into P. haemolytica serotype 1.
3. The experimental approach that successfully produced and aroA mutant of P. haemolytica required repeated passage of the bacterium transformed with a hybrid plasmid.
4. Although the hybrid plasmid replicated in P. haemolytica, improving the chance for recombination, its instability allowed recovery of a plasmidless aroA mutant.

Dr. Donald Knowles, "Progress Toward Recombinant Antigen Based Serologic Detection of Horses Infected with Babesia equi and Babesia caballi."

A monoclonal antibody (MAb) to an immunodominant 34 kDa Babesia equi merozoite surface protein was derived and used to molecularly clone and express the corresponding recombinant protein. This recombinant protein was applied in the Competuters Inhibition-ELISA format and used to serologically detect horses infected with B. equi worldwide. Sera from horses experimentally and naturally infected with Babesia caballi were shown to consistently recognize gradient purified B. caballi merozoite associated proteins with molecular masses of 110, 98, 70, 46, and 26 kDa. Thus far, the 98 kDa protein has elicited the highest antibody titer in sera from B. Caballi infected horses tested by immunoblot.

Dr. William Davis, "New Strategies in the Development of Synthetic Vaccines."

Recent advances in the understanding of the composition and function of the immune system have revealed the steps that must be taken to improve the capacity of synthetic vaccines to elicit a protective immune response. First, comparative analysis of the immune systems in ruminants and swine has shown they differ from those noted in humans, rodents, and other species. These differences emphasize the need to consider the com-
position and function of the immune system in the relevant species when developing and testing the efficacy of synthetic vaccines. Second, analysis of the immune response to whole live, dead, and synthetic vaccines has shown the development of an immune response to antigens is mediated by sets of cytokines which regulate all differentiation following antigen recognition. The cumulative data suggest the initial cytokine signals determine the patterns of T cell differentiation. Thus, the initial encounter with the pathogen/parasite at the site of entry can initiate a cascade of events that lead to protective immunity, as noted with live-attenuated vaccines or a cascade of events that lead to suboptimal or deleterious responses and disease. It is clear from these recent observations that efforts to design new synthetic vaccines and improve the preparation of old vaccines must take into account the immunogenic properties of the vaccine antigen and its capacity to elicit the appropriate set of signals at the port of entry following antigen uptake and presentation.
NEW STRATEGIES IN THE DEVELOPMENT OF SYNTHETIC VACCINES

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Recent advances in the understanding of the composition and function of the immune system have revealed the steps that must be taken to improve the capacity of synthetic vaccines to elicit a protective immune response. First, comparative analysis of the immune systems in ruminants and swine has shown they differ from those noted in humans, rodents and other species. T cells are present in high concentration in the peripheral blood of young animals. In swine, there is an additional difference. The T cell population is composed of three subsets that express CD4, CD8, or CD4 and C8, the accessory molecules associated with the development of immune responses to antigens restricted by class I and class II elements of the major histocompatibility complex. These differences emphasize the need to consider the composition and function of the immune system in the relevant species when developing and testing the efficacy of synthetic vaccines. Second, analysis of the immune response to whole live, dead, and synthetic vaccines has shown the development of an immune response to antigens is mediated by sets of cytokines which regulate cell differentiation following antigen recognition. Current studies show protective immunity is associated with the appearance of functionally mature CD4+ and CD8+ T cells that secrete specific sets of cytokines, termed ‘type 1’ and ‘type 2’ respectively. Where cell mediated immunity plays a significant role in imparting protection, the cells secrete the ‘type 1’ pattern of cytokines that includes IL-2 and IFN-γ as essential cytokines. Where humoral immunity appears to play a predominant role, the cells secrete the ‘type 2’ pattern of cytokines that includes IL-4, IL-5, IL-10, and IL-13, essential for regulating development and differentiation of B cells. Professional antigen presenting cells such as macrophages, Langerhans cells, and dendritic cells and non-professional antigen presenting cells that can express MHC class II molecules, secrete cytokines that potentially direct the pattern of differentiation of naive T cells along the ‘type 1’ or ‘type 2’ pathways through positive and negative signals. IL-4, IL-10 and IL-12 have been implicated as the main cytokines involved in signaling the pattern of differentiation of T cells. The cumulative data suggest the initial cytokine signals determine the patterns of T cell differentiation.

Thus the initial encounter with the pathogen/parasite at the site of entry can initiate a cascade of events that lead to protective immunity, as noted
DEVELOPMENT OF SYNTHETIC VACCINES

with live-attenuated vaccines or a cascade of events that lead to suboptimal or deleterious responses and disease. It is clear from these recent observations that efforts to design new synthetic vaccines and improve the preparation of old vaccines must take into account the immunogenic properties of the vaccine antigen and its capacity to elicit the appropriate set of signals at the port of entry following antigen uptake and presentation. Experimental evidence on how this might be achieved is now being obtained with the use of candidate vaccines used alone or in concert with adjuvants. Purified target antigens have tended to yield suboptimal responses and little or no evidence of the development of a protective immune response. The primary response to the antigens has been the development of antibody. When introduced with adjuvants such as ISCOMS or liposomes, the responses have been heightened to the antigen, with evidence that the responses have included the development of responses by both CD4+ and CD8+ T cells. In model systems where synthetic antigens have been coupled directly with certain types of lipids, evidence has been obtained which shows protection comparable to that obtained with challenge exposure occurs. These data clearly show that analysis of the events associated with the appearance of protective immunity elicited by modified synthetic vaccine constructs will reveal the critical events associated with the induction of protective immunity at the cellular level.
GENETIC RESISTANCE TO PORCINE POST-WEANING DIARRHEA CAUSED BY ENTEROTOXIGENIC ESCHERICHIA COLI THAT EXPRESS 2134P PILI

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From the National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA 50010 (Bosworth, Casey, Nystrom) and the Department of Animal Science, Iowa State University, Ames, IA 50010 (Rothschild).

Summary
Enterotoxigenic Escherichia coli (ETEC) expressing 2134P pili adhered to brush borders of intestinal epithelial cells of some, but not all, weaned pigs. Weaned pigs were inoculated with a 2134P+ ETEC and retrospectively phenotyped as having either non-adhesive or adhesive brush borders using an in vitro adhesion assay with a 2134P+ ETEC. Pigs with non-adhesive brush borders did not develop diarrhea or have histologic evidence of bacterial colonization. Pigs with adhesive brush borders developed diarrhea and/or had histologic evidence of bacterial colonization. Pigs with non-adhesive brush borders gained significantly more weight than did pigs with adhesive brush borders. The 2134P+ ETEC did not adhere to villous epithelium in ligated ileal loops in weaned pigs with non-adhesive brush borders, while 2134P+ ETEC did adhere in pigs with adhesive brush borders. Brush borders non-adhesive for 2134P+ ETEC were also non-adhesive for verotoxigenic Escherichia coli (VTEC) expressing the F107 pilus, while brush borders adhesive for 2134P+ ETEC were adhesive for F107+ VTEC. Examination of litters containing pigs with either non-adhesive or adhesive brush borders revealed that this trait was genetically controlled by a locus on porcine chromosome 6.

Introduction
Enterotoxigenic Escherichia coli (ETEC) cause diarrhea in newborn and weaned pigs. Pili or fimbrial adhesins (K88, F41, K99, and 987P) are important virulence factors in the pathogenesis of porcine diarrhea as pili mediate adherence to brush borders of intestinal epithelial cells and, in this way, promote intestinal colonization. Host age or genetics can influence the ability of ETEC to adhere to brush borders and colonize the small intestine.

Recently, 2 new pilus types have been identified in pathogenic E. coli isolated from weaned pigs. The F107 pilus is present in most verotoxigenic E. coli (VTEC) isolated from weaned pigs with edema disease. The 2134P pilus is found in some ETEC isolated from weaned pigs with diarrhea.
Both F107 and 2134P pili mediate bacterial adherence to brush borders and promote intestinal colonization. Susceptibility or resistance to edema disease caused by F107+ VTEC is genetically controlled and the locus controlling susceptibility or resistance has been reported to be on chromosome 6.4

The 2134P and F107 pili share some similarities. Polyclonal antisera raised against either 2134P+ ETEC or F107+ VTEC has some cross-reactivity with the heterologous strain. Both 2134P and F107 are thin pili (3.5 - 4.5 nm in diameter) and morphologically similar. The deduced amino acid sequences of the 2134P and F107 pilus subunits are > 90% identical.

Due to the similarities of the F107 and 2134P pili, we compared the adherence of F107+ VTEC and 2134P+ ETEC to brush borders of weaned pigs. Brush borders of some weaned pigs were non-adhesive for both F107+ VTEC and 2134P+ ETEC, while brush borders of other weaned pigs were adhesive for both F107+ VTEC and 2134P+ ETEC. One objective of this study was to determine if the adherence of both F107+ VTEC and 2134P+ ETEC to brush borders was controlled by a locus on chromosome 6. Another objective of this study was to determine if pigs with non-adhesive brush borders were resistant to challenge with a 2134P+ ETEC.

Materials And Methods

Bacterial strains: The 2134P+ ETEC strain, 2134, which expresses 2134P pili and was originally isolated from a weaned pig with diarrhea, was used as inocula in animal experiments and the in vitro adhesion assay. The non-pathogenic strain 123, originally isolated from a healthy pig, was used as a control inocula in animal experiments. The F107+ VTEC strain, 107/86, was used in the in vitro adhesion assay.

Brush border isolation and in vitro adhesion assay: The ability of strain 2134 or strain 107/86 to adhere to brush borders in vitro was determined as follows. Equal volumes of brush borders (approximately 1 x 10^9/ml) and bacteria (approximately 1 x 10^8/ml) were mixed, incubated at 37 C for 30 to 60 minutes and then washed by centrifugation. On the basis of the in vitro adhesion assay, pigs were designated as either non-adhesive (< 1 bacterium/brush border) or adhesive (> 3 bacteria/brush border).

Animal experiments: Pigs from 3 litters (17 to 23 days of age) were weaned, fasted overnight, orally intubated, and then inoculated intragastrically with 10^10 CFU of strain 2134 (principals) or strain 123 (controls). Seventeen pigs served as principals and 4 pigs served as controls. Brush borders were collected at necropsy and used to designate pigs as non-adhesive or adhesive using the in vitro adhesion assay. Mean weight gain of non-adhesive principals, adhesive principals and controls was compared using Student's t-test.

A fourth litter (21 days old) was used in studies to determine the in vivo adherence of bacteria in ligated ileal loops which were created in each of the 8 pigs. Three loops were inoculated with 10^8 CFU of the enterotoxigenic
strain 2134, and 3 additional loops were similarly inoculated with the non-pathogenic strain 123. Pigs were sacrificed 18 hours post-inoculation.

Histopathology, Immunocytochemistry, and Bacteriology: Ileal samples from the intra-gastrically inoculated pigs and ligated ileal loops were processed for histopathology. Formalin-fixed sections of ileum from intra-gastrically inoculated pigs and ligated ileal loops were analyzed by immunohistochemistry using polyclonal anti-2134P antisera as previously described.

Linkage analysis: Sires, dams and offspring were genotyped for glucose phosphate isomerase (GPI), 6-phosphogluconate dehydrogenase (PGD) and for polymorphisms in the ryanodine receptor gene. Pigs homozygous for polymorphisms in the RYR gene are susceptible to porcine stress syndrome.

Offspring were designated as either non-adhesive or adhesive for 2134P+ ETEC and F107+ VTEC using the in vitro adhesion assay. The genotype of dams and sires was predicted on the basis of in vitro adhesion results of their offspring. Statistical analysis using the decimal logarithm of the odds ratio (lod score) for genetic linkage and the most probable genetic map was determined using the Mapmaker program.

Results

Intra-gastric inoculations: Of the 17 principals inoculated with strain 2134, 7 were designated non-adhesive and 10 were designate adhesive. The 7 non-adhesive principals did not develop diarrhea (Table 1). Seven of 10 adhesive principals developed diarrhea 1 or 2 days post-inoculation (Table 1). The 4 controls, 2 non-adhesive and 2 adhesive, did not develop diarrhea following inoculation with the non-pathogenic strain 123. Histologic evidence of bacterial colonization was not seen in either controls or non-adhesive principals (Table 1). Four of the 10 adhesive principals had histologic evidence of bacterial colonization (Table 1).

The weight gains of non-adhesive and adhesive principals at 24 and 48 hours post-inoculation are shown in Table 1. The mean weight gain (+/- S.E.) of non-adhesive principals was 0.58 kg (+/- 0.06) and 1.17 kg (+/- 0.09) at 24 and 48 hours post-inoculation, respectively. The mean weight gain (+/- S.E.) of adhesive principals was 0.21 kg (+/- 0.06) and 0.76 kg (+/- 0.14) at 24 and 48 hours post-inoculation, respectively. Non-adhesive principals gained significantly more weight than did the adhesive principals at 24 and 48 hours post-inoculation (P < 0.001 and < 0.05, respectively). Controls had a mean weight gain (+/- S.E.) of 0.48 kg (+/- 0.09) and 0.85 kg (+/- 0.13) at 24 and 48 hours post-inoculation, respectively. There was no significant difference between weight gain in the controls and non-adhesive principals at 24 and 48 hours post-inoculation. Controls gained significantly more weight than did the adhesive principals at 24 hours post-inoculation (P < 0.05), but not at 48 hours post-inoculation.
Ligated ileal loops: Of the 8 pigs used in the intestinal loop experiments, 3 were retrospectively designated non-adhesive and 5 were designated adhesive on the basis of the in vitro adhesion assay. No evidence of bacterial adhesion was noted in ileal loops of non-adhesive pigs other than an occasional association of bacterial cells with goblet cell secretions. Four of the 5 adhesive pigs had evidence of bacterial adhesion in ileal loops.

Linkage analysis: All 29 pigs (3 weeks old) used in the intra-gastric challenges and ileal loop experiments described above plus an additional 15 offspring (6 months old) were phenotyped as non-adhesive or adhesive for both 2134P+ ETEC and F107+ VTEC using the in vitro adhesion assay. Pigs from both age groups were non-adhesive for both 2134P+ ETEC and F107+ VTEC, or adhesive for both 2134P+ and F107+ VTEC.

Five sires and 8 dams were used in producing the 8 litters used for linkage analysis. Three litters were derived from heterozygous (Ss) by heterozygous (Ss) matings and 5 litters were derived from heterozygous (Ss) by homozygous (ss) matings. A total of 25% (4 of 16) of offspring from Ss by Ss matings were non-adhesive (ss) and 46% (13 of 28) of offspring from Ss by ss matings were non-adhesive (ss).

Only offspring that were non-adhesive (ss) or adhesive offspring (Ss) from Ss by ss matings were used for linkage analysis. Linkage analysis demonstrated that the locus controlling non-adhesion or adhesion had recombination rates of 3.7%, 3.7%, and 7.4% relative to GPI, RYR, and PGD, respectively. There was genetic linkage between the locus controlling non-adhesion or adhesion with both GPI and RYR (P < 0.001), but linkage could not be shown with PGD (P > 0.001). However, PGD was clearly linked to both GPI and RYR (P < 0.001).

Discussion

ETEC adhere to brush borders and colonize the small intestine of susceptible pigs resulting in diarrhea and a loss of weight. We demonstrated that 2134P+ ETEC did not adhere to the brush borders of some 3-week-old pigs and that these pigs were resistant to intra-gastric challenge with a 2134P+ ETEC. Non-adhesive principals did not develop diarrhea and had no histologic evidence of colonization. Non-adhesive principals gained significantly more weight than did adhesive principals following intra-gastric inoculation. Adhesive principals developed diarrhea and/or had histologic evidence of colonization.

The ligated ileal loop experiment demonstrated that 2134P+ ETEC did not adhere to villous epithelium in vivo in pigs designated non-adhesive using the in vitro adhesion assay, but 2134P+ ETEC did adhere to villous epithelium in vivo in 4 of 5 pigs designated adhesive using the in vitro adhesion assay. Taken together, the intra-gastric inoculations and ligated ileal loop experiments demonstrated that 2134P+ ETEC did not adhere in vivo in non-adhesive pigs and none of these pigs were colonized or devel-
oped diarrhea. Conversely, adhesive pigs usually had evidence of bacterial adherence in ligated ileal loops and were susceptible to intra-gastric inoculation on the basis of at least one of the following criteria; colonization, diarrhea or decreased weight gain.

A total of 109 pigs from 3 different herds with different genetic backgrounds were phenotyped using the in vitro adhesion assay. Pigs were either non-adhesive for both 2134P+ ETEC and F107+ VTEC or adhesive for both 2134P+ ETEC and F107+ VTEC. This correlation has also been observed in Swiss pigs. These data indicate that both pili recognize a common intestinal receptor encoded at the same locus. Similarities in morphology and amino acid composition of 2134P and F107 pili provide further evidence of a common intestinal receptor encoded at the same locus.

The locus controlling non-adhesion or adhesion for 2134P+ ETEC and F107+ VTEC was linked to RYR and GPI loci in the animals used in this study. Genetic linkage between GPI and the locus controlling susceptibility or resistance to intestinal colonization by F107+ VTEC has been previously reported.4 The similarities in location of the locus in the present study and the study of Vogeli and others provide further evidence that a single locus encodes for a common intestinal receptor for both 2134P+ ETEC and F107+ VTEC. We observed no significant linkage between the locus controlling non-adhesion and adhesion to the PGD locus (P > 0.001), but PGD was clearly linked to both RYR and GPI.

Selection of pigs resistant to both 2134P+ ETEC and F107+ VTEC may be a desirable method of disease prevention and our linkage analysis suggests that marker-assisted selection is useful in identifying resistant pigs. However, selection based solely on disease resistance may allow for the selection of undesirable traits. Pigs in this study that were non-adhesive (resistant) for 2134P+ ETEC and F107+ VTEC were frequently NN at the RYR locus and susceptible to porcine stress syndrome, an undesirable condition. Discovery of genetic markers more tightly linked to disease resistance than the RYR locus will identify pigs that are not susceptible to porcine stress syndrome and resistant to disease caused by both 2134P+ ETEC and F107+ VTEC.

References
4. Vogeli P, Delacretaz AS, Kuhn B, et al. Associations between H blood...
group system and the GPI red cell enzyme system and the locus specifying receptors for an *Escherichia coli* strain expressing fimbriae 107 in the pig. *Internat Confer Animal Genetics 1992;Interlaken/Switzerland.*

**TABLE 1.**  
Resistance and susceptibility to challenge with a 2134P* ETEC

<table>
<thead>
<tr>
<th>Pig#</th>
<th>Diarrhea**</th>
<th>Histology+</th>
<th>Weight Change++ 24/48 hours</th>
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<td>-</td>
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<td>0.5/0.8</td>
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<th>Diarrhea**</th>
<th>Histology+</th>
<th>Weight Change++ 24/48 hours</th>
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<tr>
<td>676</td>
<td>+</td>
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<td>0.6/1.8</td>
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*On the basis of the in vitro adhesion assay, pigs were designated non-adhesive or adhesive if mean bacterial adherence was <1 or >3 2134P* *E. coli* brush border, respectively.

"At least 1 bowel movement was observed in each pig daily. Pigs were scored + if watery diarrhea was noted at 24 and/or 48h and - if normal feces was noted at both 24 and 48h post-inoculation.

*Pigs were designated + if bacteria were seen adhering to ileal epithelium and - if no adherent bacteria were seen histologically.

**Delta weight change (in kg) from 0 hours post-inoculation at 24 and 48 hours post-inoculation, respectively.
CHARACTERIZATION OF A RESTRICTION ENDONUCLEASE FROM PASTEURELLA HAEMOLYTICA SEROTYPE 1 AND CONSTRUCTION OF A GENE-REPLACEMENT AROA MUTANT.

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Ames, Iowa 50010

Abstract

A new restriction endonuclease, Phal, was isolated from \textit{P. haemolytica} serotype 1, strain NADC D60, obtained from pneumonic bovine lung. \textit{Phal} recognizes the 5 base non-palindromic sequences 5'-GCATC-3' and 5'-GATGC-3'. Cleavage occurs 5 bases 3' from the former recognition site and 9 bases 5' from the latter. A gene encoding for a methyltransferase which protects against \textit{Phal} cleavage was cloned from \textit{P. haemolytica} into \textit{E. coli}. Whereas unmethylated plasmid DNA containing a \textit{P. haemolytica} origin of replication was unable to transform \textit{P. haemolytica} when introduced by electroporation, the same plasmid DNA obtained from \textit{E. coli} which contained cloned \textit{Phal} methyltransferase could do so. The \textit{aroA} gene of \textit{P. haemolytica} serotype A1 was cloned and sequenced. A \textit{P. haemolytica} ampicillin-resistance fragment was cloned into the unique \textit{Ndel} site of \textit{aroA}. A hybrid plasmid was constructed by joining the \textit{aroA} replacement plasmid with the 4.2 kb \textit{P. haemolytica} plasmid which encodes streptomycin resistance. Following \textit{Phal} methylation, the hybrid plasmid was introduced into \textit{P. haemolytica} by electroporation. Allelic exchange between the replacement plasmid and chromosome of \textit{P. haemolytica} gave rise to an ampicillin-resistant mutant which was unable to grow on medium deficient in tryptophan. Although transformation efficiency with methylated hybrid plasmid was $< 10^5 / \mu g$, the hybrid was capable of unstable replication in \textit{P. haemolytica}, so this system may be suitable for construction of additional gene-replacement mutants.

Introduction

\textit{Pasteurella haemolytica} serotype A1 is the most common etiologic agent responsible for pneumonic pastuerellosis in cattle. The organism is prevalent in the upper respiratory tract and palatine tonsils of healthy cattle. Under stressful conditions, or with viral respiratory tract infection, \textit{P. haemolytica} can rapidly colonize the upper respiratory passages to become the predominant flora, whereupon they may be inhaled and result in pneumonic pasteurellosis. The mechanism of upper respiratory colonization and pathogenesis of pneumonia, however, are still poorly understood. Interest in elucidation of the molecular mechanisms of pathogenesis, and develop-
PASTEURella HAEMOLYTICA SEROTYPE 1 AND CONSTRUCTION OF A GENE-REPLACEMENT ARoA MUTANT

ement of improved vaccine strains, has led several researchers to investigate genetic manipulation of \textit{P. haemolytica}.

Through the use of transposon mutagenesis or site-directed mutagenesis, the role played by potential virulence factors may be assessed and improved vaccines may be designed. Efforts to introduce foreign DN into \textit{P. haemolytica} serotype 1, however, have not been particularly successful. Whereas plasmid DNA obtained from \textit{P. haemolytica} serotype 1 can be reintroduced into \textit{P. haemolytica} by electroporation without difficulty, \textit{P. haemolytica} plasmid DNA isolated from an \textit{E. coli} host does not transform \textit{P. haemolytica} under similar conditions.

In this experiment we investigate the possibility that a restriction barrier may be preventing establishment of foreign DNA in \textit{P. haemolytica}, and design an attenuated auxotrophic mutant of \textit{P. haemolytica} serotype A1. Mutants of \textit{Salmonella typhi} defective in the aromatic amino acid biosynthetic pathway were first described by Bacon \textit{et al} in 1950 as avirulent in mice. Subsequently, it has been demonstrated in widely diverse bacteria that disrupting the aromatic amino acid biosynthetic pathway produces attenuated organisms. The successful construction of an \textit{aroA} mutant of \textit{P. haemolytica} required the construction of hybrid replacement plasmids including the origin of replication of a plasmid endogenous to \textit{P. haemolytica} and methylation by \textit{Phal} methyltransferase.

Methods

\textbf{Recovery of \textit{Phal}:} \textit{Pasteurella haemolytica} serotype 1, strain NADC-D60, was disrupted by sonication, clarified by centrifugation and filtration, and injected into a prepacked heparin-sepharose column. Fractions were eluted by linear NaCl gradient and tested for nuclease activity on bacteriophage lambda DNA. Active fractions were pooled, concentrated, and stored at -20 C in storage medium containing 50% glycerol.

\textbf{Recognition Specificity and cleavage site:} Two of the four recognition sites in pBluescript (Stratagene) were identified by double digestion with \textit{Phal} and either XhoI or ScaI, which cut at opposite ends of the polylinker. Additional double digestions of pBluescript, ÖX174, and pUC19 confirmed the recognition site. Single-stranded ÖX174 DNA was digested to determine if \textit{Phal} has activity on this substrate. The cleavage site was identified by digestion of a primed-synthesis reaction on pBluescript derivatives containing the \textit{Phal} recognition site in each orientation. Reactions containing no dideoxy terminator were extended through the \textit{Phal} site using 32P-endlabelled primer. \textit{Phal} or SfeNI (New England Biolabs) was added to the unterminated reactions and allowed to digest the DNA for 2 minutes. Standard reactions were electrophoresed alongside those cut with endonuclease.

\textbf{Cloning \textit{Phal} methyltransferase:} One μg of \textit{P. haemolytica} cosmid library (pLAFR2) was digested with \textit{Phal}, then electroporated into \textit{E. coli} AP1-200-9. This strain of \textit{E. coli} was designed by Piekorowicz \textit{et al} to give color selection for functional DNA-modifying genes. Briefly, mrr and mcr,
linked to temperature-sensitive promoters, induce damage to modified DNA at the permissive temperature. SOS promoters linked to α-galactosidase allow color selection. In this study, the transformed AP1-200-9 cells were incubated at 42°C for 18 hr, then 30°C for 4 hr, then 42°C. Blue colonies, indicating the presence of a cloned methyltransferase gene, were isolated and analyzed. The colonies were screened for restriction activity, and DNA from positive colonies was analyzed for resistance to digestion by Phal. E. coli DH10B transformed with cosmid which imparted resistance to digestion was designated PhalMtase.

Methylation of hybrid shuttle vector and transformation of P. haemolytica: A hybrid plasmid was constructed consisting of pBluescript, the aroA gene of P. haemolytica with the Cmr gene of pBR325 inserted, and pD80 (4.2 kb ampicillin' plasmid from P. haemolytica). The resultant plasmid, approximately 11 kb in size, contained a P. haemolytica ori, amp', and Cmr. The hybrid plasmid was electroporated into E. coli DH10B with or without Phal methyltransferase. Methylated plasmid from E. coli was passed through P. haemolytica for comparison. P. haemolytica was transformed by electroporation with 1 μg hybrid plasmid from each of the three sources at 15,000 V/cm, 800 Ω, 25 μF. Transformants were enumerated after appropriate dilution and plasmid content confirmed.

Molecular cloning and Southern blot analysis of P. haemolytica aroA: Genomic DNA from P. haemolytica was fractionated by gel electrophoresis and Southern blotting revealed a positive signal corresponding to a 3.2 kb HindIII fragment using a radioactive 1.3 kb E. coli aroA probe. HindIII fragments from 3.0-3.4 kb in length were electroeluted from a gel and ligated into pBluescript. A recombinant plasmid, pPharoA1, complemented growth of the E. coli auxotroph AB2829. A 2.2 kb HindIII-ClaI fragment when ligated into the HindIII-Accl sites of pBluescript resulted in pPharoA2, which also complemented growth. The latter insert was sequenced by the dideoxy method and transferred into pBC SK+ (Stratagene) resulting in pPharoA3.

Construction of hybrid replacement plasmid: A 2.2 kb fragment, the product of partial Sau3A digestion of pD80, containing amp' was ligated into pBC SK+. This plasmid imparted ampicillin-resistance to E. coli up to 200 μg / ml. The 2.2 kb amp' fragment was excised, made blunt, and ligated into the unique Ndel site, made blunt, of aroA in pPharoA3 to form pPharoA-amp'. To produce a hybrid plasmid, pPharoA-amp' was digested with HindIII and ligated to HindIII-digested pD70 (4.2 kb streptomycin-resistance plasmid of P. haemolytica) to generate pPharoA-amp'pD70.

Production and characterization of P. haemolytica aroA mutant: Plasmid pPharoA-amp'pD70 was electroporated into E. coli PhalMtase and then recovered by CsCl gradient purification. One μg plasmid was mixed with 100 μl of electroporatable P. haemolytica strain NADC-D60 in a 0.1 cm cuvette and immediately electroporated. Eight amp' transformants were recovered on medium containing 10 μg / ml ampicillin. These colonies
PASTEUR&LA HAEMOBYPICA SEROTYPE 1 AND CONSTRUCTION OF A GENE-REPLACEMENT AROA MUTANT

were gassed daily for 3 days into fresh medium containing 1 µg/ml ampicilln then plated onto medium containing 10 µg/ml ampicilln. The colonies were replica plated onto medium containing 10 µg/ml chloramphenicol, medium containing 50 µg/ml streptomycin, and defined medium for growth of P. haemolytica with and without tryptophan. Clones unable to grow without tryptophan were presumed to be aroA. Genomic DNAs isolated from colonies with amp', Cm', Sm', aroA- phenotypes were analyzed by Southern blotting and hybridization.

Results and Discussion

Isolation and characterization of Phal: Under our experimental conditions, endonuclease activity was eluted from heparin-sepharose columns by 275 to 325 mM NaCl. A single pass through these columns was sufficient to allow identification of both the DNA recognition specificity and cleavage site. Results of these studies indicated Phal is an isoschizomer of SfaN1, a type Ills enzyme isolated from Streptococcus faecalis. The type Ills restriction enzymes, like the more common type II enzymes, recognize specific sequences and cleave at predetermined sites. Type Ills enzymes, however, do not recognize palindromic sequences nor cleave internally to the recognition sequence.

3'...GCATCNNNNN \downarrow NN...3'
3'...CGTAGNNNNN \uparrow NN...5'

Cloning of Phal methyltransferase: After digestion with Phal and transformation of AP1-200-9 E. coli, 15 cosmid clones of P. haemolytica genomic DNA were tested for endonuclease activity. The nine clones which were endonuclease-positive were tested for Phal methyltransferase activity. All nine expressed methyltransferase activity in addition to endonuclease activity, as evidenced by resistance to digestion by Phal of genomic DNA recovered from transformed E. coli. The selective recovery of clones containing methyltransferase was due to digestion of the cosmid library with Phal prior to transformation of E. coli.

Transformation of P. haemolytica: Hybrid plasmid pHaarACm'pD80 passed through E. coli containing Phal methylase on cosmid was able to transform P. haemolytica serotype 1. Plasmid pHaroACm', probably because it contains only a ColE1 ori, was unable to transform P. haemolytica serotype 1. The hybrid plasmid was stably maintained through multiple passages under selective pressure. Whereas DNA not exposed to Phal methylase did not transform P. haemolytica in this experiment, DNA modified by Phal methylase yielded 10² transformants per µg plasmid (Table 1). Plasmid DNA passed through P. haemolytica yielded 10⁶ transformants per µg plasmid. Plating efficiency was approximately 100-fold higher using ampicillin selection than with chloramphenicol selection. Less than 10¹ transformants per µg plasmid were recovered under chloramphenicol se-
lection using DNA passed through *E. coli* containing *Phal* methylase. All transformants recovered, however, were Amp' and Cm' upon passage.

**Nucleotide sequence and analysis of *P. haemolytica* aroA:** Sequence analysis on *P. haemolytica* aroA revealed an open reading frame of 1302 bases with a coding capacity of 434 amino acid residues. The deduced molecular weight is 47,296 and the G+C content of the aroA coding region is 43%. The predicted amino acid sequence of *P. haemolytica* aroA showed 75, 70, 69, and 68% identity with *P. multocida*, *K. pneumoniae*, *Y. enterococolitica*, and *E. coli* respectively.

**Inactivation of aroA with *P. haemolytica* ampicillin-resistance gene:** A series of replacement plasmids were constructed, each considered an improvement on the previous one which failed to produce an aroA mutant. The successful replacement plasmid contained the *P. haemolytica* ampicillin-resistance cassette from plasmid pD80. This 2.2 kb Sau3A fragment was cloned into pBC SK, passed through *Phal*Mtase, and electroporated into *P. haemolytica*. Since the methylated plasmid did not confer ampicillin-resistance to *P. haemolytica*, we concluded that it did not contain the pD80 origin of replication. The 2.2 kb amp' fragment was used to insertionally inactivate aroA upon introduction into the unique Ndel site of aroA. The resulting plasmid, after passage through *Phal*Mtase, failed to generate ampicillin-resistant colonies of *P. haemolytica*.

**Construction of hybrid replacement-plasmid:** To increase the likelihood of selecting an aroA mutant arising by homologous recombination between inactivated aroA of the deletion plasmid and the wild-type gene of the *P. haemolytica* chromosome, we constructed a hybrid plasmid. This hybrid construct joined the 4.2 kb *P. haemolytica* plasmid encoding streptomycin-resistance (pD70) with the replacement plasmid above. Because pD70 is not entirely stable in *P. haemolytica*, we hypothesized that the hybrid plasmid pPharoA-amp'pD70 should eventually give rise to plasmidless amp' mutants arising via homologous recombination. The hybrid plasmid, after passage through *Phal*Mtase, gave rise to 8 amp' *P. haemolytica* colonies following electroporation. Southern blot analysis of DNA preparations from these colonies indicated intact hybrid plasmid was present in each transformant.

**Selection and characterization of an aroA mutant of *P. haemolytica*:** After daily passage for approximately 100 generations, a single colony was selected from each of the 8 above cultures. Two of the 8 were found to have amp', Cm', Sm' phenotypes. Further, the same 2 colonies failed to grow on defined medium which lacked tryptophan, but grew with supplemental tryptophan (as did the *E. coli* auxotroph AB2829). The parent isolate grew on both media. Southern blotting indicated that both *P. haemolytica* colonies contained insertionally inactivated aroAs. Moreover, Southern blotting confirmed that both pD70 and pBC SK sequences were no longer present in the aroA mutants.
Summary

1) *Pasteurella haemolytica* serotype 1 was found to contain a type IIs restriction endonuclease and modification system, *Phal*.

2) In our hands, protection of DNA against the restriction-modification system is a critical step prior to introduction of exogenous DNA into *P. haemolytica* serotype 1.

3) The experimental approach that successfully produced an *aroA* mutant of *P. haemolytica* required repeated passage of the bacterium transformed with a hybrid plasmid.

4) Although the hybrid plasmid replicated in *P. haemolytica*, improving the chance for recombination, its instability allowed recovery of a plasmidless *aroA* mutant.

Table 1. Transformation efficiency of *P. haemolytica* NADC-D60 with hybrid plasmid pPhÃÁAroACm'-pD80 purified from various sources

<table>
<thead>
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<th>Source of DNA</th>
<th>Amp' transformants(\times 10^6)</th>
<th>Cm' transformants(\times 10^5)</th>
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<td><em>E. coli</em> DH10B</td>
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<td>nd*</td>
</tr>
<tr>
<td><em>E. coli</em> PhalMtase</td>
<td>(1\times 10^3)</td>
<td>5</td>
</tr>
<tr>
<td><em>E. coli</em> GM2163</td>
<td>(5\times 10^2)</td>
<td>nd</td>
</tr>
<tr>
<td><em>P. haemolytica</em> NADC-D60</td>
<td>(1\times 10^5)</td>
<td>nd</td>
</tr>
</tbody>
</table>

*One µg DNA introduced by electroporation using same competent cell preparation.

Purified by CsCl-ethidium bromide gradient centrifugation.

\(\times\) CFU/µg DNA, on plates containing 10 µg/ml ampicillin, cells recovered 2 hours prior to plating.

\(\times\) CFU/µg DNA, on plates containing 2 µg/ml chloramphenicol, cells recovered 1 hour prior to plating.

*Not done.

References


PROGRESS TOWARD RECOMBINANT ANTIGEN-BASED SEROLOGIC DETECTION OF HORSES INFECTED WITH BABESIA EQUI AND BABESIA CABALLI

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Summary

Recombinant antigens are ideally suited for use in the competitive inhibition ELISA (CI-ELISA) format (1) since the specificity of the CI-ELISA depends solely on the monoclonal antibody (mAb) used. A mAb to an immunodominant 34 kDa Babesia equi merozoite surface protein was derived and used to molecularly clone and express the corresponding recombinant protein. This recombinant protein was applied in the CI-ELISA format and used to serologically detect horses infected with B. equi worldwide. Sera from horses experimentally and naturally infected with Babesia caballi were shown to consistently recognize gradient purified B. caballi merozoite associated proteins with molecular masses of 110, 98, 70, 46 and 26 kDa. Thus far, the 98 kDa protein has elicited the highest antibody titer in sera from B. caballi infected horses tested by immunoblot.

Babesia equi and Babesia caballi are hemoprotozoan, tick-transmitted parasites of horses (2,3). Infection of horses with B. equi or B. caballi is a problem for the importation and exportation of horses. Many countries, including the United States, do not allow importation of infected horses (4). In 1969, the U.S. Department of Agriculture adopted the complement fixation test (CFT) (5-7) as the official test to identify horses infected with B. equi and B. caballi. Limitations associated with the CFT are that (i) sera with anticomplement activity are not testable by the CFT; (ii) sera which react with CFT control erythrocyte antigen cannot be evaluated by the CFT, and (iii) sera containing specific immunoglobulin G(T) [IgG(T)]g antibody may yield false-negative results because IgG(T) does not fix complement by the classical pathway (8). Also, until recently it has not been possible to continuously cultivate B. equi or B. caballi in vitro, and antigen for the CFT had to be produced by the infection of splenectomized horses. However, the in vitro cultivation of both B. equi and B. caballi have been described (9,10).
Recombinant Antigen Based Serologic Detection

In an effort to develop an improved serologic test for the detection of equine anti-\textit{B. equi} antibodies, the antibody response of infected horses to \textit{B. equi} merozoite surface proteins was initiated (11). A monoclonal antibody (mAb), that bound specifically to a merozoite surface protein epitope was derived (11) and the corresponding protein molecularly cloned and expressed (12). The \textit{B. equi} specific mAb and recombinant protein were applied in a competitive inhibition ELISA format and tested for their ability to detect equine anti-\textit{B. equi} antibody in the sera from infected horses worldwide (12,13). A 94% concordance was found when the results of the CI-ELISA were compared with those of the CFT. The CI-ELISA is specific for anti-\textit{B. equi} antibody in that sera CFT positive for \textit{B. caballi} and CFT negative for \textit{B. equi} was also negative for \textit{B. equi} by the CI-ELISA (13). Sera yielding discrepant results between the CFT and CI-ELISA were retested by immunoprecipitation of $^{35}$S-methionine labeled in vitro translation products of \textit{B. equi} mRNA. The results clearly showed those sera that were CFT negative and CI-ELISA positive to be positive by immunoprecipitation (13).

Sera from horses experimentally and naturally infected with \textit{Babesia caballi} were shown to consistently recognize gradient purified \textit{B. caballi} merozoite associated proteins with molecular masses of 110, 98, 70, 46 and 26 kDa. These results are similar to those reported using sera of horses infected with \textit{B. caballi} from Brazil (14). Thus far, the 98 kDa protein has elicited the highest antibody titer in sera from \textit{B. caballi} infected horses tested by immunoblot. Monoclonal antibodies are currently being produced to these proteins.

References

MECHANISMA OF ABDOTION IN BRUCELLA ABORTUS INFECTED CATTLE

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Bovine Brucellosis was originally called bovine contagious abortion. Even prior to Bang’s discovery of the bacteria responsible for this condition, the primary symptom of the disease, late term abortions, was well established. Despite over 100 years of brucellosis research, the mechanisms responsible for abortions have not been adequately explained. Early Brucellosis researchers recognized that sexual maturity represented an important factor in host susceptibility to infection. They recognized that sexually immature calves were resistant to infection with virulent B. abortus and that pregnant cattle were most susceptible to infection. Early efforts at prevention of the disease by vaccination called for exposing non-bred heifers to the virulent bacteria to prevent abortions if the cattle were later exposed to bacteria as bred heifers¹.

Susceptibility has been found to be dependent upon sex and age. It is generally accepted that sexually immature calves are less susceptible to brucella infection than sexually mature males. Sexually mature males are less susceptible to infection than sexually mature females which are less susceptible than pregnant cows. Further, cattle less than 157 days of gestation are less susceptible than cattle over 157 days of gestation². Interestingly, aged cattle which no longer cycle become resistant to experimental brucella infection. Collectively these observations suggest that reproductive hormones must play some role in the susceptibility of cattle to brucella infection. Despite this overwhelming circumstantial evidence for the influence of hormones on susceptibility to the infection, few studies have attempted to investigate the role of hormones in this disease. There are two reasons for the general belief that hormones play an insignificant role in the susceptibility of cattle to this infection. First, in 1960 prolonged treatment of virginal heifers with progesterone failed to make those animals susceptible to infection⁹. The second reason is related to the discovery of the role erythritol plays in the localization of brucella in the pregnant uterus. This four carbon polyol was found in high concentrations in the pregnant uterus and was shown to stimulate the growth of B. abortus, B. melitensis, and B. suis. The unrestricted growth of Brucellae in placental tissues and the fetus was thought to result in endotoxic shock and/or fetal death and result in abortions¹⁰,¹¹,¹².
Several factors concerned with the nutritive requirements of brucella and with the pathogenesis of fetal infections with these bacteria have raised unanswered questions concerning the role of erythritol in the disease and role of endotoxin in the abortion process. *B. ovis* infects sheep and has been demonstrated to cause placental and fetal lesions and abortions. This Brucella species differs from other members of the genus for it is incapable of utilizing erythritol. Strain 19, the attenuated *B. abortus* biotype 1 used as a vaccine for prevention of Bovine Brucellosis, is inhibited by the presence of erythritol. Strain 19 lacks the enzyme D-erythrulose 1-phosphate dehydrogenase which is thought to result in the build up of D-erythrulose 1-phosphate, an intermediate in the catabolism of erythritol, which may be toxic for the bacteria. Strain 19, however, administered to pregnant cattle will infect the pregnant uterus and result in abortions. Several studies conducted within the last eleven years have clearly demonstrated that *B. abortus* preferentially replicates within placental trophoblastic cells of infected mice despite the absence of erythritol in these tissues. The bacteria replicate within the rough endoplasmic reticulum (RER) of placental trophoblastic cells just as they replicate within the chorionic trophoblasts of ruminants.

The pattern of abortion or premature delivery of brucella-infected fetuses is somewhat unique. Typically, cattle, sheep, goats and sows experimentally infected during the last half of gestation deliver near-term viable, infected fetuses. The initiation of parturition and the stages of parturition differs little in these infected animals from that which occurs in normal parturition. Frequently, it is suggested that placental damage due to inflammation associated with Brucella infections results in placental insufficiency and is responsible for death of the fetus and its delivery. Placental vascular damage and fetal death are assumed to be due to brucella endotoxin. Few experimental studies have been conducted in which brucella lipopolysaccharide (LPS) and/or endotoxins have been evaluated as an abortifacient in cattle. Such experiments have resulted in the rapid expulsion within 3 to 5 days of dead fetuses. Fetal death and the resulting abortions in these cases differ little from the patterns of fetal death and abortions caused by experimental inoculation of pregnant cattle with LPS from other gram negative bacteria. While there is little doubt that brucella LPS may in some cases result in rapid delivery of dead fetuses, such processes are very different from the usual pattern of abortions in a majority of natural and experimental infections of pregnant cattle with *B. abortus*.

Experimental infection of bovine fetuses with *B. abortus* generally results in prolonged infections lasting an average of 12 days prior to delivery. The infected fetuses develop active inflammatory responses; many mount immune responses to the bacteria; and most demonstrate adrenal cortical hyperplasia and elevated cortisol levels. Following in utero *B. abortus* infection, fetuses demonstrate the typical stress response observed in mature cattle. There is a fetal leukocytosis with a neutrophilia and monocytosis.
accompanied by lymphopenia and eosinopenia. Similar results observed in experimentally infected sheep and goats’ fetuses led to the conclusion that fetal stress played a role in the initiation of premature parturition of infected fetuses. The fetal stress theory as a cause for premature delivery of brucella-infected fetuses was, however, not supported by in utero brucella infections of fetal goats using a stable rough mutant of \( B.\ abortus \) strain 2308. In these studies, the rough mutant replicated in fetal lung tissues and in placental trophoblasts. Fetal inflammation was typical of that observed with the smooth parent strain of \( B.\ abortus \); however, infected fetuses were carried to term and delivered alive. Accidental double exposure of a pregnant cow prior to 150 days of gestation with this mutant also resulted in the term delivery of an infected calf. At the time we considered the absence of smooth LPS associated with this mutant to be responsible for the failure to induce abortions in both infected goat and bovine fetuses. The inability to cause abortions in the obviously stressed fetuses indicated to us that our understanding of the whole process of abortion was deficient.

We initially chose to examine the replication of various strains of \( B.\ abortus \) in explanted chorioallantoic membranes (CAM) obtained from cattle averaging from 180 to 210 days of gestation. Our assumptions were that the virulent smooth strain 2308 \( B.\ abortus \) would preferentially infect the CAM trophoblasts and replicate at an accelerated rate and that the replication rate of the attenuated smooth strain 19 \( B.\ abortus \) and the attenuated rough strain RB51 \( B.\ abortus \) would be depressed in these cells. Interestingly, it was noted that the rates of infection and replication were the same for all three strains. Further, the cytotoxic effects of \( B.\ abortus \) strain 2308 infection on CAM trophoblast cells were approximately twice that observed with strain 19 and strain RB51 infected explants. This study also suggested that factors other than erythritol may be responsible for the replication of \( B.\ abortus \). Strain 19 replicated at a rate equal to the other two strains which utilize erythritol. Both the stock strain 19 used to infect these explants and the strain 19 recovered from the explants following 24 hours of culture were inhibited by erythritol. A filtered lysate of heat-killed strain 2308 was demonstrated to contain large amounts of \( B.\ abortus \) LPS by the limulus amebocyte lysate assay. This heat-killed extract caused minimal cytotoxic effects on the CAM explants. Further, equal degrees of toxicity were observed in CAM explants infected with strain 19 and strain RB51. Strain 19 is a rich source of smooth LPS while Strain RB51 possesses only rough LPS.

These \textit{in vitro} studies and \textit{in vivo} studies on the pathogenesis of \( B.\ abortus \) in ruminants and laboratory animals generated more unresolved questions concerning the pathobiology of brucella infections. Why are pregnant cattle more susceptible? Why do abortions occur after midgestation? What pathophysiological events are responsible for the premature delivery
of brucella infected fetuses?

While conducting the in vitro studies outlined above, CAM explants derived from 90 to 100 day pregnancies were infected with B. abortus. Bacterial infection rates of these early pregnancy cases were similar to that noted in midgestation and late gestation derived CAM explants. However, bacterial replication over a 24 hour to 48 hour period was significantly depressed in 90 day CAM explants.

The studies in explants also demonstrated that following infection with B. abortus, profound changes were induced in the ability of the explanted tissues to produce various steroidal hormones and prostanoids. Both light microscopic and ultrastructural studies clearly demonstrated that the brucella were infecting trophoblast cells of the extraplacentomal chorioallantoic membrane.

Further studies were initiated using bovine trophoblastic cell lines derived from the trophoblastic vesicles of an 11 day gestation bovine embryo, from the placentomal tissues in the fifth month of gestation, and from extraplacentomal CAM from the eighth month of gestation.

Materials and Methods

Cell lines - Each of the three cell lines was passaged, grown, and maintained in HAM F-12 and DMEM media supplemented with 10% fetal bovine serum, and various growth factors. Equal numbers of cells were present at the time of infection and thereafter during the experiments. The cell cultures were infected with 1.0 x 10⁶ CFU of B. abortus strain 2308 in DMEM. The ratio of bacteria to trophoblastic cells was 100-200:1. The bacteria were incubated with the cells for 10 hours at 37°C in 5% CO₂. Following the incubation, the monolayers were washed twice with DMEM and incubated for one hour with complete media containing 40 μg/ml of gentamicin sulfate. Media containing the gentamicin sulfate was removed and replaced with complete media without the antibiotic. Zero time in these studies refers to the time at which the gentamicin containing media was removed and replaced with fresh media. Cultures were then allowed to incubate as above for 4, 8, 16, 20, 24 and 30 hrs. At each of these time points, the media was removed from the wells; and the monolayers were washed twice with DMEM and lysed with 0.1% deoxycholate acid in water for 15 minutes. The lysates were then diluted and plated for determination of colony forming units of bacteria. Paired, similarly treated cells were stained with trypan blue dye to determine cell numbers and percent viability following infection with B. abortus. Non-infected cells were treated as above and served as controls for culture conditions, washes and antibiotic treatment.

Bacterial Strains - The virulent strain 2308 B. abortus was used in these studies. Smooth LPS fraction 5 of B. abortus was supplied by Dr. Alex
MECHANISMA OF ABORTION IN BRUCELLA ABORTUS INFECTED CATTLE

Winter. Crude bacterial endotoxin was prepared from a log phase growth of strain 2308 B. abortus grown for 24 hours at 37°C in DMEM with 10% FBS. The growth suspension was centrifuged at 10,000 X g for 20 minutes and the supernatant collected and filtered through a 0.2 µm filter. The filtrate was aliquoted and stored at minus 70°C. Both fraction 5 and the crude endotoxin filtrate were assayed using a chromogenic limulus amebocyte lysate test kit. Both demonstrated endotoxin activity 3 to 4 times that of the standard endotoxin supplied in the kit as a positive control.

Hormone and Prostanoid Assay: Prostanoids, PGF₂α, and 5'HETE concentrations in non-infected and infected trophoblastic cell lines were determined using a commercial radioimmunoassay (RIA) kit. Steroidal hormone (progesterone, estrogens, and cortisol) concentrations in non-infected and infected trophoblastic cell lines were also measured using RIA procedures. The trophoblastic cell lines were cultured as above and infected as previously described. For prostanoid assays, 24 hours following attachment, complete culture medium was discarded; and after a rinse with DMEM without serum, the cells were maintained in media without serum. In each experiment monolayer cultures were inoculated with either 1.0 x 10⁸ CFU B. abortus strain 2308 or with B. abortus LPS fraction 5 in DMEM.

Erythritol Determinations: Chorioallantoic membranes were collected aseptically from 3, 5, and 8 months normal pregnant cattle at slaughter. Between 1 to 2 gm of tissue were weighed and frozen at minus 20°C until assayed. Monolayers of trophoblastic cells were resuspended and washed in DMEM and counted. The cell numbers for each of these cell lines were adjusted to contain 3.0 x 10⁶ cells. The cells were then submitted for assay as suspension cultures or as centrifuged cell pellets. Erythritol was extracted from both CAMs and culture cells using a C-18 matrix solid-phase dispersion procedure and fractionated with various solvents. Solvent eluates were then derivatized with BSTFA and subjected to gas chromatography/ spectrometry analysis. All runs were compared to a 1 µg erythritol standard. The concentrations of erythritol in tissues and/or cells were determined by the ratio of the areas of the erythritol standard to the area of the erythritol peak for test samples and reported as µg of erythritol.

Results

The intracellular replication rates of B. abortus strain 2308 were determined in three trophoblastic cell lines by comparing the bacterial number recovered from the various cell lines at 4, 8, 16, 24, and 30 hours following the removal of extracellular B. abortus. Viability of the cells in each of the three lines remained high; greater than 95% of the infected cells, the cells treated with B. abortus fraction 5 and non-treated control cells excluded trypan blue dye at the conclusion of the experiment. Initially all 3 cell types were infected with equal numbers of brucella. By 12 hours significant growth
of bacteria occurred in cell lines derived from placentomal and extraplacentomal tissues at mid and late gestational ages. At each interval after 12 hours, significantly more bacteria were present in the cell line derived from the 8 months of gestation CAM than from the line derived from the fifth month of gestation. Significant bacterial growth failed to occur in the embryonal cell line. At the conclusion of the experiment, microscopic examination of the cell lines inoculated with *B. abortus* demonstrated that 6% of the embryonal cell line were infected while 34% and 40% of midgestation and late gestation derived cell lines were infected with the bacteria.

All three cell lines produced low levels of PGF$_2\alpha$ during culture. *B. abortus* LPS failed to stimulate significant increases in PGF$_2\alpha$ production by any of the three cell lines. Following *B. abortus* infection peak PGF$_2\alpha$ production occurred in the cell line derived from the 8 month gestational CAM at 16 hours. The three fold increase of PGF$_2\alpha$ production occurring at this time was significantly higher than the PGF$_2\alpha$ production occurring in the other two cell lines. All non-infected cell lines produced high levels of progesterone initially. The levels of progesterone declined over the 20 hour culture interval at equal rates in the three cell lines. Following infection progesterone production in the embryonal cell line dropped precipitously at 4 hours. The level of progesterone in the other two cell lines dropped at a slower rate and were not significantly different from one another.

Non-infected cells in all three lines produced very low levels of cortisol throughout the course of the study. Increased cortisol production occurred in the brucella-infected late gestational cell line within 4 hours. Significantly increased cortisol production in the midgestational cell line was not observed until 16 hours. At 16 and 24 hours, the late gestational line produced more cortisol than the midgestation line. The embryonal line failed to produce significant increases in cortisol following infection.

All non-infected cell lines produced estrogens during the culture period. The level of estrogen production gradually increased in these cells. No differences between non-infected cells related to estrogen production were noted. Following infection with *B. abortus*, all three cell lines increased the level of estrogens produced; however, the largest increases were noted in the midgestation and late gestation cell lines. At 16 and 24 hours the late gestational cell line produced significantly more estrogen than the midgestational cell line.

Erythritol was detected in CAMs derived from 3, 5 and 8 months of gestation. The highest concentration of erythritol was noted at midgestation. Owing to the small sample size, the significance of this difference is not known. As expected, each of the three cell lines also produced erythritol; and as observed in the CAMs, the midgestational cell line produced larger quantities of this compound.
Discussion and Conclusions

With reference to bacterial replication, the experiments conducted with trophoblastic cell lines demonstrated that preferential replication of *B. abortus* occurred in mid and late gestational cells. This same pattern of replication of the bacteria was observed in CAMs derived from extraplacentomal tissues at 3, 5, and 8 months of gestation. The growth of brucella in trophoblasts appears to be independent of erythritol concentrations in these cells. Peak erythritol concentrations were noted in cells and/or tissues derived from the middle stage of gestation, yet brucella replication is consistently greater in cells and tissues derived from the late stage of gestation.

Trophoblastic cells of the placenta are known to be among the most metabolically active cells in the body. These cells vary their production of proteins and steroids throughout gestation and play an important role in fetal development and in the maintenance of pregnancy in the cow.7 This study clearly indicates that the intracellular growth of brucella in these cells is associated with changes in the baseline production of prostanoids, progesterone and estrogens. Interestingly, trophoblastic cells infected with brucella produce cortisol, a steroidal hormone not normally produced by the placenta. The increased levels of PGF2α and decreased production of progesterone coupled with increased production of estrogens and cortisol in *B. abortus* infected trophoblasts derived from placental tissues at mid and late stages of gestation are identical to the changes in these substances which occur at term with the initiation of parturition in normal cattle.

The molecular events associated with the preferential growth of *B. abortus* in mid and late gestational trophoblasts are not known. Preliminary results suggest that estrogen stimulates the growth of these bacteria. It is possible that both estrogens and erythritol are responsible for stimulating *B. abortus* replication in ruminants. Interestingly, blood levels of estrogens are increased in the pregnant cow at the same time uterine localization of *B. abortus* occurs. Erythritol is not present or is at extremely low concentrations in the nonreproductive tissues of the cow. Increased estrogens levels may be responsible for increasing *B. abortus* numbers in these tissues and increasing the likelihood of the bacteria reaching the pregnant uterus. Washko's experiments in the early 1950s suggested that estrogens may be important in allowing the persistence of *Brucella abortus* in cattle 19.

References


REPORT OF THE COMMITTEE ON BRUCELLOSIS

Chairman: Dr. J. Lee Alley, Montgomery, AL
Vice Chairman: Dr. C. E. Barton, Nashville, TN

Dr. L. Garry Adams, TX; Mr. John Adams, VA; Mr. John B. Armstrong, TX; Dr. Terry L. Beals, TX; Dr. Paul Becton, FL; Dr. Valerie E. Benson, FL; Mr. Neal Black, MN; Mr. Philip E. Bradshaw, IL; Mr. John S. Cargile, TX; Dr. Max E. Coats, TX; Dr. Thomas Dees, GA; Dr. B. L. Deyoe, IA; Dr. John C. Doyle, OK; Dr. Steven R. England, NM; Dr. Fred M. Enright, LA; Dr. Brian H. Espe, OK; Dr. G. H. Frye, MD; Dr. Michael J. Gilsdorf, MD; Mr. Francis D. Gregerson, CO; Mr. Ted Hickerson, TX; Dr. Bob R. Hillman, ID; Mr. James E. Horne, OK; Mr. Majon Huff, CO; Dr. Fred S. Idtse, WI; Mr. Jon Johnson, TX; Mr. Denis Joyce, ND; Mr. Alfred W. Keating, IL; Dr. John D. Kopec, MD; Dr. Beth Lautner, IA; Dr. Maxwell Lea, Jr., LA; Mr. Larry D. Mark, VA; Dr. Bret D. Marsh, IN; Dr. Charles Massengill, MO; Dr. Andrea Mikolon, CA; Dr. Harry F. Moberly, Jr., IL; Mr. Richard E. Nelson, VT; Dr. Tomas A. Neuzil, IA; Dr. Donald L. Notter, KY; Dr. Roger J. Odenweller, KY; Mr. J. O. Pearce, Jr., FL; Dr. James P. Quigley, GA; Dr. Frank Y. Rogers, MS; Dr. Robert B. Sanders, AR; Dr. John C. Sawyer, CA; Dr. John Schiltz, IA; Dr. Roy A. Schultz, IA; Dr. Gerhardt Schurig, VA; Mrs. Sherry Seubert, WI; Dr. Clarence J. Siroky, MT; Mr. Glenn Slack, KY; Dr. Barrett D. Slenning, NC; Mr. Walter E. Stemler, IL; Dr. Arnold C. Taft, MD; Dr. Lewis P. Thomas, WV; Dr. E. Tom Thorne, WY; Dr. Daryl K. Thorpe, SD; Dr. Kenneth J. Throlson, CO; Dr. Robert J. Velure, ND; Dr. Gary M. Weber, DC; Mr. Raleigh Wilkerson, AL; Dr. Richard D. Willer, AZ; Dr. Larry L. Williams, NE; Dr. Ernest W. Zirkle, NJ.

The Brucellosis Committee met on October 31, and November 1, 1994. Listed below are the presentations given and the actions taken by the committee.

Dr. Donald Luchsinger, Deputy Administrator, APHIS, VS, gave a brief discussion of the plan to complete the eradication of bovine brucellosis by December 31, 1998. He commended the group on the progress that has been made up to this time. In his discussion he included fiscal support from Congress for the brucellosis program and specific improvements to strengthen the program so that eradication can be completed by 1998.

Dr. Granville Frye, APHIS, VS, presented the committee with the current status report of the National Brucellosis Program. He reported that there were only 172 Brucellosis infected herds at the end of August 1994.

Dr. Bob Hillman, Idaho State Veterinarian, gave a report on the brucellosis situation in bison and elk in the Greater Yellowstone Area. He reported on the establishment of the Greater Yellowstone Interagency Brucellosis Committee (GYIBC) at a meeting of involved federal agencies.
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held at Bozeman, Montana, on January 10-12, 1994. The first major objective of the GYIBC was to conduct a national symposium on Brucellosis in the Greater Yellowstone Area. This symposium was held in Jackson, Wyoming, September 26-27, 1994, with 216 registered participants in attendance. There were 34 presentations relating to the issues involved given at the symposium.

Mr. John Lawrence, IDEXX Laboratories, presented data on the HerdChek Brucellosis Milk ELISA Test that compared it to the Brucellosis Ring Test. Mr. Lawrence made the recommendation that it be accepted as an official test.

Dr. Fred Enright, brucellosis researcher from LSU, gave a paper on research trials on the use of the mutant B. abortus strain RB51 as a possible alternate to strain 19. Early results are promising that RB51 may produce very serviceable immunity without the complicating antibody production that often results with strain 19 vaccination.

Dr. Garry Adams, brucellosis researcher from Texas A&M University, reported on research results using several different killed and live mutant brucella vaccines in cattle. Two of these vaccines gave promising results in the research phase, but need further field evaluation to determine their potential value as vaccines.

Mr. Robert Dieterich, California, presented data on the validation of brucellosis serologic tests in reindeer.

Dr. Julia Bevins, Fairbanks, Alaska, reported on the reindeer brucellosis symposium held in Anchorage, Alaska, in July 1994. The following research and management priorities were identified: to improve the existing vaccine with a new adjuvant; to validate serologic tests for B. suis biovar 4 in reindeer; to research vaccination procedures that would allow improved animal ID; to establish the host range for B. suis biovar 4; to develop assays for natural immunity; to use DNA fingerprinting to assess cross-transmission of brucellosis from caribou to reindeer; and to assess venereal transmission.

Dr. Paul Becton, Tallahassee, Florida, and Dr. Terry Beals, Austin, Texas, reported on field procedures that had been implemented in the States of Florida and Texas to hasten the eradication of brucellosis and shorten the period required to move from Class A to Class Free.

Dr. Larry Williams, Lincoln, Nebraska, reported on problems encountered in his state during the countdown period in achieving Brucellosis Free Status.

Dr. John Kopec, APHIS, VS, reported on brucellosis in Llamas. In a limited study of 11 animals he reported that the standard plate test, the BAPA test, the card test, and the rivanol test appeared accurate in Llamas. Since 48 percent of the samples were anti-complimentary the complement fixation test was not recommended.

Dr. Donald Davis, brucellosis researcher, Texas A&M University, gave
a summary of findings on various diagnostic tests in bison and a report of a limited study on an oral vaccine made from *B. neotomae* in bison. He stated that all the serologic tests used in cattle appear to be satisfactory in bison, but indicated that a battery of tests should be used rather than a single test. He further stated that strain 19 vaccination was not as effective as that observed in cattle. In one study of pregnant bison vaccinated with strain 19, approximately $\frac{2}{3}$ aborted. Therefore, vaccination of pregnant bison with strain 19 is not recommended.

Dr. Charles Massengill, Jefferson City, Missouri, presented data from Texas and Missouri on the use of the card test in livestock markets. He requested the committee to consider changing the card test from an official test to a presumptive test because of the number of animals detected at livestock markets disclosing card test positive titers that have negative results to supplemental tests. This request was referred to the Scientific Advisory Subcommittee.

Dr. John Kopec, APHIS, VS, presented the proposed Uniform Methods and Rules (UM&R) for Cervidae. There was considerable discussion especially on the matters of indemnity, movement of animals, and validation of serologic tests. The proposed UM&R was not accepted and the committee requested that USDA work further with industry in developing a more workable document.

Dr. Francisco Gurria, Mexico, gave a brief report on the status of brucellosis in that country. Since January 24, 1994, the Mexican government has initiated brucellosis eradication efforts primarily involving testing and vaccination. Two hundred and forty Mexican veterinarians are employed to conduct the eradication efforts.

Dr. Jim Alexander, Austin Texas, reported on the use of the *Brucella abortus* field ELISA test in a large brazilian beef herd. He presented the cost benefits of using this test in conjunction with infected herd management. He stated that the value of this test appears to be in its ability to detect antibody titers earlier than other serologic tests.

USDA, APHIS, VS, presented a draft of their policy position concerning the use of calfhood vaccination in the future. The key elements in the draft are as follows:

1. Encourage calfhood vaccination in herds and areas where there is a high risk of exposure to infection.
2. Encourage States to rescind laws and regulations that mandate vaccination, such as those requiring vaccination for importation or sale.
3. Educate herd owners and veterinary practitioners regarding vaccination so their decisions on its use will reflect the advantages, disadvantages, and appropriateness in the herd under consideration.
4. Not encourage routine calfhood vaccination in states and areas that are classified as free of brucellosis.
5. Eliminate federal funding for the purchase and application of brucella vaccine, with the exception being infected or designated high risk herds.
6. Emphasize the importance of proper calfhood vaccination as related to age, dosage, identification, and reporting.

Report of the Swine Brucellosis Subcommittee
The subcommittee met on October 30, 1994, with eight members of the subcommittee and 30 guest attending the meeting.

Dr. Joe Annelli, APHIS, VS, reported that when the indemnity regulations were amended in March, providing for payment at fair market value for whole herds depopulation, there were 34 infected herds in the country. Two of those herds were out of business and two others were found to be negative, leaving 30 infected herds.

All have been depopulated except one, plus all newly infected herds, and as of October 28, 1994, there were only four known infected herds in the country, containing a total of about 300 animals. He said the trouble spots remaining include the Dallas-Fort Worth area, southern Arkansas, and south Florida. He said newly discovered infected herds are being depopulated within a few weeks.

State reports highlighted the types of complexes typically being found infected across the south—small herds with old cars used for housing and wooden pallets and bed springs used for fencing. These complexes are impossible to clean and disinfect and difficult to manage from a regulatory standpoint.

It was pointed out that the herd which remains infected in Florida feeds about half the enormous amount of food waste created at the Disney World complex. The owner has refused depopulation, but is setting up a new herd and gradually closing out the infected herd.

Dr. Maxwell Lea of Louisiana discussed a new development for disposing of carcasses in herd depopulation. Called air curtain incineration, the method blows air at a high velocity over a fire in a pit or a portable container, almost eliminating pollution from smoke or ash escaping from the fire. He said the system, developed in Florida, was tested in a herd depopulation in Louisiana and it took 20 to 25 minutes to burn a 250-lb. hog in the pit. He estimated that 15 to 20 hogs could be burned in an hour or less in the portable metal fire box that is an alternative to the pit. The committee urged additional studies on the system as a possible solution to problems with other methods of disposal of carcasses in herd depopulations.

Dr. Tom McGinn of North Carolina reported on the outbreak of brucellosis among workers at a packing plant in that state which played a part in stimulating the change in the program to provide for whole herd depopulation. During the two-year period 1991-'93, there were 30 additional cases of human brucellosis connected to the plant. During 1994, two additional cases
associated with the plant have been reported, one of which appears to have existed before the victim began working at the plant. He said the North Carolina Department of Labor has issued several citations and penalties, which are on hold pending court challenges.

Dr. Beth Lautner of the National Pork Producers Council encouraged risk-based slaughter surveillance to find infection, prompting a discussion on surveillance.

The subcommittee approved the following recommendation: That regional and state epidemiologists develop plans for surveillance in high-risk areas that will maximize surveillance efforts, with such plans to be implemented by the states this year.

Proposals presented by Dr. Arnold Taft, APHIS, VS, to revise the brucellosis UM&R were approved.

The subcommittee report was accepted.

Report of the Subcommittee on Brucellosis Education

The Subcommittee on Brucellosis Education met on October 31, 1994, with eight persons in attendance. Two items were discussed at length: (1) Production of a video which will stress the importance of finishing the task of complete brucellosis eradication, and (2) the need to develop better communication between designated epidemiologists in the various states.

The subcommittee recommends that a video of less than 20 minutes be produced which would be suitable for use at cattlemen meetings, Vo-Ag classes, livestock marketing associations, 4-H groups, and any other groups with an interest in brucellosis eradication. The focus on the economic aspects of the disease, the ramifications of failure to complete eradication, and the benefits to the industry which eradication will bring.

Because it is essential that this video be produced in a short period of time, it is requested that APHIS, LPA, explore contracting out the production if that will shorten the production time. One group that might be considered is LCI which has previous experience in such productions.

The second proposal is that VS develop a uniform method of information sharing among designated brucellosis epidemiologists. A monthly format was suggested. Information on newly affected herds would be routinely shared as well as any other problems, concerns, etc. It was agreed by the committee that this information sharing network should be a priority of the new brucellosis position which will report to the Deputy Administrator.

The subcommittee report was accepted.

Report of the Scientific Advisory Subcommittee

The subcommittee met on October 30-31, 1994, with four members in attendance. The following items were reviewed by the subcommittee.

1. IDEXX HerdChek Milk ELISA Test

The Brucellosis Scientific Advisory Committee has evaluated the
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Phase I, Phase II, and Phase III data sets submitted by IDEXX Laboratories to support adoption of the enzyme-linked immunosorbant assay-based HerdChek B. abortus antibody test kit as an official presumptive milk test for brucellosis equivalent to the Brucellosis Ring Test (BRT). The Brucellosis Scientific Advisory Subcommittee determined that the HerdChek Test was equivalent to the BRT in Phase I (Preliminary Comparison), Phase II (Double-Blind Critical Comparison) and the Phase III (Experimental Comparison) testing conditions. The Brucellosis Scientific Advisory Subcommittee recommends adoption of the HerdChek test as an official brucellosis milk test equivalent to the BRT.

2. Reduction of Minimum Age for Calfhood Vaccination to Two Months

After thoroughly searching the literature for data evaluating reduced dose strain 19 calfhood vaccination induced protective immunity in 2 month calves, no experimental data were found. Additionally, use of the former “high dose” 50-90 billion cfu strain 19 vaccine at lower ages produced the following results:

1-month-old calves - no perceptible protection
2-month-old calves - marginal, but sufficient protection
3-month-old calves - acceptable protection

Thus, these results indicate that reducing the age of vaccination to 2 months under field conditions even using the former “high dose” 50-90 billion cfu strain 19 vaccine would be very risky much less using the current reduced dose. Furthermore, there are no data supporting the concept that reduced doses protect more than higher doses of strain 19 vaccine in calves or adult cows.

Therefore, until the current strain 19 dose is determined to induce adequate protection against virulent challenge in 2-month-old calves in valid experiments, the Brucellosis Scientific Advisory Subcommittee cannot recommend adoption of its use in this manner.

3. Changing of the status of the card test in livestock markets from an official test to a presumptive test.

The Scientific Advisory Subcommittee recommends that no change be made in the official status of the card test until adequate data are accumulated, or developed to address the issue.

The three recommendations in the report of the Scientific Advisory Subcommittee were accepted and approved.

Dr. Jim Quigley, Georgia State Veterinarian, recommended that the age for official calfhood vaccination be reduced from 12 months to 8 months. The recommendation was not approved by the committee.

Dr. Bob Hillman, Idaho State Veterinarian, recommended that USDA, APHIS, VS, examine current data available for the serologic detection of Brucella suis biovar 4 in reindeer, using CFT, SPT, BBA, RIV, ELISA, PCFIA,
and mercaptoethanol tests, and that this evaluation, if it provides sufficient data, serve as the basis for designating official tests for the serologic detection of *Brucella suis* biovar 4 infection in reindeer and be used in the regulation of interstate movement of reindeer. The recommendation was approved.

Dr. Bret Marsh, Indiana State Veterinarian, recommended that a suspense status be created for Brucellosis Class Free states, similar to the procedure used in the tuberculosis program, when brucella infection is disclosed until such time as the source of infection is determined. The recommendation was approved.

Dr. Paul Becton, Florida Assistant State Veterinarian (retired), recommended that the following changes shown in bold be made in the Uniform Methods and Rules for brucellosis eradication.

Page 66. Item E. Calves From Affected Herds (Class Free Status)
1. The calves must be “S” branded neutered, or;
2. **Sexually intact** heifer calves must be quarantined and held separate and apart from the affected adult herd after weaning until they are negative on an official test no sooner than 30 days following completion of their first calving, or;
3. **Sexually intact** heifer calves may remain in the affected adult herd, but the entire herd shall not be released from quarantine until all such heifer calves have matured and calved, after which the entire herd must be tested negative for brucellosis no sooner than 30 days after the last heifer has calved to qualify for quarantine release.

Page 77 - Item E. Calves From Affected Herds (Class A Status)
Page 88 - Item E. Calves From Affected Herds (Class B Status)
Page 100 - Item E. Calves From Affected Herds (Class C Status)
Should read exactly as the item above on page 66.
This recommendation was approved.

Dr. Paul Becton, Florida Assistant State Veterinarian (retired), recommended that USDA, APHIS, VS, change CFR 78.8 (c), (1) as follows:

(i) delete
(ii) delete
(iii) **Sexually intact** heifers, vaccinated against brucellosis with an approved vaccine, held under quarantine separate and apart from the affected adult herd and negative to an official brucellosis test no sooner than 30 days after completion of their first calving, or;
(iii) **Sexually intact** bull calves, which will retain the status of the affected herd. Any movement will be in accordance with items (a) or (b) above, or they will be neutered and allowed to move unrestricted.

The recommendation was approved.

There were five resolutions forwarded from the Committee on Brucellosis to the Committee on Resolutions.

The Committee adjourned at 4:30 p.m.
REPORT ON THE NATIONAL SYMPOSIUM ON BRUCELLOSIS IN THE GREATER YELLOWSTONE AREA AND THE GREATER YELLOWSTONE INTERAGENCY BRUCELLOSIS COMMITTEE

Presented to the USAHA Committee on Brucellosis October 31, and November 1, 1994 by Bob Hillman, DVM

Last year Dr. Don Ferlicka reported to this committee on the Tri-State Interagency Brucellosis Task Force, the development of the Tri-State Interagency Brucellosis Committee and its Mission, Goal and Objectives. The Mission, Goal and Objectives are included at the end of this paper.

This report will briefly review these activities and bring you up to date on activities since the last USAHA meeting.

The Tri-State Interagency Brucellosis Committee was established in the summer of 1993 by the Governors of Wyoming, Idaho and Montana. The original committee was made up of representatives of animal health and fish and game agencies of the three states. These representatives developed a mission, goal and objectives that would fulfill the needs of the state agencies relative to brucellosis in wildlife in the Greater Yellowstone Area (GYA). GYIBC REPORT

Those persons present at this initial meeting felt strongly that efforts to find solutions to the brucellosis problem had to be initiated at the state rather than the national level since the states had to bear the consequences of the disease. The representatives also recognized that federal agencies had to participate if the problem was to be solved since much of the disease problem is in animals under federal control.

With these factors in mind a meeting was set for January 10 - 12, 1994 in Bozeman, Montana. All of the federal agencies (National Park Service, U.S. Fish and Wildlife Service, Forest Service, Bureau of Land Management, and Veterinary Services) that had jurisdictional authority over animals or lands in the GYA were invited to participate with the state animal health and fish and game representatives of the three states to address the problem of brucellosis in wildlife in the GYA.

At the Bozeman meeting the federal agencies accepted the mission, goal and objectives that had been developed by the state agencies, with a few modest changes in wording but not meaning. Those in attendance felt that the name of the committee should be changed to Greater Yellowstone Interagency Brucellosis Committee (GYIBC) to reflect the participation of the federal agencies. The participants at this meeting also developed a draft Memorandum of Agreement to be signed by the Governors of the three states and the Secretaries of the Department of Interior and the Department of Agriculture.
The structure of the GYIBC includes an Executive Committee, consisting of a representative of each of the state and federal agencies, and two subcommittees which will serve the Executive Committee. The Executive Committee members are to be decision makers for the agencies. The subcommittees include a Technical subcommittee, which will serve to depoliticize the brucellosis information base and develop strategies to solve the wildlife brucellosis problem, and an Information and Education Subcommittee, which will distribute objective information concerning GYIBC's purpose, manage public involvement and communicate to the public the need to solve the problem of brucellosis in wildlife.

The first major objective of the GYIBC was to conduct a National Symposium on Brucellosis in the GYA. The Technical and Information and Education Subcommittees developed the agenda and made arrangements for the symposium. The symposium was held at Jackson, Wyoming on September 26 and 27, 1994. There were 216 registered participants and a number of guests in attendance.

Thirty four presentations covering the entire gamut of the issue were made at the symposium. Major topics included: Human Dimensions of Brucellosis in the GYA; Brucellosis from State and National Perspectives; Cattle, Elk, Bison and Brucellosis of the GYA; Brucellosis - The Disease; Brucellosis in the GYA; Brucellosis from Federal Agency Perspectives; and, Brucellosis Eradication and Future Needs. Speakers included Governors Sullivan, Andrus and Racicot and representatives of the Secretaries of the Department of Agriculture and the Department of Interior.

The symposium was highly successful in bringing all of the issues to the table and allowing all of the major interests to participate. It is hoped that the symposium would provide information and data necessary to solidify support for efforts of the GYIBC to find solutions to the problem of brucellosis in wildlife of the GYA. We believe that this was accomplished.

The day following the close of the symposium the GYIBC held its first official meeting. All federal and state members were represented at the meeting. Topics discussed included the status of the Memorandum of Agreement, development of funding for the function of the Committee, status of the Montana/Yellowstone National Park Bison Environmental Impact Statement, an update on the Jackson Bison Environmental Impact Statement and the Intertribal Bison Council's desire to obtain YNP bison. The following actions were taken by the Committee:

1. Approved the charters for the function of the Technical and Information and Education Subcommittees.

2. Directed the Technical Subcommittee to:
   a. Evaluate the role of bull bison in the transmission of brucellosis.
   b. Develop a standardized protocol for collection of samples from bison that are available for sampling to assure uniformity and acceptability of research results.
   c. Draft feasibility and protocol for the quarantine of exposed bison and eventual transfer of clean bison from the GYA to Na-
tive Americans. This protocol will not address the regulatory aspects of the movement of bison.

3. Directed the Information and Education Subcommittee to develop and distribute a news release on the Symposium and first meeting of the GYIBC.

4. Approved a position statement on wildlife feedgrounds. This position statement discourages the feeding of elk and recommends against the development of new feedgrounds and the development of emergency feedgrounds for other ungulates that could attract bison or elk in the GYA.

We believe the GYIBC has made a good start. All member agencies appear willing to fully participate and seek equitable solutions to the problems of brucellosis in wildlife of the GYA. The next meeting of the GYIBC is planned for February 1995 in Idaho Falls, Idaho.

GOAL, MISSION, and OBJECTIVES of the Greater Yellowstone Interagency Brucellosis Committee [GYIBC]

It is the Goal of the Greater Yellowstone Interagency Brucellosis Committee to protect and sustain the existing free-ranging elk and bison populations in the Greater Yellowstone Area (GYA) and protect the public interests and economic viability of the livestock industry in the three states.

Toward this end it is the Mission of this Committee to facilitate the development and implementation of brucellosis management plans for elk and bison in the GYA.

This will be accomplished by subscribing to the following management Objectives which will, in turn, guide the Committee.

1. Recognize and maintain existing state and federal jurisdictional authority for elk, bison and livestock in the GYA;
2. Maintain numerically, biologically and genetically viable elk and/or bison populations in the respective states, national parks and wildlife refuges;
3. Maintain the brucellosis free status of Wyoming, Montana and Idaho and protect the ability of producers in the respective states to freely market livestock;
4. Eliminate brucellosis related risks to public health;
5. Eliminate the potential transmission of *Brucella abortus* among elk, bison and livestock;
6. Coordinate brucellosis related management activities among all affected agencies;
7. Base brucellosis related management recommendations on defensible and factual information while encouraging and integrating new advances and technology;
8. Aggressively seek public involvement in the decision making process;
9. Communicate to the public factual information about the need to prevent the transmission of brucellosis, the need for its eradication and the rationale for related agency management actions; and
10. Plan for elimination of *Brucella abortus* from the GYA by the year 2010.
Fiscal year (FY) 1994 marks the fifth year that the Cooperative State-Federal Brucellosis Program has operated under the Rapid Completion Plan. The exceptional progress made during this period clearly shows that this plan, if properly used, is capable of eradicating brucellosis from the United States by the 1998 goal. During FY 1994, the number of reactors, the number of newly infected herds, and the number of herds under quarantine were all less than the previous year. California attained Class Free status during the year, but later reverted to Class A when two infected herds were found in the Chino valley area. A significant program milestone was reached in March when Texas, the last Class B State, advanced to Class A status. Four Class A States are presently in the qualifying period for Class Free and are expected to reach that status in FY 95. Six additional States, each with less than five known affected herds, should enter the qualifying period for Class Free during the coming year.

Uniform Methods and Rules for a brucellosis program in Cervidae were developed during FY 1994 and circulated for comment. They are expected to be implemented later in the year. Two brucellosis evaluation studies on llamas were carried out in cooperation with the North American Llama Association. Also carried out were comparative studies of 4 brucella milk tests and a sensitivity comparison of the old and new BRT antigens. The results of these studies are presented elsewhere.

On August 31, 1994, there were 172 herds under quarantine for brucellosis in the United States with more than half of these in Texas. This is a 44 percent reduction from the 306 herds under quarantine a year earlier. Only 7 of the quarantined herds are dairy herds.

The Environmental Impact Statement (EIS) on brucellosis in Yellowstone National Park (YNP) that was referred to in the FY 1993 report is still in preparation by representatives of the Interior Department, the Forest Service, the State of Montana, and APHIS-VS. Park officials agree with the goal of eradication, however, there has been no agreement on what eradication procedures will be permitted in the Park. The Greater Yellowstone Interagency Brucellosis Committee (GYIBC), formed by the governors of Idaho, Montana, and Wyoming, is developing a brucellosis eradication plan whose goal is the elimination of brucellosis from the Greater Yellowstone Area by the year 2010.

During the year, 46 bison were intercepted as they left YNP. Only 3
were tested for brucellosis and all were negative. In a related issue, 1,359 elk calves were vaccinated on 13 Wyoming feed grounds by the Wyoming Game and Fish Department using bio bullets containing Strain 19. This project, and efforts to improve the elk's habitat to reduce their dependency on feed grounds, are funded by APHIS.

Due to normal reporting delays from the field stations, the following graphics contain estimated data for the last 2 months of the FY. On September 30, 1994, 32 States, Puerto Rico, and the Virgin Islands held Class Free status and 18 States were Class A. Texas advanced from Class B to Class A during the year (Figure 1).

Thirty-eight percent of the Nation's 35 million beef cows that have calved are located in Class Free States and 62 percent in Class A States (Figure 2). Of 9.6 million dairy cows, 63 percent are in Class Free States and 37 percent are in Class A States (Figure 3). The 7 dairy herds under quarantine for brucellosis on August 31, 1994, were located in 4 States: 1 in Kansas and 2 each in Texas, New Mexico, and California (Figure 4).

Of all beef and dairy cattle, 43 percent are in Class Free States and 57 percent are in Class A States (Figure 5).

A total of 442 brucellosis affected herds were found in FY 1994. This was a decrease of 26 percent from the 601 affected herds found in FY 1993 (Figure 6).

These 442 herds were in 18 States, with 94 percent located in 9 States and 6 percent in the remaining States. There were no affected herds in 32 States. Texas, with 240 brucellosis affected herds, represented 54 percent of the national total. The States of Arkansas with 12 reactor herds, Florida with 41, Georgia with 9, Kansas with 58, Louisiana with 26, Mississippi with 20, and Tennessee with 12 together represented 40 percent of the total for the year. Iowa, Oklahoma, South Dakota, Nebraska, Alabama, Kentucky, Colorado, New Mexico, and California had the remaining 24 herds (Figure 7).

This year we will again explain the two preceding figures to clarify this traditional, but confusing, method of presenting annual reactor herd data. As shown, the data implies that all of the herds were found during the FY covered by the report. However the reactor totals in these figures includes not only those herds found infected this year but also those found last year in which reactors were found during FY 1994. If the herds carried over from FY 1993 are subtracted, the number of infected herds actually found in FY 1994 was 282 in 15 States (Figure 8). Eleven of the fifteen States were successful in reducing their newly infected herds in FY 1994 from the number they had the previous year. Colorado had no infected herds during the year and is expected to qualify for Class Free status in October.

The number of herds under quarantine for brucellosis dropped dramatically during the year from 325 on July 31, 1993, to 198 on July 31, 1994; a reduction of 40 percent (Figure 9).

Three brucellosis affected dairy herds were located in FY 1994 by
Brucellosis Ring Test (BRT) surveillance. A total of 657 suspicious BRT laboratory reports resulted in 473 herds being blood tested for a herd test rate (HTR) of 72 percent. The HTR in FY 1993 was 62 percent (Figure 10). There were 12.3 million Market Cattle Identification tests conducted in FY 1994 was 500,000 more than the number collected the previous FY. Of these, 7.0 million samples (57 percent) were collected at slaughter plants and 5.3 million (43 percent) were collected at stockyards (Figure 11).

The total number of cattle tested for brucellosis in FY 1994 was 15.7 million, an increase of 1,300,000 over FY 1993. Of these, 3.4 million (22 percent) were sampled on farms or ranches and 12.3 million (78 percent) were tested under the MCI program. Although the total number of tests increased by 9 percent, there was a 22 percent decrease in reactors from 16,746 in FY 1993 to 13,000 in 1994, 3300 of which were found on farms (Figure 12).

The 7.6 million calves vaccinated for brucellosis in FY 1994 was an increase of 600,000 over the number vaccinated during the previous FY (Figure 13).

Forty-nine herds were depopulated because of brucellosis in FY 1994. These herds were located in 9 States and had a total of 2,005 animals. Depopulation continues to be the preferred method of handling infected herds under the Rapid Completion Plan.

The brucellosis program made outstanding progress during FY 1994, but this progress must be accelerated if the 1998 goal of eradication is to be achieved. In recognition of this, APHIS assembled a knowledgeable committee to review the program and recommend enhancements that would achieve this acceleration. The enhancement recommended by the committee in its White Paper include: an increase in indemnity rates to encourage herd depopulation; an emphasis on whole herd vaccination of herds not depopulated; a strengthening of State program technical reviews; an increase in brucellosis training; the maintenance of a high level of surveillance; the control of brucellosis in other species of animals; and the improvement of national program coordination by the appointment of a brucellosis program director. The committee also recommended that APHIS develop a national policy on brucellosis vaccination.

A meeting sponsored by Livestock Conservation Institute was held in Memphis, Tennessee, in August to discuss the recommendations and their implementation. They were generally well received by the State, Federal, and industry people in attendance. APHIS is currently taking steps to comply with the recommendations of the White Paper and some are, or will soon be, in operation.

On March 17, 1994, USDA, APHIS amended the swine brucellosis indemnity regulations, by interim rule, providing for payment at fair market value for whole herds of swine depopulated because of brucellosis. At the time of publication, there were 34 infected herds in the United States (FL-
18, TX-12, MS-1, LA-1, OK-1, SC-1). Since its publication APHIS and State field personnel have been aggressively contacting herd owners to make them aware of the new policy and arranging for depopulation and disposal.

When active contacts began, two herds (FL) were found to no longer raise hogs, and two others (FL) were never tested after initial slaughter traceback and have since been found negative. One herd (SC) had previously tested negative twice and with the third negative test, was released from quarantine. These developments reduced the infected herd population to 30 without the need to pay indemnity. After some initial disorder regarding appraisals, administrative procedures, appropriate euthanasia, and disposal, the "buy-out" program exceed expectations. Within 60 days of the implementation of the interim rule, the United States was left with only 9 infected herds (FL-4, TX-3, LA-1, and AR-1), most of which were already appraised and ready for depopulation.

Just over six months later, there is currently six infected herds in the United States (FL-3, LA-1, AR-1, TX-1) only one herd owner that is reluctant to accept the "buy-out". APHIS' goal is to have all currently known infected herds depopulated by July 1, 1994. Any newly discovered infected herd will attempt to be depopulated within 30 days of discovery. Because of this rule change and the States successful implementation of it, it appears likely that the goal of freedom from swine brucellosis by 1996 is achievable.
BRUCELLOSIS ERADICATION PROGRAM
OCTOBER 1994

Figure 1
Brucellosis Eradication
Distribution of Beef Cattle by Brucellosis Status

CLASS A
62%

CLASS FREE
38%

September 1994

Figure 2
Brucellosis Eradication
Distribution of Dairy Cattle by Brucellosis Status

CLASS FREE
63%

CLASS A
37%

September 1994

Figure 3
DAIRY HERDS UNDER QUARANTINE
AUGUST 1994

TOTAL 7

Figure 4
Brucellosis Eradication
Distribution of All Cattle by Brucellosis Status

CLASS FREE
43%

CLASS A
57%

September 1994

Figure 5
## Brucellosis Eradication

### Number of Reactor Herds Found (According to State Classification)

<table>
<thead>
<tr>
<th>Year</th>
<th>Class Free</th>
<th>Class A</th>
<th>Class B</th>
<th>Class C</th>
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<tr>
<td>1994</td>
<td>32</td>
<td>18</td>
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</tbody>
</table>

*Estimated* 

![New State Classification Graph](image-url)

*Figure 6*
Brucellosis Eradication

Percent of Total Reactor Herds Found

Fiscal Year 1994 *
Total herds: 601

54%
States: 1
Herds: >200
Total reactor herds = 240

28%
States: 3
Herds: 25 < 200
Total reactor herds = 125

6%
States: 43
Herds: <10
Total reactor herds = 24

12%
States: 4
Herds: 10 < 25
Total reactor herds = 53

* Estimated

Figure 7
Brucellosis Eradication

New Reactor Herds

October 1993 through September 1994 - 282*
October 1992 through September 1993 - 356

*Estimated
HERDS QUARANTINED BECAUSE OF BRUCELLOSIS
AS OF AUGUST 31, 1994 - 172 - RATE 0.14
AS OF AUGUST 31, 1993 - 306 - RATE 0.24

Figure 9
Brucellosis Eradication

Milk Ring Test Results (BRT)

- Total Suspicious BRT Tests
- Follow-up Herd Blood Tests
- Infected Herds Found

Fiscal Year

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Suspicious BRT Tests</th>
<th>Follow-up Herd Blood Tests</th>
<th>Infected Herds Found</th>
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<td>2,276</td>
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<tr>
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<td>657</td>
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</tbody>
</table>

Figure 10
Brucellosis Eradication

Blood Testing: Cattle

Figure 11
REACTORS FOUND (thousands)

![Bar chart showing reactors found from 1985 to 1994. The X-axis represents the years, and the Y-axis represents the number of reactors found, in thousands. The chart shows a decreasing trend from 1985 to 1994. The bars are divided into two categories: Farm or Ranch and MCI.]

Figure 12
Brucellosis Eradication

Calves Vaccinated

*Estimated

- Figure 13
REPORT OF THE COMMITTEE ON ENVIRONMENTAL RESIDUES

Chairman: Dr. Henry M. Stahr, Ames, IA
Vice Chairman: Dr. J. C. Reagor, College Station, TX

Mr. John Adams, VA; Dr. William B. Buck, Urbana, IL; Dr. Howard H. Casper, ND; Dr. Delmar R. Cassidy, IA; Dr. Colleen Y. Erbel, TN; Mr. L. Wayne Godwin, FL; Dr. Carl H. Graham, MO; Dr. John P. Honstead, MD; Dr. William T. Hubbert, MD; Dr. William E. Ketter, MD; Dr. Tari P. Kindred, MD; Dr. Gavin Meerdink, IL; Dr. Gary D. Osweiler, IA; Dr. Jane F. Robens, MD; Dr. Frank Ross, IA; Dr. Larry G. Sullivan, MI; Mr. William Thomas, ME; Dr. Manuel A. Thomas, Jr., TX; Dr. Janice Webb, FL.

The Environmental Residues Committee of the United States Animal Health Association met at 1:30 p.m., Tuesday, November 1, 1994, at the Amway Grand Plaza Hotel, Grand Rapids, Michigan. There were 11 committee members and 9 visitors present.

Presentations at the committee meeting were as follows: Vernon J. Feil from the USDA/ARS Metabolism Laboratory, Fargo, N.D. discussed dioxin in the environment, our food supply, and the four-year trial in progress to help define the physiological deposition of dioxin in beef cattle. Major sources of dioxin are: industrial processes, chlorophenolic compounds, past spraying of 2-4-5 T and Agent Orange, municipal and hazardous wastes, metal refining, hospital incinerators unless equipped with modern scrubbers, the smoking of cigarettes, and the burning of any carbon products (wood etc). Automobile exhaust was an important source when leaded fuel was used. More dioxin is formed at 400°F burning temperature.

Dioxins are very stable compounds. They are destroyed by burning at 2,000°F. The present background level of dioxin is 10ppT. Human serum levels peaked in 1988.

The dioxin 2, 3, 7, 8 TCDD is a very potent compound. It is given a toxic value of 1, and others are given an equivalency toxic value relative to 2, 3, 7, 8 TCCD. At sufficient dosage levels dioxins cause wasting and death, and are carcinogens.

Low dose exposure to dioxins over a long period of time is of greater risk than a single high dose exposure. It accumulates in fats and lipids. Beef, dairy products, and fish are the sources of dioxin in our food products.

The committee deliberated on the reported information in the 1994 EPA Dioxin Reassessment Report. Copies of the summary will be circulated to committee members for information evaluation.

In Lynn Willet's absence, committee chairman, Mike Stahr, gave a brief presentation of work on chlorinated hydrocarbons in cows at Ohio Agricul-
Lynn Willet discovered that chlorinated hydrocarbon compounds are mobilized in pasture soils by rainfall, volitized, and then contaminate forages. Thus old pesticide contaminated sites become a source of trace contamination to livestock grazing these areas. Dr. Willet's present work is to define management practices that will minimize pesticide contamination of forages in these areas.

M. Saeed, Purdue University, West Lafayette, Indiana, provided an update on Salmonella contamination of poultry, poultry products, and the poultry environment. The environment and vertical transmission through the egg by *Salmonella enteritidis* are a major means of Salmonella transmission. Feed can be a source of contamination but is not a significant contributor to the problem.

Rodents are an important source of environmental contamination. Once the large poultry facilities are contaminated, present physical structures and management practices do not allow an economical means for eliminating the Salmonella. M. Saeed presented information on use of a serum ELISA, antibody in egg yolk, and culturing. A significant antibody titer develops by about one month after infection at which time shedding shuts down. However, the birds may still be a carrier.

C. H. Graham, Farmland Industries, Kansas City, Missouri, presented information on use of copper sulfate at above nutritional levels in swine rations for improved growth rate and feed efficiency, and as an aid in controlling some diarrheal disorders. Copper's antimicrobial activity is attributed to the combining of copper with microbial protein. Use level at 250 ppm gives the best response. The response is about 0.1 lb. improvement in average daily gain and an equal feed savings per pound gain. Copper sulfate is primarily used at these levels in swine rations for 10-50 lb. pigs with some use in rations for 50-125 lb. pigs.

Use at 250 ppm copper will result in a 10 fold greater quantity of copper in swine liver and swine manure. Application of the copper enriched swine manure to soils appears to present a minimal short-term risk. However, it is speculated that long term (50-100 years) application could lead to accumulation of toxic levels in the soil.

Respectfully submitted

Carl H. Graham for Mike Stahr
If no human ever travelled from home, by boat or vehicle or airplane, the work of this Epizootic Attack Committee would be considerably simpler. All concern for the exotic agent hazard could be focussed on migrating birds, bats, and perhaps marine organisms. However, humans do travel and always have. And the numbers of travellers and the transfer of goods from one place to another is escalating. Modern transportation technology has made travel and shipment of goods economical and fast. Recent trade agreements have escalated activity and also changed the nature of the activities.

The activities have changed for several reasons. First, the concept of regionalization has been incorporated into the trade agreements. If the most general meaning of regionalization prevails, an area as small as a farm premise can expect to be evaluated for regionalized exports. Second, the demands made on the importing country have changed. Information used to evaluate the acceptability of an import must be well-documented. If new information becomes available, the process of acceptance must account in a flexible way for the new information. The evaluation method for an export from one country or one exporter and another country or exporter must be consistent. Furthermore, the documentation must be transparent. That is, it must be clear and understandable to others. The new openness in international trade imposes requirements for sharing the documents used in making decisions about acceptability for the proposed export.

All these changes (frequency and rapidity of travel of people and goods, new trade agreements which accelerate movement of commodities, demands for openness) increase the challenge for those of us concerned with the introduction of exotic agents. Fortunately, there have been developments in science and technology which help us manage these challenges. The developments in science have been in the field of risk analysis. In technology, support comes from the availability of powerful computers, construction of databases, and accessibility of information through Internet.

Risk analysis is the term used to mean risk assessment, risk management, and risk communication. Each part of the risk analysis trio has an important contribution to make to the process of evaluating the acceptability of imports. These tools, along with the new technologies mentioned
ability of imports. These tools, along with the new technologies mentioned earlier, will allow us to compete freely in the international marketplace and yet maintain the high standards of animal health we have attained through hard work over the years. Risk analysis is the key.

Risk analysis is the term used to mean risk assessment, risk management, and risk communication. Each part of the risk analysis trio has an important contribution to make to the process of evaluating the acceptability of imports. First a brief definition of each term is in order, then the discussion will focus on risk assessment specifically in the context of evaluating animal health.

Risk assessment answers three questions: What can go wrong? (hazard identification). What is the probability of this occurring? What is the magnitude of the consequences should the hazard occur? In a formal sense, these last two questions are defined as risk. Risk assessment is the domain of the biological sciences in which the pertinent information is structured to assist the risk manager in making the decision about a particular import.

Risk management refers to the decisions made about the acceptability of the risk and the use of mitigation to decrease the risk. Larger decisions of policy, diplomacy and politics involving economics and legal issues may also be referred to as risk management. Risk communication is the open, all around sharing of information among all concerned parties: scientists, managers, clients and consumers.

The long tradition among countries which have actively traded in animals and animal products has been to aim for "zero risk" in all import situations. Certainly this philosophy was strongly influenced by the Tariff Act of 1930 in which trading with countries whose livestock were affected with the FMD agent was forbidden. However, the condition of "zero risk" is unattainable on several grounds. Scientifically, one can never prove a negative. One can demonstrate very low probability, but zero probability is a theoretical construct, not a practical one. On another level, claiming one extreme, zero risk, implies the opposite, or total probability of 100%. This is somewhat akin to the dichotomous way in which we have viewed the risk of incursion of exotic agents into this country: like a light switch, on-off, 100%-0%. In this analogy, risk assessment is somewhat like a rheostat. It allows us to provide fine gradations in the amount of illumination, a more flexible way to provide light to a room.

In order to better understand risk assessment, let's take a look at a hypothetical example of how risk assessment can support import product and animal decision making. You are the risk manager for a South American country which has certain strains of foot-and-mouth disease (FMD). However, your country does not have strains Asia 1, 2 or 3. One of your citizens wishes to import raw cattle hides from Asia (where these strains occur).
In this example a technique called Scenario or Event Tree Modelling is used. It is a very flexible technique that permits one to use of all the information available. There are other techniques and models, each fitted for particular purposes. But for the illustration today, the Scenario Model is quite useful.

Example:

Hazard identification: FMD, Asian strains 1, 2, 3 coming from an Asian country in rawhides imported into Country X.

"Facts":
--30% of cattle have Asian FMD
--50% of rawhides from affected cattle sow the agent 6 months later
--time from slaughter of cattle to arrival of rawhides is about 6 months
--cattle from which hides come are distributed all over the originating country

The Risk Questions:

Of 100 hides imported from this oriental country, what is the likelihood (probability that and FMD Asian strain will enter Country X? What is the magnitude of the consequences should this Asian strain FMD enter Country X? All sorts of economic and animal health analyses could be made, but in veterinary medicine, there is a sort of "default assumption" made in answer to this question. It is that any disease on the OIE List A will be of sufficient magnitude that the consequences are "very large".

Therefore in this example we will deal only with the probability of entry (assuming that the consequences would be unacceptably high).

Given the "facts" above, the probability is \((100)(0.3)(0.5)\) or 15. If Country X imports 100 rawhides a year, this suggests that 15 will be carrying virus capable of multiplying and causing an outbreak of Asian strain 1, 2, and/or 3. As the Risk Manager, ask yourself the question: Is this safe enough?

Likely your answer as protector of the animal health of Country X was "NO". However, the importer persists and asks please to find a way to allow the import. There is a new process that kills 90% of the agent, but leaves the hides in their desirable "raw" condition. This is a mitigation measure, something which reduces the likelihood of the hazard occurring. Is this safe enough? The analysis gets more complicated and the point is not to discuss the mathematics of the issue. Rather it is to point out how this method, or any other can be used in animal importation.

An actual risk assessment must be presented to define the hazard and to show the actual model used. All assumptions used must be clearly shown. For each step in the model, the evidence and the source of the evidence used to make a decision must be shown. The best estimates or the actual data used at each step must be clearly shown. The outcome of the analysis along with an appropriate set of graphs must be presented to the risk manager/decision maker. This document must become a perma-
nent part of the record whether the decision is to allow the import, allow the import with mitigation measures (specified), or forbid the import. Only by retaining and making available such well-documented analyses can a country provide the "open-ness" which is demanded by the new trade treaties. An example of this new "open-ness" is the plant health group in New Zealand. All their risk assessments will be available to anyone through Internet. Therefore, all trading partners can immediately compare their status with New Zealand.

I believe that this vision of risk analysis is what it will take to be competitive in the new world of the twenty-first century. Good science-based risk assessment using the best data and information available well-documented in a format that is flexible and transparent to demonstrate that treatment of one country is consistent with that of another. This risk assessment guides the decision making of the risk management and along with clear, unambiguous and open risk communication among all stake-holders, we have the key to free world trade and world-wide improvement in animal health.
In November 1993, the U.S. Congress ratified the North American Free Trade Agreement (NAFTA). This Agreement, in effect since January 1994, is aimed at reducing and eliminating barriers to trade, investment, and services between the United States, Canada, and Mexico. NAFTA creates the largest free trade area in the world with 360 million people and a gross national product (GNP) totalling $6 trillion.

The Uruguay Round of the General Agreement for Tariffs and Trade (GATT), which lasted seven years, came to completion in December 1993. The Agreement was officially signed by member countries in Morocco on April 15, 1994. If approved by Congress, GATT would go into effect in January 1995.

GATT is aimed at reducing and eliminating barriers to trade, investment, and services between the 100-plus signatory countries. Besides eliminating traditional barriers such as quotas and tariffs, both GATT and NAFTA attempt to control the use of non-tariff barriers, such as unjustified technical health standards. For example, sanitary and phytosanitary (S&P) measures can and have been used for protectionist purposes. With this in mind, GATT and NAFTA negotiators established a set of principles to prevent the use of S&P standards as disguised barriers to agricultural trade. The following are key S&P provisions contained in both Agreements:

- The use of science-based measures (i.e., using risk assessment)
- Recognition of pest- and disease-free areas and areas of low pest or disease prevalence and allowing trade from those areas.
- Participation in the international standard setting organizations and wherever possible basing import requirements on international standards.
- Recognition of equivalent treatments and quarantine practices to facilitate trade.
- *Dispute settlement process which begins with a technical consultations step and proceeds, if necessary, to the use of a formal dispute settlement system.*

**Key Provisions**

NAFTA's animal and plant health provisions are found in Chapter Seven, Section B of the NAFTA text. The Uruguay Round provisions on sanitary and phytosanitary commitments are found in the "Agreement on the Application of Sanitary and Phytosanitary Measures" of the Final Act. The S&P
provisions in these Agreements, which are nearly identical, are as follows:

1. **Basic Rights** *(NAFTA: Article 712 and GATT: Article 2)*

   Both GATT and NAFTA make explicit a country's right to adopt and maintain any measures it believes are necessary to protect the health of its plants and animals, as long as these measures are: (1) based on science, (2) transparent in the way it was developed and implemented, and (3) do not arbitrarily or unjustifiably discriminate between its goods and like goods another party or between goods of another party where identical or similar conditions prevail.

   Both NAFTA and GATT encourage countries to their base import requirements on international standards, but preserve all countries' right to adopt health measure that are more stringent than existing international standards, as long as they are scientifically based.

   While countries have the right to chose and adopt their desired level of protection, NAFTA and GATT encourage countries to take into account the objective of minimizing negative trade effects and to avoid arbitrary or unjustifiable distinctions in the level of protection in different circumstances if such distinctions result in unjustifiable discrimination against goods from another trade partner.

   One must keep in mind the basic right of countries to maintain measures to protect its agriculture, including measures that may be more stringent than existing international standards. However, both NAFTA and GATT seek to minimize the variations in the levels of protection by requiring countries to participate in international standard setting organizations. It is hoped that the development of international standards, a long-term goal, will help reduce unjustified variations in levels of protection chosen by different countries.

2. **Risk Assessment** *(NAFTA: Article 715 and GATT: Article 5)*

   The requirement that measures be based on science means that import decisions must be based on a risk assessment which uses scientific data and methodologies. GATT and NAFTA describe a number of factors which should be considered in a risk assessment, including:

   - Risk assessment methodologies and techniques developed by the OIE, IPPC or regional standards organizations.
   - Relevant scientific evidence.
   - Relevant processes and production methods.
   - Relevant inspection, sampling, and testing methods.
   - Prevalence of disease or pests of concern, including the existence of pest- or disease-free or low-prevalence areas.
   - Relevant ecological and environmental conditions.
   - Relevant treatments, including quarantines.
   - *Relevant economic factors such as production or sales losses and control costs if a particular disease or pest were to be introduced.*
3. **Equivalence (NAFTA: Article 714 and GATT: Article 4)**

This provision commits GATT and NAFTA signatory countries to treat an exporting country's S&P measures as equivalent to its own when the exporting country demonstrates, using scientific evidence, that its measures achieve the importing country's desired level of protection.

Equivalence encourages countries to recognize that different procedures could and can be used to achieve the level of protection demanded by the importing country. Equivalence focuses on procedures (such as inspection, certification, surveying, trapping, fumigation, and other treatments or practices) that countries use to ensure or certify the safety of a particular commodity.

4. **Role of International Standards Organizations (NAFTA: Article 713 and GATT: Article 3)**

Basic to GATT and NAFTA are the provisions encouraging countries to base their S&P measures as much as possible on international standards, whenever such standard exists. Animal health measures should be based on Office of International Epizootics (OIE) standards and plant health measures are to be based on the International Plant Protection Convention (IPPC) standards.

The reason for encouraging the development and use of international standards is to harmonize, to the greatest extent possible, countries' S&P measures. This is intended to reduce unnecessary variances between countries' technical health standards—differences which are oftentimes the cause of trade disputes.

The actual body of international standards in both the plant and animal health area is not fully developed. However, the trend, driven by the GATT and NAFTA as well as the explicit policy of APHIS management, is to be an active participant in the development and elaboration of these international standards.

5. **Regionalization (NAFTA: Article 716 and GATT: Article 6)**

GATT and NAFTA commit countries to recognizing disease and pest-free areas as well as areas of low pest and disease prevalence. This concept, known as regionalization, is perhaps the most significant policy and regulatory issue facing APHIS. It is expected to create new opportunities for the United States to export from areas which are demonstrated to be free of particular diseases even though diseases of concern may exist elsewhere within the national territory.

The Agency has been working through the OIE to develop internationally consistent regionalization principles, particularly risk assessment standards. APHIS believes that a standardized and transparent risk assessment process needs to include criteria for evaluating an exporting country's veterinary infrastructure, including its disease control capabilities, surveillance and monitoring systems, and animal import policies. Such assess-
ments are necessary to help verify disease-free area claims and assure the importing country that the integrity of the specified area can be adequately maintained.

APHIS' proposed approach discards the notion of an area as being free or not-free, replacing it with the concept of levels of risk. Six levels of risk have been identified, ranging from Risk Class RO (lowest risk)...R1...R2...R3...R4...and RU (highest risk). Risk factors considered in developing these classes include: (1) proximity of the area to infected areas, (2) historical disease incidence within the area, (3) vaccination policies and practices within areas, (4) type of physical separation from higher risk areas, (5) type of importation of animal and animal products from higher risk areas, (6) disease surveillance within the area, (7) disease control policies and resources within area, and (8) demography and infrastructure within area. This approach of recognizing different levels of risk or health status is consistent with the GATT and NAFTA provision requiring countries to recognize not only disease-free areas, but also areas of low disease prevalence.

STATUTORY CHANGES TO IMPLEMENT REGIONALIZATION: Until recently, the 1930 Tariff Act restricted the Department's ability to recognize disease-free zones. This Act was changed as part of the NAFTA enabling legislation to allow the United States to fulfill this NAFTA and GATT commitment. In preparing the GATT implementing legislation, the 1930 Tariff Act was again amended to include concept of recognizing "areas of low-disease prevalence." Both NAFTA and GATT regionalization provisions include both "disease-free" and "low-disease prevalence" concepts.

6. Transparency (NAFTA: Article 718-719 and GATT: Article 7)

NAFTA and GATT include various provisions which encourage greater transparency of the domestic rulemaking process which affects trade. For example, NAFTA and GATT require member countries to provide advance notification of any new or modified regulation which may negatively affect the exports of another country. An exception to this advance notification rule exists for emergency disease or pest situations.

Also, both NAFTA and GATT require signatory countries to identify a contact point where other countries can access information and documents regarding procedures, decisions, and other information related to the establishment of S&P measures. The National Institute of Standards and Technology (NIST) at the Department of Commerce has been designated to serve as the U.S. inquiry point as required by this provision. The NIST is expected to forward inquiries on U.S. sanitary and phytosanitary measures to APHIS. This provision is meant to ensure that regulatory agencies, including APHIS, have a known contact point where trade partners can obtain information regarding risk assessment procedures, decisions, and related documents.

7. Sanitary and Phytosanitary Committees (NAFTA: Article 722 and
Both NAFTA and GATT call for formation of S&P Committees. APHIS as well as USDA's Foreign Agricultural Service (FAS) are participants in these committees.

The basic function of the Committees is to oversee and monitor implementation of the Agreements' S&P provisions. Furthermore, the GATT and NAFTA S&P Committees are expected to play a vital role in (1) facilitating consultations on specific S&P trade problems, (2) encouraging technical cooperation, and (3) addressing standard setting issues as they arise.

8. Consultations and Dispute Settlement (NAFTA: Article 72 and Chapter 20 and GATT: Article 11 and Annex 2)

Both NAFTA and GATT establish two separate and distinct dispute settlement systems. Both systems share the common feature that disputes begin with technical consultations at the S&P Committee level. If no resolution is achieved through technical consultations at the S&P Committee level, the complaining party may elevate their complaint by invoking the formal NAFTA or GATT dispute settlement process.

Generally, in both the GATT and NAFTA dispute settlement systems, panels may be formed to review the matter and make recommendations. Panels, formed to review complaints, may seek recommendations and advice from the relevant international standard setting organizations as well as form a board of experts to evaluate an issue.

Four points about the process should be underscored:
1. Emphasis will be placed on resolving technical disputes and complaints at the technical level (i.e., S&P Committee level) in order to prevent setting-off the more formal and complex dispute settlement process.
2. The challenging party has the burden of proving that a particular S&P measure or standard is in violation of GATT or NAFTA.
3. Panels which are formed to review a dispute are advisory. Panels will determine whether the measure subject to dispute is based on a scientific risk assessment and whether data and the process used in that assessment was:
   - collected in a scientific fashion
   - based on international standards if such standards exist
   - not unnecessarily and unfairly discriminatory in nature
   - transparent
4. If a panel issues an opinion that a standard is in violation, that country has the option of either changing the offending measure or keeping the GATT or NAFTA inconsistent measure and compensating the complaining party for the value of impaired trade. If compensation is not provided, the complaining party would be permitted to suspend some trade concessions of equivalent value to lost trade.
GATT AND NAFTA

Discussions with the office of the United States Trade Representative (USTR) suggest that great effort will be made to resolve issues at the S&P Committee level. This will focus attention on regulatory agencies such as APHIS to assist those U.S. commodity groups seeking to challenge other countries' technical trade practices. APHIS, through the offices of its Administrator and the APHIS Trade Support Team is actively involved in educating government and commodity groups on how this process works as well as in assisting U.S. groups present a case or defend/clarify a particular U.S. sanitary and phytosanitary position.

Implications of GATT and NAFTA

Trade Impact: It is not expected that NAFTA will lead to an immediate surge of imports as quotas and tariffs are eliminated gradually over time. In any event, APHIS has already been experiencing and responding to an increased volume of Mexican agricultural imports over the past 5 years. Monitoring systems are in place to collect information on pest interceptions and import flows. As this information enters the system, APHIS evaluates current and future priorities and adjusts inspection resources accordingly.

Mexico Requests Under NAFTA: NAFTA may provide Mexico with a renewed basis for seeking U.S. approval for the entry of certain commodities into the United States. For example, Mexico has tried vigorously to argue for the entry of avocado and citrus shipments to the northeastern states. Under NAFTA, Mexico may have greater leverage to request a consideration of these issues under new terms.

On the animal health side, Mexico is actively seeking U.S. approval of a hog cholera free-area and/or a cattle tuberculosis-free area similar to the Sonora pest-free zone. APHIS is working with the Mexico Secretariat and Agriculture Canada on recognition of such zones using the NAFTA regionalization provisions. Similarly, Current APHIS requirements prohibit the importation of poultry products because of VVND. Mexico may also seek U.S. recognition of a VVND-free area or zone.

New U.S. Export Opportunities: APHIS is currently experiencing difficulty in reestablishing trade in U.S. hogs because of Mexico's claim that U.S. swine present a significant risk of exposing Mexican swine herds to Porcine Reproductive and Respiratory Syndrome (PRRS). Under NAFTA, APHIS can demand Mexico respond to this issue under more favorable timeframes and could challenge Mexico's actions at the NAFTA S&P Committee as well invoke the dispute settlement process if progress is not achieved at the technical consultations level.

Regionalization: Examples of countries requesting U.S. recognition of disease- or pest-free zones or areas include Mexico's current effort to gain U.S. approval of hog cholera-free and cattle tuberculosis-free zone within its territory. APHIS must publish criteria and standards which Mexico would have to meet before any importations could take place. NAFTA requires APHIS to make the necessary regulatory changes by January 1995.
to implement the Agreement's regionalization commitments.

Other examples of pending regionalization requests stem from two recently signed Memorandums of Understanding (MOU) between the United States and Uruguay and another with Argentina. These MOU's, negotiated as side agreements to the GATT in April 1994, promise Argentina and Uruguay each a 20,000 metric ton quota of fresh de-boned beef in the U.S. market once these countries meet U.S. sanitary requirements. To this end, the MOU's commit APHIS to establishing a formal bilateral working group with each country to resolve U.S. sanitary concerns as they relate to their fresh beef exports to the United States.

Bilateral discussions with Uruguay and Argentina began with a high level USDA delegation visiting both countries in July 1994 and will proceed with technical group meetings according to a timetable set in July. Many countries are watching the United States closely with regard to implementation of these MOU's.

**Beyond NAFTA:** The trade agreement with Mexico is considered an initial step in a process for building a hemispheric free trade area (which has been known as the "Enterprise for the Americas Initiative"). At this point Chile is considered the most likely next candidate for a free trade agreement with the United States. Chile's intense interest in a trade pact should give the United States added leverage to get Chile to resolve existing trade problems including S&P trade issues.

**New Agency Tasks Under GATT and NAFTA**

- **Technical Working Groups**
  APHIS will be to be an active participant in the S&P Committees established under GATT and NAFTA as well as technical working groups which may be established to address specific sanitary issues and complaints.

- **Dispute Settlement**
  APHIS staff will be key players in assisting U.S. industry groups seeking to use the NAFTA or GATT dispute settlement procedures. APHIS technical staffs will be called upon to perform the technical review and analysis to these groups wishing to challenge other countries' health-related barriers.

  APHIS technical personnel will also be needed to clarify and/or defend U.S. regulatory positions when another country has a complaint about a particular U.S. import decision, requirement, or policy.

- **International Harmonization**
  APHIS will have to increase its involvement and presence at the international and regional standard setting organizations, such as the OIE, IPPC, and NAPPO. The current trend toward harmonization will require APHIS to be particularly active in order to ensure that future international standards are developed in a manner that reflects U.S. health concerns, interests, and objectives.
Regionalization and Risk Assessment
Implementing the concept of regionalization will require the following actions:

-- Development of criteria and standards for evaluating regionalization requests.
-- Promulgation of regulations for publication in the Federal Register.
-- Sharing and promoting U.S. regionalization criteria as model for deriving OIE-based standards for regionalization.

Annual Report to Congress
The NAFTA Implementing Bill requires the Secretary to provide an annual report to Congress, beginning in January 1995. This report must address NAFTA's impact on border inspections of meat, animals, and plant commodities. APHIS is prepared to provide input and information regarding its border inspection activities (e.g., pest and disease interceptions, transhipment problems, and volume of commodities inspected).

Conclusion: Contribution of Veterinary Medicine to International Trade

Soon, GATT will no longer be known as the GATT. Instead, the Uruguay Round Agreement establishes the World Trade Organization (WTO)—the new name for the transnational body which will be responsible for facilitating implementation of the Uruguay Round obligations and administering the dispute settlement system.

The challenge, as Uruguay Round and NAFTA negotiators saw it, was how to create an acceptable approach to resolve disputes involving differences in regulatory systems or measures which were the cause of disruptions in trade.

The NAFTA and Uruguay Round solution is to encourage harmonization through the widest possible use of international standards and the creation of a dispute settlement system.

Central to the dispute settlement process is the use of expert panels or boards, which may be convened to review disputes and provide findings and recommendations. Basic questions the panels are likely to ask when reviewing an issue or complaint are: (1) what is the relevant international standard or guideline, (2) what is the expert advice of the relevant international standard setting body, and (3) what available scientific data exists to support the measure subject to dispute?

The GATT and NAFTA panels established to review disputes and complaints will rely on international sources of expertise for advice on technical trade matters, such as veterinary expertise available at the OIE. In fact, the international standard setting bodies, such as the OIE, are likely to have direct representation on the GATT's Committee for Sanitary and Phytosanitary Measures. Given the emphasis on international standard setting bodies and the advisory role of veterinary officials in the operation...
of the GATT and NAFTA dispute settlement process, officials and practitioners in the field of veterinary science can make the following contributions to international trade policy:

First, the actual body of international standards in both the plant and animal health arena is not fully developed. To some extent, the international standard setting organizations, including the OIE, are still developing their own institutional and organizational roles and structures. An important contribution of veterinary science will be to help accelerate the development of technically and operationally feasible international standards, including standard methodologies for conducting risk assessments.

Second, veterinary expertise will play an important advisory role in technical trade disputes which come under review under the GATT or NAFTA dispute settlement processes. For example, we are likely to witness disputes arise over the levels of protection imposed by importing countries, particularly sanitary measures viewed as overly stringent and which unfairly impede trade. This issue over the "appropriate level of protection", also known as the "acceptable level of risk", is likely to be the basis of many health-related disputes. Veterinary science can play a key role in discussions which attempt to clarify and define levels of risk.

In particular, the Uruguay Round and NAFTA commit countries to recognizing areas of low disease prevalence. It may be necessary to widen and deepen the level of discourse among regulators and veterinary scientists around the world to begin defining prevalence and establishing standard criteria for identifying different levels of risk.

Finally, veterinary research scientists can continue to play a vital role in advancing free trade objectives while ensuring against the spread of diseases by focussing on research which may yield new technologies to reduce animal health related risks to levels which allow for safe trade in animals and products. In particular, emphasis has shifted from zero-risk based regulations and decision-making to the development of risk assessment tools and the application of new and/or varied risk management strategies to allow trade in low-risk commodities. Given the shift away from the "zero-risk" paradigm, increasing emphasis will be placed on developing technologies, strategies, and tools to allow for trade while ensuring that risk is reduced to minimum levels. The veterinary field will be an important contributor to developing these new tools.
The committee on epizootic attack met at 1:30 p.m. on Thursday, November 3, 1994, in the Haldane Room of the Amway Grand Plaza Hotel, Grand Rapids, Michigan. There were 19 committee members in attendance and 32 guests.

Animal Carcass Disposal

Dr. Cal J. Flegal, Professor, Department of Animal Science, College of Agriculture and Natural Resources, Michigan State University, reported on the Michigan poultry industry carcass disposal problems and what the Michigan industry needed to do to make composting legal, results of research, and legislative experience. Dr. Flegal reviewed the traditional techniques of dead bird disposal and discussed the feasibility, mechanical procedures, and legislative processes that were instrumental in allowing the Michigan poultry industry to adopt composting of dead birds. Prior to 1993, there were only three legal methods available to Michigan livestock and poultry producers to dispose of dead carcasses. Those methods were incineration, burial, and rendering.

A study was begun in 1988 to determine if poultry composting would operate in Michigan climatic conditions, if it would produce a pathogenically safe product, and whether it would be legal under current Michigan law at that time. Composting procedures took place for 12 consecutive months using two stage batch composters. The process took 56 days from initiation until the compost was spread. Internal temperatures were recorded during the entire procedure and observations were made on insect and
predator activity. Microbial culturing and virus isolation was done on the finished compost. In addition, laboratory compost samples artificially inoculated with Hemorrhagic Enteritis (HE) virus and compost containing poults previously vaccinated with HE virus were collected for virus isolation.

Results indicated the compost temperature varied between 120-140 degrees F. No pathogenic bacteria or virus was present in the finished product. Flies and predators were not a problem if the composting material was covered with at least 6 inches of litter cake or straw. Odor was minimal except during turning of the cake. The operation time was 15-30 minutes daily. Grinding or chopping of the carcasses prior to composting appeared to speed up the process.

The Michigan Attorney General's office determined the Bodies of Dead Animals Law would need to be amended and a set of rules and regulations developed to include poultry composting. The state veterinarian appointed a committee to develop rules and regulations regarding composting to be agreed upon by representatives of government, university, and the poultry industry in Michigan. The Bodies of Dead Animals Law was amended and signed into law November 4, 1993, after 36 months of work, and the rules and regulations have been accepted by all interested parties at this time.

Dr. Dale Rozeboom, Assistant Professor and Extension Swine Specialist, College of Agriculture and Natural Resources, Michigan State University was the next speaker and discussed what the Michigan swine industry is doing regarding carcass disposal problems. Current methods of disposal have environmental drawbacks or are not available to small producers. On farm demonstration projects were started to demonstrate the effectiveness of composting dead pigs and afterbirth in Michigan and to provide evidence to support legislative approval of swine composting. Controlled experiments are being conducted to monitor Pseudorabies virus (PRV) and \textit{Actinobacillus pleuropneumoniae} (APP) bacterial survival during composting.

On farm demonstration composting sites are located away from wells, surface water, and neighbors. Composting facilities are built to minimize precipitation entering and run-off. Bins approximately 8 x 8 foot are constructed and approximately 1.25 square feet per inventoried sow are needed to accommodate normal mortality. Compost piles are turned from primary to secondary bins after approximately 2-3 months. When turned, carcasses are almost completely composted and no carcass remnants remain after 2 months in secondary bins. Compost pile internal temperatures are monitored with temperatures reaching 130 to 150 degrees fahrenheit. One swine farm in Michigan is currently operating a compost facility and construction of three others is underway.

Feeder pigs were inoculated with PRV and APP and composted to determine microbial survivability. Microbiological testing on this is pending. A second controlled experiment is planned to feed this composted product to pigs not previously infected with PRV or APP.
Dr. Adam Grow, Senior Staff Veterinarian, Emergency Programs, USDA, APHIS, VS presented a paper submitted by Mr. William Ford, Staff Officer, Emergency Programs, USDA, APHIS.

He discussed large animal carcass disposal utilizing an air curtain incinerator system. High temperature incineration may provide a disposal method that is effective, controls disease spread, and is environmentally acceptable. The air curtain incinerator forces air out restricted inlets at up to 165 mph angled across and downward into a trench or pit. The air swirls down through the fire and creates a forge effect increasing temperatures to 1800 to 2800 degrees fahrenheit. The curtain of air across the top traps organic compounds and allows them to completely burn with little or no ash or smoke escaping into the air above. An additional fuel source is critical and the high-burn temperatures are maintained by addition of fuel to the incineration trench as needed. Front-end loading equipment is necessary to supply fuel and carcasses to the fire. Carcasses from brucellosis infected and exposed swine in Louisiana were used to test a Model T-39 Air Curtain Incinerator for on-site incineration. Approximately 15 minutes was necessary to incinerate one 250 pound sow carcass. Incineration of thirty four hogs weighing 225 lbs. each required less than 3 hours and one 850 lb. cow incinerated in less than 1 hour. Examination of ash revealed no traces of teeth or bones. The air curtain system would likely be adequate to dispose of average size swine herds, small cattle herds, and moderate size poultry flocks. A self-contained system is also available which weighs 22 tons and is transported on a Lo-Boy.

The next speaker was Dr. Don A. Franco, Director, Scientific Services, National Renderers Association. Dr. Franco gave a report on how renderers assure that toxic or infectious disease material is not in finished rendered product. Rendering product is under computer control from beginning to end. The heat inactivation of infectious product used by the rendering industry is based on the thermal death point of Bacillus anthracis spores. The final temperature in the finished product ranges from 250 to 270 degrees fahrenheit. The Animal Protein Producers Industry has initiated a Salmonella reduction/education program since 1984 involving training, sampling, educational meetings, and a structured response. Approximately 76 percent of all renderers participate. Renderers use pesticide screens and require veterinary contact on multiple death submissions. Industry leaders and technical staff are active on several committees of the USAHA and serve on national committees for scrapie and BSE control. The rendering industry infrastructure could be readily prepared to cooperate in natural disasters and disease emergencies.

Renderers in the United States pick up approximately 91 million pounds of waste material each day and turn it into usable product. Without the recycling services of renderers, inedible animal disposal would pose serious environmental consequences. As environmental concerns increase,
EPIZOOTIC ATTACK

ous environmental consequences. As environmental concerns increase, rendering facilities could play a significant role in the disposal process.

Updates

Dr. John L. Williams, Chief Staff Veterinarian, Emergency Programs, USDA, APHIS, VS, presented the committee with an update on bovine spongiform encephalopathy (BSE) surveillance in the United States. In July 1989, a ban was placed on importation of cattle from countries infected with BSE. From January 1981 through July 1989, 499 United Kingdom cattle were imported into the United States. As of August 29, 1994, tracebacks indicate that 137 imports are known to be alive, 232 are known to be dead, 69 are being traced through VS area offices, and 53 are being traced through breed associations. Eight of the imports have been exported to Mexico and Canada. From the onset of the domestic surveillance program in May 1990 through August 18, 1994, 1740 bovine brain tissue specimens have been submitted to the National Veterinary Services Laboratory in Ames, Iowa. All of the samples have been negative for histopathological evidence of BSE. In October 1993 a pilot project was initiated by APHIS, VS and the Food Safety Inspection Service (FSIS) to collect bovine tissue specimens from selected FSIS plants. Five slaughterhouses in selected states have agreed to contact VS when non-ambulatory adult cattle with CNS disorders are condemned during ante-mortem inspection.

Dr. Adam Grow, Senior Staff Veterinarian, Emergency Programs, USDA, APHIS, VS, gave an update on emergency programs. During Fiscal year 1994, veterinarians from USDA, APHIS, VS and the states conducted 283 FAD investigations. The investigations included 93 vesicular conditions, 58 encephalitic conditions, 37 avian diseases, 36 swine septicemic conditions, 13 mucosal disease conditions, 11 due to excessive death loss, 8 myiasis/ascariasis, 7 pox like conditions, 3 unusual respiratory conditions, and 2 abortions cases. All investigations were negative. A memorandum of understanding with the AVMA was prepared to establish a mechanism for cooperation during an animal health emergency. Emergency program staff held many educational meetings during the year. Four issues of the foreign animal disease report were published in Fiscal year 1994 and over 25,000 copies distributed worldwide.

Dr. Owen Hester, AVIc in Alabama, presented a paper by Dr. Donald G. Cheatham, USDA, APHIS, VS. The paper was titled "Foreign Animal Disease (FAD) Awareness Among Veterinary Practitioners in Alabama." In May 1994, a Foreign Animal Diseases awareness survey was conducted among veterinarians licensed to practice in Alabama. A two page questionnaire was mailed to 888 veterinarians. The response rate was 31 percent. The intent of the survey was two-fold:

1. To obtain practitioner input so their level of FAD awareness and
degree of reporting could be determined along with learning the needs veterinarians have in increasing their knowledge in this important area.

2. By the simple act of the practitioner receiving the questionnaire, written material, and informational brochures, it was anticipated awareness would be elevated to some degree by bringing the subject to mind.

Seventy-five percent of the responses mentioned foot and mouth disease as the first disease they think of when FAD is mentioned. When asked how knowledgeable the veterinarian's thought they were in recognizing clinical signs or necropsy findings of FADs, 8 percent felt very knowledgeable, 30 percent moderately knowledgeable, and 62 percent stated they needed a refresher. Three percent felt very knowledgeable in differentiating between domestic diseases and FADs, 36 percent felt moderately knowledgeable, and 61 percent stated they needed a refresher. Twenty-four percent were familiar with the Regional Emergency Animal Disease Organization (READEO), 67 percent were not, and 9 percent did not respond to this question. Fourteen percent did not know who to report FAD suspicions to, 86 percent would report FAD suspicions to state or federal regulatory veterinarians or a state diagnostic laboratory. Only 77 percent of the practitioners responded that they had a readily accessible telephone number to report suspicion of FADs. Seventy-three percent said it would be beneficial if more FAD topics were on the agenda of state and local meetings. Forty-seven percent asked for more FAD informational mailings, 22 percent wanted more FAD presentations at meetings, 11 percent said a combination of mailings and presentations at meetings was desirable, 15 percent felt a need for more personal contacts with state and federal officials, and there were 5 percent with miscellaneous suggestions.

Dr. Lynn M. Siegfried, Associate Director, National Veterinary Services Laboratory (NVSL), Ames, Iowa, reported that NVSL had been working on strengthening areas identified by an independent team of national and international veterinary laboratory administrators and scientists. The following accomplishments were noted:

1. Improvements in development and introduction of new diagnostic techniques.
2. Use of best methods for diagnosis of foot and mouth disease.
3. Increased communication and collaborative efforts between the Foreign Animal Disease Diagnostic Laboratory and the Plum Island Agriculture Research Service staff.
4. Enhanced abilities to accurately perform diagnostic testing for foreign animal diseases that are not frequently tested for.

At the 1993 USAHA meeting, a suggestion was made to establish a strategic planning task force to address the key points made by the technology review task force. This has been delayed to await the final report of a
USDA task force addressing biocontainment in APHIS facilities. This report will provide additional information that can be used by the task force for which USAHA will be asked to provide a member for this task force.

In 1993-1994 there were over 15,000 virus isolation attempts for velogenic Newcastle disease and highly pathogenic avian influenza virus. Neither of these viruses were isolated from domestic birds. In January 1994, NVSL parasitologists identified primary screwworms collected from a leg wound of a horse being imported to the United States from Argentina. Also in January 1994, five specimens of Haemaphysalis sulcata, a cause of tick paralysis and a species exotic to the U.S., were identified from trophy hides of Asian wild sheep.

The Foreign Animal Disease Diagnostic Laboratory is nearing the date when it can occupy its newly renovated facility on Plum Island. The estimated date for completion is January 1995.

Risk Assessments And Trade Issues

Dr. Alwynelle (Nell) Ahl, USDA, APHIS, Policy and Program Development gave a presentation and example of risk assessment in the evaluation of animal health. Risk analysis is the term used to mean risk assessment, risk management, and risk communication. Risk assessment answers three questions: What can go wrong? What is the probability of this occurring? What is the magnitude of the consequences should the hazard occur? Risk management refers to the decisions made about the acceptability of the risk and the use of mitigation to decrease the risk. Risk communication is the open, all around sharing of information among all concerned parties: scientists, managers, clients, and consumers.

Dr. Dan Shessley, Director, Trade Support Team, APHIS, International Services presented a paper on GATT and NAFTA: Impact on USDA and Agriculture. In November 1993, the U.S. Congress ratified the North American Free Trade Agreement. This agreement is aimed at reducing and eliminating barriers to trade, investment, and services between the United States, Canada, and Mexico. NAFTA creates the largest free trade area in the world with 360 million people and a gross national product (GNP) totalling $6 trillion.

The Uruguay Round of the General Agreement for Tariffs and Trade (GATT) came to a completion in December 1993. The agreement was officially signed by member countries on April 15, 1994, and is waiting approval by Congress. GATT is aimed at reducing and eliminating barriers to trade, investment, and services between the 100 plus signatory countries. Besides eliminating traditional barriers such as quotas and tariffs, both GATT and NAFTA attempt to control the use of non-tariff barriers, such as unjustified technical health standards. There are key sanitary and phytosanitary measures in both NAFTA and GATT as described below:

1. BASIC RIGHTS—A country has a right to adopt and maintain any measures it believes are necessary to protect the health of its plants and
animals as long as these measures are based on science, transparent in the way they were developed and implemented, and do not arbitrarily or unjustifiably discriminate between its goods and like goods, another party, or between goods of another party where identical or similar conditions prevail.

2. RISK ASSESSMENT--The requirement that measures be based on science means that import decisions must be based on risk assessment which uses scientific data and methodologies.

3. EQUIVALENCE--This provision commits GATT and NAFTA signatory countries to treat an exporting country's sanitary and phytosanitary measures as equivalent to its own when the exporting country demonstrates, using scientific evidence, that its measures achieve the importing country's desired level of protection.

4. ROLE OF INTERNATIONAL STANDARDS ORGANIZATIONS--Basic to GATT and NAFTA are the provisions encouraging countries to base their sanitary and phytosanitary measures as much as possible on international standards, whenever such standards exist. Animal health measures should be based on Office of International Epizootic (OIE) standards and plant health measures are to be based on the International Plant Protection Convention (IPPC) standards.

5. REGIONALIZATION--GATT and NAFTA commit countries to recognizing disease and pest free areas as well as areas of low pest and disease prevalence.

6. TRANSPARENCY--NAFTA and GATT include various provisions which encourage greater transparency of the domestic rule making process which affect trade. This requires member countries to provide advance notification of any new or modified regulations which may negatively affect the exports of another country.

7. SANITARY AND PHYTOSANITARY COMMITTEES--Both NAFTA and GATT call for formation of sanitary and phytosanitary committees. APHIS as well as USDA's Foreign Agricultural Service (FAS) are participants in these committees to oversee and monitor implementation of the agreements of the sanitary and phytosanitary provisions.

8. CONSULTATIONS AND DISPUTE SETTLEMENT--Both NAFTA and GATT establish two separate and distinct dispute settlement systems. Both systems share the common feature that dispute settlement begins with technical consultations at the sanitary and phytosanitary committee level.

Veterinary research scientists can continue to play a vital role in advancing free trade objectives while ensuring against the spread of diseases by focusing on research which may yield new technologies to reduce animal health related risks to levels which allow for safe trade of animals and products. In particular, emphasis has shifted from zero-risk based regulation and decision making to the development of risk assessment tools and the application of new and/or varied risk management strategies to allow
trade in low-risk commodities. Given the shift away from the "zero-risk" paradigm, increasing emphasis will be placed on developing technologies, strategies, and tools to allow for trade while ensuring that risk is reduced to minimal levels. The veterinary field will be an important contributor to developing these new tools.

Dr. Althea Langston, USDA, APHIS, Policy and Program Development, gave a report on Aquaculture, Aquatic nuisance species, and non-indigenous aquatic species. Aquaculture in the United States includes aquarium/ornamental species, food products, aquatic plants, and other aquatic segments. The total value of this industry encompasses 6,400-6,700 producers and 1.5 billion dollars not including allied segments. Import of non-mammalian aquatic animals is currently controlled by individual states except for species designated as injurious wildlife or live salmonids which could carry viruses injurious to wild fish in the United States. Aquaculture has historically been regulated by state departments of fish and game and departments of natural resources but this is gradually changing to departments of agriculture. The introduction of the zebra mussel into the Great Lakes raised concern over exotic aquatic species introduction and the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 was passed and a multi-agency task force was developed. State and Federal departments of agriculture will have expanded roles in aquaculture in the future. Aquaculture and wild or stocked aquatic animals often share water systems and this may lead to increased environmental concerns. Acquisition, treatment, and disposal of water are major concerns as is disease.

Dr. John Huntley (State Veterinarian, New York), Dr. Kenneth Thomazin (Chief, Animal Health Branch, California), Dr. George Edwards (State Veterinarian, North Carolina), Dr. Bret Marsh (State Veterinarian, Indiana), and Dr. John Williams (USDA, APHIS, VS, Emergency Programs) conducted a panel discussion concerning the states animal carcass disposal concerns and environmental issues. Legal ways to dispose of animal carcasses were discussed. Dr. Williams urged all state veterinarians to become acquainted with officials in their respective states who are responsible for environmental protection prior to the need to dispose of a large number of carcasses or diseased carcasses. It is easier to work with individuals you know than to meet individuals in the face of a crisis.

There were no resolutions brought before this committee and the committee adjourned at 5:20 p.m.
FOREIGN ANIMAL DISEASE AWARENESS AMONG VETERINARY PRACTITIONERS IN ALABAMA

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Veterinary practitioners are considered the first line of defense in the detection and reporting of Foreign Animal Diseases (FAD). However, there continues to be a rather low level of reporting of such suspected conditions by practitioners in many states annually. This is due in part to the fact that in the work day of busy practitioners, among the many disease conditions seen, those resembling a FAD are seldom encountered. Therefore, there is a concern as to the level of FAD awareness and reporting as well as the current state of knowledge of those foreign diseases that pose a threat.

In May 1994, a Foreign Animal Disease Awareness Survey was conducted among veterinarians licensed to practice in Alabama. A two-page questionnaire was mailed to 868 veterinarians with a cover letter explaining the purpose of the survey, a separate note to be completed if the practitioner desired a personal visit to discuss FAD and a return addressed postage paid envelope. Also, included were several informational color brochures describing selected diseases of foreign origin that affect farm and companion animals and avian species.

The intent of the survey was two fold:

A. To obtain practitioner input so their level of FAD awareness and degree of reporting could be determined along with learning the needs veterinarians have in increasing their knowledge in this important area.

B. By the simple act of the practitioner receiving the questionnaire, written material and informational brochures, it was anticipated awareness would be elevated to some degree by bringing the subject to mind.

The response rate of veterinarians to the survey was 31% (266 of 855 deliverable questionnaires). Two hundred and twenty-nine (229) of those responding were determined to be in active practice attending to species of animals or birds susceptible to foreign animal diseases with 64% of these being farm animal veterinarians and 36% small animal/avian practices. Twenty-four veterinarians (9%) asked for a visit to their clinic to discuss FAD matters.

Veterinarians were asked which disease they thought of first when FAD was mentioned. Seventy-five percent of those responding to the question (162 of 216) stated foot and mouth disease. Exotic newcastle disease and
CHEATHAM

screwworm myiasis were the next two in order. Several domestic diseases rather than FAD were indicated such as bluetongue, anthrax and tuberculosis. Only 29 veterinarians said they had opportunity to see a disease that resembled a FAD in the last 12 months.

In assessing how knowledgeable veterinarians thought they were in recognizing clinical signs or necropsy findings of FAD, 11 diseases were listed and the veterinarian was asked whether they felt very knowledgeable, moderately knowledgeable or needed a refresher. The diseases listed were exotic newcastle disease, African horse sickness, foot and mouth disease, vesicular exanthema, swine vesicular disease, African swine fever, hog cholera, heartwater, screwworm myiasis, rinderpest and bovine spongiform encephalopathy. Averaging the overall totals, only 8% of practitioners felt very knowledgeable, 30% moderately knowledgeable and 62% stated they needed a refresher. Those conditions where the greatest knowledge deficiency was expressed was African swine fever, African horse sickness and heartwater. Those that felt most comfortable in diagnosis were foot and mouth disease, hog cholera and screwworm myiasis.

Similarly, when asked the ability to differentiate between domestic diseases and the 11 listed foreign diseases, only 3% (7 of 245) felt very knowledgeable, 36% (88 of 245) felt moderately knowledgeable and 61% (150 of 245) stated they needed a refresher. The non-response rate to the knowledge questions was less than 8%.

Fifty-four percent of veterinarians thought that an introduced vesicular disease would be the one most apt to affect the wildlife population. Most respondents, 90%, were correct in stating that vesicular stomatitis, almost indistinguishable from foot and mouth disease, occurs periodically in the U.S.

Veterinarians were asked whether they were familiar with the Regional Emergency Animal Disease Organization (READEO). Twenty-four percent were aware of the organization, 67% were not and 9% did not respond to the question. A further analysis showed a higher percentage of farm animal veterinarians had heard of the READEO than small animal/avian practices.

An all important question asked was to whom the veterinarians would report if a condition were seen that resembled a FAD and did they have that person's phone number readily accessible. Fourteen percent either did not know to whom to report or did not respond to this question. Eighty-six percent would report to a regulatory source such as the state veterinarian or USDA-VS central office, a state or federal veterinary medical officer or a state diagnostic laboratory. Concerning having a readily accessible phone number, 77% of practitioners responded in the affirmative, but 23% either did not have a phone number or did not provide an answer to the question.

One of the main objectives of the survey was to ascertain the veterinarians' desires as to receiving FAD information and increasing knowledge in
FOREIGN ANIMAL DISEASE AWARENESS IN ALABAMA

this field. Overall, 73% of practitioners said it would be beneficial if more FAD topics were on the agenda of state and local meetings. When asked specifically how state and federal animal health officials could assist in FAD awareness, 137 of 266 (52%) responded to the question. Of the 137 respondents, 47% (65) asked for more FAD informational mailings such as newsletters and articles on current developments, 22% (30) wanted more FAD presentations at meetings, 11% (15) said a combination of mailouts and FAD presentations at meetings was desirable, 15% (20) felt a need for more personal contact with state and federal officials and 5% (7) were miscellaneous suggestions.

Findings And Recommendations:
The findings of the survey indicate a genuine interest among veterinarians in increasing their knowledge in FAD information and diagnosis. Although few report seeing conditions resembling foreign diseases, most know to whom to report and have a phone number. Those veterinarians who expressed they either lack sufficient knowledge in certain foreign diseases or would not know to report the condition poses a risk in missing an early diagnosis of a FAD. Depending on the disease, a missed diagnosis could result in disastrous consequences to our animal or avian population.

The following recommendations are intended to be implemented in Alabama:

- Provide a copy of this survey report to veterinarians practicing in Alabama and to Colleges of Veterinary Medicine at Auburn and Tuskegee, Alabama.
- Visit those 24 veterinarians from this survey requesting such visit, discuss FAD and leave informational material.
- Issue an updated list of reportable disease to practitioners.
- Provide a specially designed magnetic sticker to each veterinary clinic with the wording “Foreign Animal Disease Alert” and the phone number of the state-federal office.
- Submit an article on a selected foreign animal disease and any current developments for each issue of the quarterly Animal Health Newsletter for practitioners.
- Offer FAD presentations at the annual Food Animal Health Conference and at state and local VMA meetings.
- Increase personal contact between state and federal veterinary medical officers and practitioners to assist in FAD awareness.
- Be available to make presentations on FAD topics to the senior class of the two Colleges of Veterinary Medicine in Alabama.

I would like to acknowledge and thank the veterinarians in Alabama for their participation in this study and for their valuable input that made this effort a success.
IMPORTATION (AND MOVEMENT) OF NON-MAMMALIAN AQUATIC ANIMALS: AQUACULTURE, AQUATIC NUISANCE SPECIES, AND NONINDIGENOUS AQUATIC SPECIES.

Althaea Langston, D.V.M, M.P.V.M.
United States Department of Agriculture
Animal and Plant Health Inspection Service
Policy and Program Development

At the present time importations of live non-mammalian aquatic animals are controlled by the individual states. This is true except for those species designated in Title 50 (by authority of the Lacey Act) as injurious wildlife, and for live salmonids (trout, salmon, Arctic char) (fish and eggs) which could carry viruses transmissible to wild fish in the United States. These viruses are viscerotrophic hemorrhagic septicemia (VHS), infectious pancreatic necrosis (IPN), infectious hematopoietic necrosis (IHN), and oncorhynchus masou virus (OMV).

The present system of regulation occurred because fish and wildlife were not mentioned in the Constitution, and thus became a states right. In the early days of aquaculture development in this country, fish were regulated by state departments of fish and game or natural resources, and usually not by state departments of agriculture. This situation is changing now, however, as aquaculture is recognized as an important and growing segment of agriculture. Some states have transferred responsibility for aquaculture to the state department of agriculture, and in many states responsibility for aquaculture is shared between departments of agriculture and natural resources. Increasingly state veterinary laboratories are serving the aquaculture industry, both within the state and from other states.

Aquaculturists and researchers for many years have been concerned about the ease with which fish pathogens can be brought into this country. Indeed, the Title 50 regulations specify that the viruses cannot be brought in in live fish or their eggs; pure cultures of exotic fish pathogens are not restricted in their entry; nor are noninfected salmonids and all other fish which are not designated as injurious wildlife restricted in entry.

Introduction of the zebra mussel into the Great Lakes, however, raised concern about exotic aquatic species introductions, and the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 was passed. A multi-agency federal Aquatic Nuisance Species Task Force was created, co-chaired by the National Oceanic and Atmospheric Administration and the U.S. Fish and Wildlife Service; federal committee member agencies are the State Department, the Army Corps of Engineers, the Environmental Protection Agency, and the U.S. Department of Agriculture's Animal and...
IMPORTATION (AND MOVEMENT) OF NON-MAMMALIAN AQUATIC ANIMALS

Plant Health Inspection Service (APHIS). Additionally, non-federal ad hoc members represent Indian tribes, water and electric utilities, boating interests, and aquaculture. Several activities were mandated by the act, among these the examination of intentional introductions, and the development of a protocol to prevent introductions caused by research on nonindigenous aquatic species. The Intentional Introductions Policy Review Committee examined presently used and potential methods for regulating or controlling the introduction or importation of nonindigenous species into the U.S., and made several recommendations to Congress in its report. Among these is the recommendation that a formal permit system be established to control the entry of nonindigenous aquatic species into the United States. This was proposed by APHIS and the Agricultural Research Service, and included a provision for joint signoff on permits by the Fish and Wildlife Service and/or the National Marine Fisheries Service as applicable.

Regulation of aquaculture in the U.S. is in a transition from natural resources to agriculture, and from total state regulation to a combination of state and federal regulation. This change is driven by the needs of the aquaculture industry, by import requirements of other countries and trading blocs such as the European Union, and by public concern over the destructive economic and environmental potential of exotic species. This concern was addressed by a recent report from Congress' Office of Technology Assessment entitled "Harmful Non-Indigenous Species in the United States."

In the future both state and federal departments of agriculture will have new and considerably expanded roles in aquaculture and in the monitoring and regulation of entry of nonindigenous species. APHIS' present activities in aquaculture include licensing of vaccines for use in aquaculture and other animals, animal damage control activities, import restrictions on aquatic plants to protect against importation and dissemination of plant pests and noxious weeds, a limited amount of diagnostic laboratory consultation, program development and planning, and export health certification for aquatic plants and animals. Export animal health certification for aquatic animals has been done by APHIS on an ad hoc, as needed basis as a service to the industry for many years; this year it has become a formal activity, with primary users presently the tropical fish industry in Florida, and the salmonid broodstock industry in the Pacific northwest. These are both large valuation industries with major export segments; the 1993 estimated value (domestic and export) for the salmon and trout egg industry was more than $60 million, and the U.S. retail ornamental and aquarium fish industry was valued at over $600 million.

An aquaculture plan for the veterinary side of APHIS was recently developed, and includes expanded health certification activities, expert and reference laboratory services, production and standardization of non-commercially available aquaculture references and reagents, development of new testing methods of aquatic animal disease, import aquatic animal dis-
ease and pest safeguards, cooperative state/federal/industry aquatic animal improvement programs, identification and disease reporting systems, disease control programs, and expanded international animal and plant health activities.

Future aquatic nuisance species-related activities include import permitting, sampling and inspection of ballast water for living organisms or to verify that exchange has taken place (Plant Protection and Quarantine officers would have this responsibility), and programs to certify that transported live aquatic organisms (such as bait fish) are not carrying certain aquatic nuisance species (such as zebra mussel larvae) which could be damaging to the receiving environment.

Environmental considerations will also be a part of aquatic organism import permitting. Because aquaculture and wild or stocked aquatic animals often share the same water systems, and in some cases are the same species, environmental concerns are more intense for the aquaculture industry than they have been for terrestrial livestock industries. Acquisition, treatment and disposal of water is a major concern, as is the disease or infection status of seed stock or fish introduced into the system, including not only aquaculture fish, but also fish stock into common waters by natural resource agencies.

All this leads to new roles and new interactions for department of agriculture, fish and wildlife/natural resources, and environmental protection, both state and federal. Most states have aquaculture coordinators, usually in the department of agriculture, sometimes in natural resources, but always in a coordinating and facilitating role. There are aquaculture associations in almost every state, and industry associations exist, both general and species based. Several state veterinary diagnostic laboratories provide service to aquaculture. Animal aquaculture in the U.S. is a multisegmented $1.4 billion industry (see table) which will benefit and con-
**Figure 1**

Estimated Value of Some Segments of the Aquaculture Industry

(Farm gate value for all but aquarium/ornamental fish, which is retail)

(Value is for food animals except aquarium, ornamental, bait fish, eggs, plants)

(Fry, fingerlings not included except for bass; egg sales salmon & trout only)

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Produce (number)</th>
<th>Production (in pounds)</th>
<th>Value</th>
<th>States (primary listed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquarium/ornamental</td>
<td>1993</td>
<td>750-1000</td>
<td>—</td>
<td>&gt;$800 million</td>
<td>FL, CA, TX, MO, AR, MD, all</td>
</tr>
<tr>
<td>Catfish</td>
<td>1993</td>
<td>1400</td>
<td>466 million</td>
<td>$370 million</td>
<td>MS, AL, AR, LA others</td>
</tr>
<tr>
<td>Oysters, Clams, Mussels</td>
<td>1992-93</td>
<td>8007</td>
<td>—</td>
<td>&gt;$171 million</td>
<td>WA, CT, CA, OR, NV, FL others</td>
</tr>
<tr>
<td>Trout</td>
<td>1993</td>
<td>457</td>
<td>55 million</td>
<td>$69 million</td>
<td>ID, many others</td>
</tr>
<tr>
<td>Salmon</td>
<td>1993</td>
<td>50</td>
<td>26 million</td>
<td>&gt;$60 million</td>
<td>ME, WA, others</td>
</tr>
<tr>
<td>Bass Fish</td>
<td>1993</td>
<td>280-300</td>
<td>50,000 acres</td>
<td>$55 million</td>
<td>80% AR, MN, AL, LA, WI, others</td>
</tr>
<tr>
<td>Crawfish</td>
<td>1993</td>
<td>2000</td>
<td>60 million</td>
<td>$30 million</td>
<td>90% LA, TX, AR, AL, NC, SC, GA, FL</td>
</tr>
<tr>
<td>Salmon &amp; Trout Eggs</td>
<td>1993</td>
<td>34</td>
<td>—</td>
<td>$21-26 million</td>
<td>WA, ID, ME, Midwest, Tribes, CA, NE</td>
</tr>
<tr>
<td>Striped bass/hybrids</td>
<td>1993</td>
<td>50-60</td>
<td>&gt;6 million</td>
<td>$21.2 million</td>
<td>CA, SE, TX, FL, MS, VA, MD</td>
</tr>
<tr>
<td>Shrimp</td>
<td>1992</td>
<td>26**</td>
<td>5.6 million</td>
<td>$15 million</td>
<td>TX, SC, HI</td>
</tr>
<tr>
<td>Tilapia</td>
<td>1993</td>
<td>200</td>
<td>12.5 million</td>
<td>$17 million</td>
<td>CA, AR, ND, ID, MS (not AK, ME)</td>
</tr>
<tr>
<td>Alligators</td>
<td>1991-2</td>
<td>128</td>
<td>+</td>
<td>$13.1 million</td>
<td>70% LA, 25% FL</td>
</tr>
<tr>
<td>Ornamental Goldfish</td>
<td>1993</td>
<td>5 major</td>
<td>—</td>
<td>$10-15 million</td>
<td>AR, MD, PA, MO, AL, FL, KS</td>
</tr>
<tr>
<td>Ablone</td>
<td>1992</td>
<td>12</td>
<td>—</td>
<td>$2 million</td>
<td>CA</td>
</tr>
<tr>
<td>Aquatic Plants:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornamental, mitigation</td>
<td>1991-2</td>
<td>107+</td>
<td>—</td>
<td>$12 million</td>
<td>FL (most), HI, MD, TX</td>
</tr>
<tr>
<td>Waterlily</td>
<td>1993</td>
<td>110</td>
<td>—</td>
<td>$3 million</td>
<td>HI</td>
</tr>
<tr>
<td>Macroalgae</td>
<td>1992-93</td>
<td>4 major</td>
<td>—</td>
<td>$10-11 million</td>
<td>CA, HI, MD</td>
</tr>
<tr>
<td>Macroalgae</td>
<td>1992</td>
<td>5</td>
<td>—</td>
<td>$1 million</td>
<td>HI</td>
</tr>
</tbody>
</table>

Major segments of aquaculture not included: support industries (e.g., embryonated eggs other than salmon and trout, fry, fingerlings, shrimp postlarvae, feed, tanks, oxygen systems, equipment, processors, vaccine and pharmaceutical/chemical manufactures).

*Sources: Salmon, Trout, Catfish, USDA, ERS
Others: Industry associations and individuals, State Departments of Agriculture and Wildlife and Fisheries, University specialists, Bob Rosenberry (shrimp).

**Also 9 shrimp hatcheries producing postlarvae; no value available.

<table>
<thead>
<tr>
<th>Alligators:</th>
<th>Hides</th>
<th>Values</th>
<th>Meat</th>
<th>Value</th>
<th>Total Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992 LA</td>
<td>128,300</td>
<td>$6,206,128</td>
<td>577,062 lb</td>
<td>$2,569,000</td>
<td>$8,772,128</td>
</tr>
<tr>
<td>1991 FL</td>
<td>17,800</td>
<td>$3,600,000</td>
<td>163,000 lb</td>
<td>$924,000</td>
<td>$4,420,000</td>
</tr>
<tr>
<td>TOTALS</td>
<td>145,200</td>
<td>$9,806,128</td>
<td>740,062 lb</td>
<td>$3,390,000</td>
<td>$13,172,128</td>
</tr>
</tbody>
</table>

+++ Three large scale farms employ 103 people, sell into an international market

# Totals for segments of aquaculture industry in chart above.

Aquatic plants: >225 producers $26-27 million
Aquatic animals: 6150-6475 producers $1, 454-1, 464 billion
Total: 8419-8699 producers >$1, 400-1, 491 billion
Dr. Spencer Garrett, National Marine Fisheries Service (N.M.F.S.) reviewed the history of a HACCP program. The Menhaden fishmeal industry and the NMFS have participated in a federal inspection program for fishmeal products for 20 years. The program is only slightly modified from that agreed to by the USAHA nearly 20 years ago. The program is recognized for its effectiveness and held up as a model which should be made available to other segments of the animal feed industry. The program is not based on a zero tolerance rather the plan is \( n=0, c=1 \) employed three times during the six months fishmeal production periods. The program operates under a memorandum of understanding with FDA and USDA. The fishmeal inspection program is converting to the HACCP concept. HACCP is recognized as a superior approach for process control provided the "traditional" regulatory pitfalls can be overcome. To do this NMFS is asking to design, test and formulate new inspection programs based on the HACCP concept. This preliminary model is currently being tested. It may well be that the Menhaden fishmeal industry will be the first segment of the animal feed industry to have a federally recognized HACCP program to control Salmonella.
Dr. Doug Stidham related the experience with Salmonella Control in a farm-supply cooperative (West Central Cooperative). This company manufacturers a soybean meal product. In January, 1993, West Central Cooperative agreed to participate in a FDA conducted voluntary salmonella survey of its product. Initial monitoring indicated positive salmonella assays in the environment and in the finished product samples. For the next 12 months, West Central personnel worked to identify problem areas, eradicate the salmonella organism and finally to institute an effective HACCP plan.

FDA Survey results on Salmonella Contamination of Animal and Vegetable Protein Products was presented by Dr. Daniel G. McChesney, C.V.M. FDA collected samples of products (described by the manufacturer as meal) from 78 animal protein processors and 46 vegetable protein processors in 1993. Sixty-two percent of the animal protein and 37% of the vegetable protein processors had product testing positive for salmonella.

Dr. G.A. Mitchell, FDA/CVM discussed preharvest Food Safety Initiatives by FDA and USDA emphasizing unmet hazards and risk characterization assessments for feed. He indicated that it was not the intention that firms scrap functioning quality assurance programs, but rather that they review and expand in order to improve their programs. There are a number of major segments of the feed industry that has developed HACCP programs. In addition to being preventive and more efficient in nature, the HACCP approach offers additional benefits.

HACCP is being discussed in producer organizations and among federal agencies. At the federal level, the current action is as follows:

- HACCP for seafood - proposed final rule (FDA) FR 1/28
- HACCP for landfood - ANPR (FDA) FR 8/4
- HACCP for FSIS slaughter plants (FSIS) promised by the end of year
- HACCP for live animals (APHIS) Pathogen Reduction Act
- HACCP for live animals (GAO) report 9/28/94

Report of the Feed and Feed Ingredient Sub-Committee
Presented by DR. JHUNG-WON COLBY

Report of the Feed and Feed Ingredient Sub-Committee was presented by Dr. Jhung-Won Colby. She indicated that the Perdue Inc. has started to use a HACCP program in several of their mills.

Critical control points were illustrated in a flow diagram and include incoming material, conveyers, pellet mill, pellet coolers and other areas where moisture can result in material build-up. Examples were given showing how control of critical points can be improved.
FEED SAFETY

The Report of the Transportation Subcommittee
Presented by TIM MILLER

The following is the Committee’s thoughts on provisional guidelines for developing a HACCP plan for individual plants.

A Transportation Critical Control Point is defined as those points at which there is a high probability that improper control may cause, allow or contribute to contamination or recontamination.

This plan is not limited to microbial hazards but has been developed as a response to the control of salmonella in finished feeds.

I. Assessment of Hazards
   Microbiological contamination and “other”

II. Critical Control Point
   Loading of empty truck, car, trailer, hopper, barge or tanker

III. Critical Limits
   A. Car, Truck, Trailer
      Product shall be loaded only in suitable and clean cars, trucks or trailers. As a minimum requirement, vehicles shall protect product from weather and road contamination. All interior surfaces must be clean and intact. Closed doors must produce a dust-proof seal.
      1. Requirements of the Conveyance
         a. The integrity of the conveyance must be such that no external entry of insects, moisture, rain, or spray, that can compromise the load is permitted when the various covers are in place.
         b. The conveyance must be designed to be clean or cleanable when empty.
            1) Load integrity includes lack of contamination from previous loads.
            2) To reduce contaminants there must be no shelter in the conveyance that permits harborage of filth or material foreign to the load.

   B. Requirements for the Shipper of an Ingredient
      1. Before loading any conveyance, that conveyance must be inspected for integrity of closures including slide gates, hatch covers, tarp, etc.
      2. Before loading any conveyance, the interior must be inspected for residual contamination. If any is found, the conveyance must be cleaned before loading.
      3. After loading the conveyance, it must be sealed and any spillage on the conveyance cleaned thus precluding growth of microorganisms and infestation by insects.

IV. Procedures for Monitoring
   A. The individual responsible for the load will inspect empty truck for debris, moisture, etc. (as outlined in critical limits).
REPORT OF THE COMMITTEE

B. The individual responsible for the load will inspect doors, lids, tarp, tie-downs, etc. of each load.

V. Corrective Action
   A. If empty truck is not clean, all debris to be swept and cleaned out prior to loading.
   B. Faulty lids or doors on ripped tarps must be repaired prior to loading or product.

VI. Record Keeping System
   The individual responsible for the load will fill out (initial) an inspection form on every load.

VII. Verification of HACCP
   Supervisor will review "Form" for each load monthly.

VIII. Comments
   Comments on these procedures and guidelines are requested.

1994 Report of the Microbiology Subcommittee of the USAHA Feed Committee
Given by Dr. Stan Bailey

The microbiology subcommittee would like to dedicate this report to a valued member and friend, Glen Snoeyenbos who died last year.

Microbiology Subcommittee Charges

A new baseline survey is necessary to properly define the contemporary prevalence of salmonellae in the animal feed industry. The previous FDA baseline surveys did not use the committee's proposed sample unit compositing scheme which allows for more qualification and understanding of positive data. The committee's understanding is that we need to develop two sampling plans. One for the USAHA baseline study which could be used to monitor progress over time. Under this plan companies would voluntarily pull samples according to the USAHA protocol, analyze them and supply the data to Dr. Frank Jones, North Carolina State University. Dr. Jones will accumulate, code and analyze the data.

Dr. Al Rainosek of the University of South Alabama will assist in analysis of the data. An operational protocol for the conduct of the baseline survey will be written by Dr. Jones, further the FDA will be requested to provide funding for sample collection kit preparation, mailing, data analysis, etc. It is anticipated that this baseline survey will begin the Fall of 1995. All participating companies in the baseline survey will be responsible for sampling, analysis and forwarding data to Dr. Jones. Upon completion of the baseline survey, industry would know what the salmonellae prevalence rates are. Such knowledge will allow them to establish international sampling plans for routine monitoring to measure reduction of salmonellae levels. A second sampling plan for routine monitoring by regulatory agencies.
FEED SAFETY

will be developed by USAHA with specific numbers of sample units to be evaluated dependent on the results of the baseline survey. This routine monitoring plan could voluntarily be incorporated into individual plant sampling plans to predict what the regulatory agency would find if they sampled the plant.

Definition of terms:

For both the baseline and FDA monitoring plan $n$ refers to the number of subsamples taken and $c$ is the acceptance number.

Baseline prevalence survey

In order to avoid confusion, we should define terms similar to the Category I, II or III for foods [FDA (FAM, 1978)]. After much discussion, the committee recommends that for the USAHA baseline background study we establish an FDA Category II Foods approach using the ICMSF-tightened sampling plan of $n=30$. In order to understand how extensive salmonellae contamination is within lots, samples could be composited for initial analyses, but should then be examined individually if a composite tests positive.

Possible routine monitoring plan

For monitoring by a regulatory agency or others (at least on an interim basis until the baseline is established and prevalence rate of different feed types is established) the ICMSF recommended sampling plans of $n=10, c=0$ for routine (normal) or $n=5, c=0$ (reduced) depending on the product being sampled should be used. These plans as well as others could be reconsidered depending on the results of the survey. The reasoning for the recommendation of this less intensive sampling plan is as follows. FDA has currently stated that they will have a zero salmonellae as reflected by sampling plan for feeds and feed ingredients. With a zero tolerance, $c$ must always equal 0 in the sampling plan. Without a clear knowledge of what the baseline study will show it is the general observation that the prevalence of salmonellae in feed ingredients and mash feeds could easily run in the 20% to 50% positive range and grains and pelleted feeds could run up to 10% positive. With these expected high prevalence rates, more stringent sampling plans (e.g. FDA's $n=30, c=0$) will almost always test positive. With all processors failing to meet the requirement, it would be impossible to differentiate processors with <20% prevalence from those with >20%, or for that matter a processor with an 80% prevalence. Thus making it extremely difficult for processors with the highest prevalence rates of salmonellae. The percent chance of lot acceptance tables for the recommended ICMSF sampling plans and more stringent sampling plans (including FDA's sampling plan for Category I, II or III Foods) appear in Appendix II.
USAHA Baseline Study:

The USAHA baseline study will be confined to swine, poultry, beef, dairy and fish feeds and ingredients. The feed and feed ingredients subcommittee will be asked for recommendations on how best to limit the scope of the study to determine the prevalence of salmonellae in the finished feed and ingredient facilities by recommending broad product categories for analysis. The microbiology subcommittee requests the feed and feed ingredients subcommittee to make the appropriate product category recommendations by January 1, 1995. However, we commend the feed safety committee adopt this microbiology subcommittee report, to be modified by subsequent recommendations of the Feed and Feed Ingredient Subcommittee for specific broad categories. Further it should be understood that the Microbiology Subcommittee is recommending three separate surveys. One to be conducted of the feed ingredient industry, another to be conducted at feed mills to include finished feeds and their ingredients and the third of transportation vehicles. These studies will not be conducted concurrently however.

Sampling size and protocol.

Based on categories, pull 30 subsamples of a minimum 10' g each. Then form 5 composites by taking 25 g from each of six subsamples. After thorough mixing, a 25 g sample (analytical unit) from each of the composites will be analyzed for salmonellae. For each composite that is positive, we recommend that each individual sample that went into the composite then be tested individually.

Sample Sites:

Samples of 30 subsamples each should be pulled from products at the load out site.

Frequency:

The baseline survey will be for one year with samples to be collected and analyzed quarterly for appropriate categories.

Sample handling and analysis:

Individual producers will pull samples. Analyses can be conducted in house or sent to independent laboratories. The proposed BAM method will be used.

Monitoring Plan/Protocol

Sample size:

ICMSF has recommended the n=5 and n=10 routine sampling plan for routine (normal) control (See Appendix II, pages 1 and 2). We will use the
terms degrees of hazard, reduced (n=5) and normal (n=10). The terms are defined to allow regulatory discretion in deciding the degree of risk. The committee recommends using these lower n figures on an interim basis until a clear understanding of what impact these data will have on the industry and regulatory agencies. Compositing of up to 5 subsamples samples is permitted. A minimum of 100 g should be collected for each subsample. The Microbiology Subcommittee emphasizes that with any sampling plan, zero tolerance means that salmonellae will not be detected using that sampling plan, not that the sample lot is assured of being truly negative for salmonellae.

**Sample size:**
- Samples should be at load out site.

**Frequency:**
- To be decided by regulatory agency.

**Transportation**
We recommend that the Transportation Subcommittee come up with sanitation GMP's for trucks, railcars, etc. As a background survey, we recommend sampling empty transport tanks, trailers or cars using the standard drag swab method used by the SE task force for environmental sampling to assess the frequency of salmonellae contamination.

**Live Production Subcommittee Report (Poultry)**
Presented by DR. TOM HOLDER

This group emphasized their recommendations for the poultry production area which are:
1. Establish a uniform testing procedure for the environment, feed and rodents.
2. Establish the salmonella risk in the environment thru drag swabs.
3. Buy pouls and breeder chicks from a company that is participating in a salmonella risk reduction program.
4. Eliminate or control rodents, pets, wild birds, flies and beetles from your poultry operations including feed and storage bins.
5. Buy animal protein and fish by-product ingredients from APPI or NMFS member companies participating in a salmonella risk reduction program.
7. Manufacture feeds at temperatures which will eliminate salmonella.
8. Control moisture in growing facilities thru proper ventilation and water management.
10. Feed and ingredient storage bins should be water tight, rodent and bird proof.

Live Production Subcommittee (Pork)
Presented by DR. BETH LAUTNER & DR. PAULA FEDORKA-CRAY

The National Pork Producers Council is including feed sampling for Salmonella in research projects that have been funded this year. Feed is being evaluated along with the introduction of breeding stock, waste management systems, pest control measures and hygienic practices for its potential to facilitate exposure to Salmonella on farms. Four projects have been funded which will provide information that will be used to develop generic HACCP plans for various types of production systems. Control of Salmonella in feeds will be one of the key components.

Dr. Paula Fedorka-Cray from the National Animal Disease Center in Ames, Iowa reviewed her producer-funded project to survey feed and feed components submitted from swine farms in different geographic regions for Salmonella. In addition to the sample testing, questionnaires will be administered to gather information about feed handling, feed storage and other management practices. This pilot study will provide the basis for developing additional survey projects.

NOTE: One resolution was unanimously passed which relates to the Preharvest and Postharvest programs.
FSIS and APHIS are involved in a new cooperative initiative toward improving food safety and have extended their efforts to include live animal production. Pre harvest Food Safety, the concept of involving the farming producer in all aspects of food safety is new. Monitoring and regulatory action by FSIS and FDA has caused the producer to take on the responsibility for preventing contamination of the food supply with the drugs and chemicals they utilize or may expose their animals to during production. However, the responsibility for providing human pathogen free or safe meat and animal products is a real and understandable concern to the livestock industry.

Physical and chemical contamination can be considered a fairly straightforward preventable problem. Physical and chemical contaminants may accumulate with repeated exposure but do not multiply or reproduce in livestock. The producer has control of the agents involved through testing and monitoring and the management of inputs, to assure that contamination does not and has not occurred. Physical contaminants have not been of major concern in recent years with modern livestock production systems. Chemical contamination has come under control by the industry over the past decade due to government monitoring and trace back which created accountability in a system where the producer controls the inputs.

Microbiological contamination poses a much different problem. The question begging an answer is: is it possible for the producer to change the microbiological flora of livestock produced for food, so that food safety risk is eliminated or even reduced? Pathogens, in very small numbers, can be introduced at a multitude of different points during the production of livestock and then can infect and multiply to create disease and or food safety problems. Current technology and production expertise does not provide the producer with control over all the points of introduction or the total ability to prevent infection or shedding. Since any one introduction can result in a problem, prevention in this living biological system must be all or none.

The Hazard Analysis Critical Control Points (HACCP) system of assuring compliance is cited as the method to use in assuring food safety. In production of live animals, it has disadvantages. The principals can be applied to food animal chemical and physical food safety. Applying the HACCP system to the control of human pathogens in livestock production is a very different situation and raises questions. A successful HACCP program requires a process or system with defined procedures that will result in a known quality end product. We don't have the capability or the
knowledge required to modify live production systems to assure no pathogens will be present at market time. We can identify a multitude of control points, where pathogens may be introduced or where factors may influence infection or shedding, and any one of them could be the critical one (control point that will assure safe food).

Using Quality Control Points or Food Safety Control Points with the goal of reduction and minimizing pathogens may be more appropriate and more acceptable to a quality conscious industry and ultimately the consuming public. Hazard is a very negative and undesirable term to associate with food when communicating with the consumer in the market place.

Livestock producers strive to maintain healthy disease free livestock in order to compete and to be economically viable. Management procedures required to prevent or minimize the introduction and contamination of food animal production systems with animal pathogens should be appropriate with human pathogens as well.

Good and best management practices (GMP's & BMP'S) have been developed by the various livestock industries for production of healthy disease free livestock. Many voluntary and government assisted disease control and eradication programs are established and working. These documents and programs need continual updating. It is an ongoing living process. Governmental agencies alone are not going to come up with new workable management and disease control practices for the industry that will be adopted. The effort must be cooperative. Research, both public and privately funded is essential to continually improve the efficiency of livestock production and the quality of the products produced. The improvements and the programs that are effective will be adopted by the industry very quickly via the information grapevine in our very competitive world of business.

FSIS has full time on site organoleptic and procedural inspection in processing plants to enforce compliance with minimum food safety standards. A multitude of sites make this impossible in live production.

Trace back is suggested by FSIS and APHIS as a tool to improve food safety in live production. It connotes the testing of animals and animal products during processing or distribution where there is a positive identification system in place allowing identification of the source live animal production unit should a food safety contaminant be detected. The agency involved would then attempt to determine the cause and the methods needed to eliminate the problem. This system makes the producer nervous with a number of very real concerns. First of all, the accuracy and validity of results is critical and a retest procedure for verification is essential. Second, government regulatory personnel are not well grounded and up to date in industry biosecurity and management programs (which are dynamic) and do not have credibility with the industry. Third, public release of negative food quality information can cause serious economic disruption of the pro-
ducer/processor or integrator and timing and sensitivity of reporting is critical. Fourth, liability from producer to processor to consumer is a serious concern and may involve business interruption losses as well as human suffering. All of these concerns indicate the necessity of a cooperative effort between government and industry.

Monitoring and testing the end product will not in itself insure food safety. It is an important tool, however making sure the current best production practices required to assure food safety are done, is more important. The industry in total should be stimulated to do what they know needs to be done and what is required to do to minimize the risk of pathogen contamination of live animals and animal food products. A regulatory trace back, verification and information system developed with industry input, could be a major factor in accomplishing this goal.

The practical goal for microbiological food safety in livestock production must be reduction or minimization and it would seem then that pasteurization of animal products during processing is necessary to reduce the pathogen level even closer to zero, and assure food safety in the market place. Additionally, since animal products may not be sterile with respect to pathogens and spoilage organisms, consumer education in the proper handling and distribution of food is also necessary.
The Committee on Food Safety was called to order by Chairman, Dr. Joe Blair at 1:30 p.m., October 31, 1994. Approximately 100 persons including twenty-three (23) Committee members were in attendance.

Harry C. Mussman, DVM, PhD., President & CEO of Food Technology Service, Inc., spoke to the group on the use of irradiation as a food pasteurization process for the prevention of foodborne illness. He illustrated the remarkable results that can be safely obtained with relatively low doses of irradiation and outlined how the process is being utilized throughout the world. Dr. Mussman mentioned that the resistance to irradiation in the U.S. is spearheaded by a small group of people with an emotional appeal and who disseminate information that is often distorted and inaccurate. He pointed out that over 70 years ago opponents of pasteurization of milk argued that by assuring cows were healthy, the milk collection system was clean and the processing of milk was carefully controlled, a certified safe raw milk could be produced. Subsequent experience showed that even with this early HACCP approach, disease transmission through milk continued. It was not until a "definitive control point" (DCP) -- pasteurization -- was added that disease transmission ceased. Dr. Mussman then drew the parallel with processed meat and poultry products, saying that even with the best possible HACCP system in place it was unlikely, because of their animal origin, these products could ever be produced pathogen free unless -- and he underscored this point -- a DCP were added to the system. He went
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on to say the only technology available at the present to provide this DCP is food irradiation (cold pasteurization).

Colonel Dale Boyle, DVM, MPH, U.S. Army Staff Veterinarian at Defense Logistics Agency presented an overview of Hazards Analysis Critical Control Points (HACCP) application by the Department of Defense (DoD). The DoD pioneered the original use of HACCP in the food industry to support the space program. The idea of experiencing preventable foodborne illness in the confines of a space suit was devastating to the fledgling space industry. The need for absolutely safe food prompted the original work by Scientists at Natick and Pillsbury. Despite the fact the original work was done by the DoD, little was done after that. More recently the U.S. Army Veterinary Corps came to the conclusion that HACCP presented an opportunity to take food safety and quality assurance to a new level. They went about establishing the programs necessary to transition to a HACCP based system of food inspection support. Current achievements include a training program, an application manual and model plans for commissary stores. With the assistance of U.S. Army Veterinary Service Personnel the Defense Commissary Agency includes HACCP principles in their Operations Manual and has allowed the implementation of HACCP in a few stores. Members of the DoD are participating in working groups with federal regulatory agencies and industry as the transition to a HACCP based system evolves. The DoD is anxious to see HACCP applied to the entire food industry. We believe everybody wins with HACCP. Food is safe, of consistent quality and profitable to the producer.

The next topic of discussion was the proposed legislation that would allow the interstate shipment of state inspected meat and poultry products.

Dr. Alice Johnson, Director, Regulatory Affairs, American Meat Institute (AMI) outlined her organization's position on the issue. The AMI is opposed to this change. The following is a summary of the position she stated:

- Small plants already have the option of going interstate via the use of Talmadge-Akin agreements that are currently available to the states. Under these cooperative agreements, plants operate as federally inspected establishments but the inspection is done by state employees with oversight from FSIS.
- Dr. Johnson stated that it is preferred to have a single system at the national level rather than numerous systems at the state level. She expressed concern that there would be a problem resolving a variety of policy differences.
- Uniformity and consistency in Labelling would be difficult to maintain.

Mr. Michael Mamminga, Bureau Chief, Iowa Meat & Poultry Inspection Program and President of the National Association of State Meat and Food Inspection Directors presented views in support of the legislation. His points
are summarized as follows:

- The states have now had over 20 years experience in running programs and that the FSIS oversight has changed over the years to give increasing responsibility to the states. Originally FSIS made a comprehensive review of each state plant at least once each year and often more frequently. The current system consists of a periodic auditing (on a 4 year cycle) of the state system with the state having full responsibility for conducting their program. The states have proven their ability to conduct "equal-to" programs.

- Meat and poultry products are the only foods that require federal inspection for interstate shipment. He gave several examples of similar foods including non-amenable meat products that presently can move interstate under state inspection.

- FSIS maintains similar auditing oversight of foreign systems. Imported meat and poultry products are allowed to move interstate.

- The states are matching federal funds dollar for dollar and thereby provide a very cost effective system of inspection. If the federal government were to assume full responsibility for all current state programs it would require a substantially higher Agency budget -- in the neighborhood of $30 million additional money.

There were some questions and comments from the floor with each individual commenting on the issues. Unfortunately there was not enough time for an in-depth discussion of the issues. Both individuals did agree that food safety was not an issue in the debate.

Dr. Bonnie Buntain, Team Leader, APHIS Preharvest Food Safety Project Team, presented an overview of the USDA/APHIS preharvest pathogen reduction food safety and quality assurance initiatives. The Secretary of Agriculture commissioned the Pathogen Reduction Task Force in late 1993 to provide leadership, coordination and oversight of the USDA's programs to ensure a safe and wholesome food supply. The Task Force, chaired by Acting Assistant Secretary Patricia Jensen and co-chaired by representatives of FDA, CDC, APHIS and FSIS, had the responsibility for coordination of all Departmental activities associated with pathogen reduction.

The first task was to establish three technical subcommittees to focus on research priorities in the farm-to-fork continuum. The Live Animal Production (Preharvest) subcommittee identified research priorities that included ecology and epidemiology, microbial physiology, pathogenicity, virulence and genetics, HACCP, economic and risk analyses, technology transfer and risk communication, and traceback technologies. All of these research priorities, except for the latter, were also identified as priorities in the other two subcommittees, Slaughter and Processing, and Food Preparation and Consumption.

In March of 1994, Dr. Lonnie King took a leadership role in the preharvest food safety program which culminated in the publication of the "Blue Book".
This book described the core strengths and services of APHIS which could enhance the preharvest food safety program. Dr. King provided nearly three-fourths of a million dollars to define APHIS's role and abilities for preharvest food safety work.

Included in this allocation of funds was the continued support of the Animal Production Technical Analysis Group which was initiated in the FSIS Track II reengineering plan. The report of this group identifies hazards and controls, and the risks and health impacts of foodborne pathogens.

APHIS also performed studies on the methodology of culture, ecology and epidemiology of *Escherichia coli* O157:H7. Some of the studies included an evaluation of automated identification equipment, fingerprint analysis of O157:H7 isolates from 1991-1992, development of the University of California's ELISA serological test and the NAHMS projects on dairy heifers and feedlot cattle.

In addition to performing pathogen studies in poultry (*Salmonella* spp.) and swine (*Trichinella* spp.), APHIS also supported the quality assurance programs in California for both dairy cattle and poultry.

Finally, APHIS/VS conducted five regional and area food safety/quality assurance workshops nationwide. These efforts created program enthusiasm and facilitated the involvement of VS in interactive and inter-disciplinary teams that included all of the stakeholders in QA and food safety programs.

The Goals identified by APHIS for future work include:

Further define USDA's policies, roles and collaborative efforts regarding investigations, traceback, traceforward, monitoring and surveillance for human foodborne outbreaks related or suspected to be related to foods of animal origin.

Work actively with livestock groups, State and Federal agencies, and other stakeholders to develop, support, monitor and coordinate commodity QA programs and pathogen reduction efforts.

Coordinate and monitor research activities to better understand factors influencing foodborne illness related to pathogens of animal origin.

Utilize monitoring, surveillance and outbreak investigation activities to learn more about animal production risk factors that affect the incidence of human foodborne illness outbreaks, identify trends that may affect the future incidence of human foodborne illness outbreaks, and promote good production practices and QA programs.

Integrate the *Salmonella enteritidis* program into the National Animal Production (Preharvest) Food Safety Program in order to utilize APHIS resources more effectively and efficiently.

Dr. Thomas M. Gomez of the USDA/APHIS/VS, presented an update on *E. coli* O157:H7 infection. His paper was also presented at the General
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Session and a copy of his presentation is included in its entirety as part of these proceedings.

Dr. Lyle Vogel, Associate Director for Membership Services of the American Veterinary Medical Association (AVMA) gave a brief report on his organization's activities in the food safety arena. They included:

- The development, publication and wide dissemination of an AVMA Food Safety Policy statement. (Copies of the statement were given to the participants and are available to any interested party by contacting the AVMA office.)
- The AVMA Council on Public Health and Regulatory Veterinary Medicine has established a Food Safety Committee which has been funded to allow two trips per year for its members to meet in Washington, DC. This allows them to meet with top officials of FSIS, APHIS, and FDA as well as with consumer organizations and other national trade organizations headquartered in Washington, DC. They also meet with the AVMA Governmental Relations Division and with various congressmen, senators and congressional staffers. The committee recently met with Mr. Michael Taylor, Administrator of FSIS and discussed research needs to meet the intent of the Pathogen Reduction Bill and expressed that there is insufficient research to establish microbiological standards for meat & poultry products at this time.
- The AVMA has taken a pro-active role in dealing with food safety issues. This included corresponding and meeting with organizations who have been critical of the role of veterinarians in food safety activities.

The Food Safety Committee considered and approved two resolutions. The first one urged the U.S.A.H.A. to establish a working group utilizing its committee system that would be available to participate with government in formulating policy and programs to achieve the food safety goals of the U.S. The second strongly urged the USDA to select a veterinarian for the currently vacant National Veterinary Extension Program Director position.

The meeting concluded at 5:30 p.m.
Introduction

The chart below shows the organization of the agricultural and veterinary medical services in Switzerland. The Department of Economy has both an Agricultural Section, with seven research divisions, and a Veterinary Services Section, with one research division. In addition, there are two Faculties of Veterinary Medicine at the Universities of Berne and Zürich. The research division of the Veterinary Services Section is the Institute of Virology and Immunoprophylaxis (IVI).

IVI was founded (under the original name of the Federal Vaccine Institute) by the government in 1942 with the main objective of being prepared for outbreaks of foot-and-mouth disease (FMD) which were occurring at regular intervals in Europe at that time; viz. the diagnosis of FMD and production of a vaccine against the disease. Since then the tide of opinion has changed, due to the present situation with respect to FMD, and prophylactic vaccination against the disease has now been terminated in Europe. However, other animal diseases which are exotic to Switzerland are now receiving more attention. The importance of this is seen from the fact that about 75% of the agricultural economy of Switzerland stems from animal production, roughly evenly divided between dairy and meat farming.

The IVI is entirely dedicated to animal health, in particular the control of Swiss National Foundation, from research on domesticated animals or from a foundation for alternative methods to animal vivisection. Approximately 60 people are employed at the IVI, about 25 of them are paid from other
A LIST A DISEASE LABORATORY IN A DAIRY AREA

Research aimed at improving and applying diagnostic procedures is being pursued against a number of OIE list A diseases. Specifically, these diseases are currently:

- African horse sickness (AHS)
- African swine fever (ASF)
- Blue tongue (BT)
- Classical swine fever (Hog cholera) (CSF)
- Foot-and-mouth disease (FMD)
- Peste des petits ruminants (PPR)
- Rinderpest (RP)
- Swine vesicular disease (SVD)

The New Institute

The site chosen for the new institute was close to the village of Mittelhäusern in the canton of Berne, permitting close access to the Veterinary Services Headquarters at Liebefeld on the outskirts of Berne and to the Faculty of Veterinary Medicine at the University of Berne. Mittelhäusern is a small village situated about 10 kilometers to the southwest of Berne. Only after a thorough investigation into the compatibility of such an institute to the region of Mittelhäusern with its agricultural environment were the excavations for the new institute begun in spring 1989. The final installations were done during summer 1992, and the official opening was held in September 1992, after an expenditure of 60 million Swiss Francs for the total construction and installation.

The construction is essentially characterized by clear spatial divisions between the individual working areas:

- high-security building with laboratory (HTL) and animal facility (HTT)
- offices in combination with animal stalls (RT)
- staff building (WO)

The high security area is separated from the other buildings and placed at the rear of the plot. There is a direct connecting path between the two working areas.

The High-Security Building

This central part of the building complex is divided into two main parts. Firstly, an animal facility with stalls, post mortem and dissection room, sewage treatment plant and animal carcass sterilization unit (autoclave). Secondly, a laboratory section with laboratory units, staff restaurant and library.
The institute is organized on five levels. At the bottom, level 1, there are the sewage treatment plant and the animal carcass autoclave. Level 2 is the so-called installations floor wherein is found all of the sanitary installations. The ground floor, or third level, is the actual working floor with the laboratories and the animal rooms. On level 4 are found the air filtration plants which are installed over the laboratories and the animal rooms. Level 5, which is outside the high-security section, contains the ventilation plant. Computer-controlled shower-locks and airlocks, together with extensive organizational measures, guarantee an optimum level of biological safety.

Safety

At the IVI our safety objectives are aimed less at the protection of the employee than primarily at making sure that no viruses are spread. The technical, structural and organizational safety measures are so well coordinated that an optimal protection for the environment is guaranteed. Good specialized training of the staff moreover ensures that the safety regulations are understood and translated into daily practice. It goes without saying that all technical installations relevant to safety are regularly checked for efficiency by internal specialists. The practical planning of safety aspects is rendered more difficult by the fact that safety cannot be directly measured, but exists merely as an ideal concept. Safety in a laboratory such as we have in the IVI means that risks are recognized, procedures, practices and safety equipment introduced and the relevant safety buildings constructed.

Containment

The term containment describes comprehensive safety measures which
guarantee the safe handling of infectious agents in a laboratory, reduce the contact between laboratory staff and the agent, and prevent the escape of potentially dangerous agents from the laboratory. A basic distinction is made between primary and secondary containment. Primary containment is aimed at protecting laboratory staff and the immediate surroundings of the laboratory from the infectious agent; it is guaranteed by good laboratory practices and the use of appropriate safety equipment. Secondary containment aimed at protecting the surroundings outside the laboratory from the disease-producing agents; it is realized by a combination of structural and organizational measures. Thus the four elements of containment are:

- laboratory techniques and practices
- safety equipment
- appropriate building construction and installations
- organizational measures

All of the micro-organisms of highest risk which are handled at the IVI are exclusively pathogenic viruses of animals. Thus, secondary containment must operate to the highest level. In contrast, the laboratories and animal rooms within the institute require a lower level since an infection of the staff is unlikely.

Whilst primary containment consists of standardized methods and safety equipment, secondary containment is a physical barrier realized by a safety concept compromising different technical systems. This safety concept consists of building an airtight physical cover through which all movement of material must be strictly controlled. The materials in question are on the
one hand air, fluids and solid bodies, and on the other hand personnel. Air is purified by filtration, fluids such as sewage are sterilized by heat, and solid bodies are sterilized by heat or chemicals, depending on whether they are resistant to heat or not. Staff wishing to leave the area of containment must shower thoroughly. This requires a multitude of complex technical systems which will be described below.

The Box-in-the-Box Principle

For safety reasons highly contaminated rooms are surrounded by less contaminated ones; the box-in-the-box principle. Thus, animal rooms containing infected animals are surrounded laterally by other animal rooms, and vertically by the filter floor above and the technical installations floor below. Consequently the highly contaminated rooms do not adjoin an outer wall. The maintenance of a differential negative pressure system keeps the disease-producing agents in the central part of the building. That is, a flow of air in the direction of the room with the highest level of contamination is produced by means of differences in pressure. Consequently, should there be any leakage in the system, the disease-producing agents could only be sucked into the inner part of the building and could not escape through the cover of the building into less contaminated areas or the environment.

The Airtight Box

The handling of highly infectious micro-organisms in the containment requires special building procedures. Among these are a special in situ concreting process using the Silica-Fume technique with appropriate follow-up treatment. Laminated epoxy resin surfaces, particularly in the animal stalls, special gas-tight constructions for windows and both shower- and airlocks all play their part in sealing off as tightly as possible the high-security area from the outside environment. The walls forming the boundaries of the physical containment are cast on the spot from a special concrete which must be as non-porous as possible and possess an extremely low degree of shrinkage in order to be gas-tight and without cracks. This objective is achieved by low water-cement ratios, the addition of extra chemicals (Silica-Fume), finely meshed reinforcement, careful follow-up treatment and long periods before the shuttering is stripped. All concrete surfaces are coated with a non-porous, five-layered coating of epoxy resin reinforced with glass-fibre matting in order to attain a surface structure which is chemical-resistant and easy both to clean and disinfect. For all necessary penetrations into the walls, fixations made of stainless steel are set in concrete in order to achieve a gas-tight connection between the fixations and the concrete box serving as the containment boundary. Gas-tight doors, made tight by means of lever-operated compressible seals, are constructed from stainless steel. The door frames in the containment boundaries are also concreted into the walls.
Man as the Weakest Point

In the whole safety system it is probably man who represents the weakest point. Unlike the materials, he cannot be so easily decontaminated. Moreover, as a possible carrier of the virus on his mucous membranes, he represents a certain risk. Thorough showering with soaping and hair-washing are a good method of decontamination. There is, moreover, a whole series of organizational measures, of which only the most important will be listed here. For example, all persons entering the high-security area must have a special pass and be informed about security regulations. Persons leaving the high-security area may not come in contact with cloven-hoofed animals for three days following showering out. Permanent staff may not keep animals that are susceptible to foot-and-mouth disease (FMD) as pets.

In order to prevent disease-producing agents from spreading within the interior of the high-security building, staff working under different levels of contamination wear different coloured clothes; this clothing must be changed upon passing between two areas in which the level of contamination differs. An important component of security procedures is the constant motivation of staff to keep to the strict yet imperative security regulations.

Air-Conditioning and Air Filtration Plants

Besides the supply of processed incoming air and the removal of contaminated exhaust air, the twin ventilation plants must, for safety reasons, fulfil two additional special requirements: the filtration of exhaust air in the sphere of extremely small particles and the maintenance of the differential negative pressure system. Of necessity, the uninterrupted functioning of the plan must be guaranteed. Furthermore, it must be possible to decontaminate filters from any disease-producing agents remaining in the filter upon the completion of an experiment. The air filtration plant at the IVI consists of a system of filters which can be found in any conventional filtration plant. In the IVI, however, this has been supplemented by High Efficiency Particulate Air filters (HEPA filters). The exhaust air from both animal and laboratory sections has to pass through two filtration phases; one directly outside and only serving each room in question, and the second consisting of a unit of HEPA filters which brings together all of the filtered air for re-filtration before passing the containment boundary. In the animal facility, HEPA filters have also been installed for the incoming air, in order to avoid cross-contamination between the various animal quarters. All HEPA filters, including those units combined with pre-filters, are equipped with connecting pieces and gas-tight valves in the air-pipes which allow the attachment of a mobile decontamination apparatus. This plant operates with formaldehyde which kills any infectious agents trapped by the filters, and thus permits a decontamination of the rooms allocated to the filters.

The ventilation plant maintains all rooms within the containment boundary at a determined negative pressure. The negative pressure goes in
pressure steps of 50 Pascal from -100 Pascal in the laboratories down to -300 Pascal in the sewage treatment plant. As a reference, the outside pressure is used in order that the regulation of pressure in the different rooms does not have to be interdependent.

**Sewage treatment plant**

Waste water from the whole containment area is brought together through a collection network, wherein individual rooms are isolated from one another by means of syphons. By means of gravity, the waste water flows to the storage tanks located at the lowest point of the animal facility. The conduits are situated in the installation floor of both laboratory and animal sections, and are equipped with back-pressure indicators at their upper ends as a safeguard against blockage. From the storage tanks, waste water passes through a cutter unit with a separator for metal parts before reaching the sterilization plant. This plant consists of two independently operating units of two sterilizers and a pair of heat exchangers each. Following sterilization, the contents of both units as well as contaminated water from the storage tanks are passed separately but simultaneously through heat exchangers. As a precautionary measure, a liquid barrier is sandwiched between the sterilized and contaminated waste water. The latter is thus pre-warmed, and subsequently heated and sterilized. Before flowing into the sewage, the already sterilized waste water is further coded by another heat exchanger, the heat thus extracted being used to produce the warm water for the showers.

**Material sterilizers**

Material that is to be taken out of the high-security section, be it organic or non-organic, is classified as contaminated and must be sterilized. Steam autoclaves are constructed in such a way that the chamber under pressure has two doors which are bolted towards each other, and functions like an airlock chamber. This unit has a welded front panel made of stainless steel, which, like all fixation plates, is permanently resilient but connected in a gas-tight manner to the surrounding walls; the unit forms the so-called germ barrier. Sterilization takes place at a temperature of 121°C for 20 minutes. All programs are processor controlled. Process parameters and program cycles are visible on an integrated screen and are additionally recorded on a documentation printer. The door on the outer side of the sterilization chamber cannot be opened until the full program has been effected correctly and all prescribed values been observed. Heat-sensitive material such as plastics or complicated technical and electronic appliances are made germ-free in an ethylene oxide gas sterilizer, which works at a temperature of only 40°C. Large airlock cabins handle the big, bulky objects by means of sterilization with formaldehyde.

Animal carcasses are sterilized in a special autoclave, which consists
of a double-shell cylinder with a built-in paddle wheel. The filling dome is located in a post-mortem room. Goods to be treated, approximately two tons of animal carcasses per charge, are sterilized at 134°C during five to eight hours and reduced to a residual water content of 12%. Both the shell and the paddle are heated with steam. The program runs automatically, parameter and process cycles being recorded on a documentation printer. This autoclave, together with the filling dome cover and withdrawal section, also forms an airlock; the interior belongs to the containment area before the sterilization process, but to the non-contaminated area following complete sterilization. Thus can the autoclave be unloaded from outside.

Energy Supply
The supply of the electricity consumed requires a high performance transformer station in both the office building and the high-security section each capable of delivering approximately one Megawatt of power. Each transformer is supplied with 16 KV from the medium voltage circuit of the local power station.

A power cut would endanger the integrity of the whole containment facility, particularly due to the loss of the differential negative pressure system. Therefore, the basic supply of the two transformers is effected from two different main distributors. The maintenance of negative pressure and the supply of compressed air provide the capacity to control and regulate for a power cut lasting up to one minute. Consequently, a powerful emergency back-up power system comprising two diesel generators functioning in parallel with a total performance of 0.5 MW has been installed. Should both public sources of mains power fail, the circuit control system would immediately turn on the emergency electricity plant. The diesel generator starting first supplies electricity within 10-15 seconds of the power failure to those systems which have been given top priority.

A maximum level of energy recycling is effected through modern heat recovery systems. Approximately 34 per cent of the annual energy consumption of the IVI is recovered from the exhaust air, sewage treatment, autoclaves, and cooling water systems.

Final Remarks
Modern civilization depends on a high degree of technical systems which have been able to increase public welfare enormously in the last few decades. However, in parallel to this development risk for men and environment are also related to those technical systems. Risk became a leading word in public discussions about essential individual and social existence. Today many different groups of specialists are concerned about the recognition of risk: scientists and engineers determine residual risk, lawyers ask about liability and politicians decide about the political acceptance of risk. Bankers, politicians, insurance people and economists have a different view
of risk from that of psychologists and philosophers. This clearly shows that risk is a construct meaning that risk is not only an object of risk perception but also a concept of risk perception. As concept of risk perception it is a kind of “filter”: what is seen as risk is not reality but rather depends on the kind of “filter”.

In 1966 the term technology assessment has been introduced by the American Academy of Science and today technology and risk assessment is common in the industrialized world. Such risk assessment should cover comprehensive analysis of the system including its environment. This is not, by any means, an easy task and is only possible by interdisciplinary cooperation. However, one should never forget that there is no scientific recipe for making decisions and therefore the final decision remains a question of policy.

In connection with the presented high security laboratory in a dairy area of Switzerland many arguments against and in favor for the chosen site could be discussed, but, arguments can only be weighted but can never be added up algebraically. On one hand, if the laboratory is supposed to be safe, it could be built everywhere, on the other hand as it is well recognized that no absolute safety exists, it could be wise to put it in an area where the risk of a disease outbreak in case of an incident is minimal. In such “how safe is safe enough” situations, society must decide how much should be invested to reduce risk. In such a process of risk analysis and policy-making the communication of risk information among technical experts, lay people and decision makers is essential. Each side, expert and public, has something valid to contribute and each side must respect the insights and intelligence of the others. Without such understanding, a high security laboratory in a dairy area could lead to dramatic opposition and evoke irrational fear.
A MOLECULAR APPROACH TO STUDIES OF HEARTWATER DNA TECHNOLOGY ON THE TRAIL OF THE REAL HEARTWATER DISEASE AGENT

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History
The first written record of heartwater appears to have been made by Louis Trichardt in South Africa in 1838, when he described multiple deaths in his flock of sheep following a serious tick infestation 3 weeks previously. The next record emerged in Natal nearly half a century later, by which time the disease was apparently common in South Africa and was known as heartwater. In 1900 Lounsberry, working in Capetown, confirmed that heartwater was a tick-borne disease carried by Amblyomma hebraeum but it was not until 1925 that the causative organism was identified. That advance was made by Cowdry, a rickettsiologist from the Rockefeller Institute in New York, during a visit to the Veterinary Institute at Onderstepoort (Cowdry, 1925). He initially named the organism Rickettsia ruminantium, later changed to Cowdria ruminantium, and it was subsequently found to be carried by no less than 12 species of Amblyomma.

Distribution and Importance
Heartwater is an extremely serious constraint to domestic ruminant production throughout sub-Saharan Africa, causing losses which are ranked third in importance, after East Coast fever (ECF) and tsetse-transmitted trypanosomiasis (Provost & Bezuidenhout, 1987). In those areas of Africa where East Coast fever is absent and where there is only a limited distribution of tsetse, heartwater is almost certainly the most important livestock disease (Camus and Barré, 1988). Heartwater also occurs on the offshore African islands of Sao Tomé, Unguja, Pemba and the Comoros and on the Indian Ocean islands of Madagascar, Reunion and Mauritius.

Heartwater-infected A. variegatum were inadvertently imported into Guadeloupe, probably in the 19th. century but possibly as early as the 18th. century, and both disease and vector have subsequently become established on several Caribbean islands (Camus & Barré, 1992). The ticks are carried as nymphae by birds migrating from island to island (Uilenberg, 1990) so there is a high probability that the disease will eventually reach the mainland. Large areas of North, Central and South America are climatically suitable for A. variegatum and, in addition, there is an even wider distribution of three other potential vector ticks among the native Amblyomma species (Barré et al, 1987; Jongejan, 1992). There would be appalling consequences for the American livestock industry in the event of such an
invasion. Heartwater has an average incubation period in susceptible ani-
mals of less than 2 weeks (Van de Pypekamp & Prozesky, 1987), adult
cattle have a subsequent mortality of up to 82% (Du Plessis & Malan, 1987)
and Merino sheep of up to 95% (Neitz, 1964).

Control
Heartwater is controlled in South Africa by infection and treatment us-
using a 'vaccine' which actually consists of viable virulent organisms in sheep
blood. This vaccine has remained virtually unaltered for over 50 years
(Oberem & Bezuidenhout, 1987) and suffers from several serious limita-
tions. It cannot be used in non-endemic areas, so it could not be used in
the event of an American outbreak of heartwater; it must be stored and
transported at -80°C; it must be administered intravenously by skilled staff
and the infection must be monitored and controlled by subsequent tetracy-
cline inoculation. Erythrolytic and/or adverse histamine reactions may oc-
cur, other blood diseases may be carried with the vaccine and the duration
of immunity is uncertain and has been reported to be short in some cases
(Van der Merwe, 1987).

Control is particularly difficult because different isolates of the heartwater
agent have been found to have different virulence and immunogenicity.
The vaccine isolate, Ball 3, was originally made in the Northern Transvaal
(Haig, 1952) and was chosen for vaccination because it produces a marked
febrile response several days before any other serious clinical signs ap-
pear. This makes it relatively easy to decide when to treat. Some isolates
give few clinical warning signs until the infection is well developed and
animals can easily be lost. Despite this advantage the Ball 3 vaccine pro-
tects against only some of the isolates which circulate in the field in South
Africa (Du Plessis et al, 1989). The isolate eliciting protection against the
widest range of other stocks is the Welgevonden isolate, but this is very
virulent and infection and treatment are difficult to control.

Diagnosis: Traditional
Definitive diagnosis has traditionally relied upon microscopic visualiza-
tion of rickettsial bodies in brain smears. A brain biopsy technique for live
animals has been described (Synge, 1978) but it is obviously impractical to
use this on a large scale. At least four serological tests have been exten-
sively investigated for routine diagnostic use; enzyme-linked immunosorbent
assay (ELISA), competitive ELISA (cELISA), indirect fluorescent antibody
(IFA), and Western blot. None of these tests is completely specific, they all
suffer to a greater or lesser extent from cross-reactions with Ehrlichia-posi-
tive sera (Jongejan et al, 1993).

Diagnosis: Serological
Indirect ELISA test. An ELISA test based on whole cell culture antigen
has been in use at Onderstepoort since 1989 (De Waal, D.T., pers. comm.).
False negative reactions are occasionally seen in this test, hence negative
sera are further examined using the mouse macrophage IFA test. Recently the sensitivity and specificity of the ELISA test was improved by replacing crude antigens with soluble antigens extracted from cultured elementary bodies (Sumption, et al, 1993). There is some indication that the use of soluble antigens may serve to reduce cross-reactivity with *Ehrlichia* positive sera, but this has not yet been tested sufficiently widely to be certain that all cross-reactivity can be eliminated.

**Competitive ELISA test.** This test utilizes competition between serum antibodies and a monoclonal antibody directed against the immunodominant MAP1 protein of *C. ruminantium* (Jongejan et al, 1991). It is more sensitive than the indirect ELISA test and can be used to monitor a wide range of animal sera. It can therefore be used for epidemiological studies in wildlife (Kock et. al, 1992) but it is still susceptible to cross-reactions with *Ehrlichia*-positive sera (Jongejan et al, 1993).

**Western blot analysis.** Serum antibodies to *Cowdria ruminantium* have been demonstrated by this method and its principal use has been for comparison with other sero-diagnostic tests (Jongejan et al, 1991; 1993; Jongejan, 1992). The technique is more sensitive than either IFA or cELISA (Jongejan et al, 1991) and Barbet et al, (1993) used it to examine sera from heartwater-endemic and heartwater-free areas of Zimbabwe. Positive reactions were seen with a large number of the supposedly heartwater-free samples, and some of the donor animals were demonstrated to be free of *C. ruminantium* by PCR and tick-transmission experiments and were fully susceptible to challenge with the Zimbabwean Crystal Springs isolate of *Cowdria* (Barbet, A.F., pers. comm.). The authors concluded that an unknown organism related to *Cowdria* exists in areas of Zimbabwe which were assumed to be heartwater-free.

**IFA test.** The mouse macrophage IFA test is currently used at Onderstepoort to confirm negative results from ELISA tests (Du Plessis, 1981). The antigen used is a preparation of peritoneal macrophages from *C. ruminantium*-infected mice. A modification has been developed which uses extracellular elementary bodies of *C. ruminantium* as the target antigen (Yunker et al, 1988; Martinez et al, 1990; Semu et al, 1992). Both of these IFA tests have been found to give positive reactions with sera from heartwater-free areas which have been attributed to anti-*Ehrlichia* spp. antibodies.

**Diagnosis: DNA probes**

**Repetitive DNA Probes.** A clone (pCS20) containing a repetitive *C. ruminantium* sequence from the Crystal Springs isolate of *Cowdria* (Waghela et al, 1991) has been used to probe for *C. ruminantium* in laboratory-infected ticks (Waghela et al, 1991; Yunker et al, 1993) and sheep plasma (Mahan et al, 1992) and was found to be highly sensitive. It is necessary, however, to incorporate blocking mammalian DNA into the hybridization solutions (Mahan, S. M., pers. comm.) failing which the probe was found to hybridize to genomic DNA from sheep, goats and cattle which
were believed to be free from heartwater. In the light of subsequent findings it is entirely possible that these supposedly heartwater-free animals did harbour organisms related to *Cowdria*. An alternative explanation may be that the pCS20 probe hybridizes to ruminant mitochondrial DNA. This possibility, which should be verified experimentally, was suggested when we screened Genbank with the pCS20 sequence and found homology to several mitochondrial sequences. Rickettsias belong to the a-subdivision of the purple bacteria, and the endosymbiont which gave rise to the mitochondrion is also believed to have arisen from this group (Yang, et al, 1985).

**The elusive Ehrlichias**

Jongejan et al (1993), using the cELISA, IFA and Western blotting tests, demonstrated that *Cowdria ruminantium* antigen gave positive reactions with sera from animals infected with *Ehrlichia canis*, *E. bovis*, *E. ovina* and *E. phagocytophila*. Despite this no-one has demonstrated which *Ehrlichia* organisms are responsible for the *Cowdria*-positive serological reactions obtained with sera from supposedly heartwater-free areas. Even more intriguing are the reports by Du Plessis (1990, 1993) of the isolation of an organism referred to as the Omatjenne agent which was microscopically indistinguishable from *E. bovis*. *Hyalomma truncatum* ticks, collected in an *Amblyomma* and heartwater-free area of Namibia, were individually homogenized and injected into mice. One mouse developed a reaction and spleen homogenate from this mouse was injected into another mouse which was used to infect clean *A. hebraeum* ticks. These ticks were used to initiate a series of sheep/tick passage cycles and after three such cycles sheep on which the ticks were fed developed clinical heartwater.

Over the last few years a number of previously unknown and very closely related *Ehrlichia* organisms have been found which cause similar diseases in humans, dogs, sheep, cattle and horses (Anderson et al, 1992; Chen et al, 1994a, 1994b; Rikihisa et al 1992). It is entirely possible that other unknown *Ehrlichia*-related organisms remain to be uncovered in or near areas in which classical heartwater is to be found.

**Looking for unknown bacteria**

Bacteria were traditionally identified after being cultured and their classification was then decided mainly upon physiological characteristics. This was recognized as being unsatisfactory the scarcity of morphological differences made it unavoidable. Over approximately the past decade, however, there has been a fundamental change in bacterial classification. Molecular genetic data are now very widely used and the molecule of choice is the small-subunit ribosomal RNA (srRNA) gene. This gene has been used with considerable success to investigate phylogenetic relationships for both prokaryotes and eukaryotes and there is now (August 1994) a very extensive database (the Ribosomal Database Project, RDP) of at least 2251 prokaryote srRNA sequences (Larsen et al, 1993). A number of studies have been conducted in which bacteriologists have 'gone fishing', not for
bacteria but for bacterial srRNA sequences, and have compared what they have found with the RDP databank. The results have been astonishing, with the presence of dozens of previously unknown organisms being inferred from the sequences detected (Ward et al, 1990; Fuhrman et al, 1993).

**Which organisms are associated with heartwater?**

In 1992 the srRNA gene sequence was reported from a Zimbabwean isolate of *C. ruminantium* designated Crystal Springs (Dame et al, 1992). We aligned this sequence with the published sequences of several other Rickettsiales and designed two oligonucleotides which would specifically amplify a 323 bp fragment of the rickettsial gene in the presence of an excess of mammalian DNA. This fragment spanned the hypervariable VI loop within which we designed an 18bp Crystal Springs-specific oligonucleotide probe. Later the srRNA sequence of the Senegal isolate of *C. ruminantium* was published (Van Vliet et al, 1992). This differed at three positions in the V1 loop from the earlier sequence.

We conducted PCR with the supposedly rickettsia-specific primers using DNA from a variety of sources. There were blood samples from clinical heartwater cases, blood samples infected with *E. bovis*, *A. marginale* and *Eperythrozoon wenyonii*, blood samples from apparently healthy sheep and goats from both heartwater-free and heartwater-endemic areas, tissue culture material infected with *Cowdria*, and tick material from heartwater-free and heartwater-endemic areas. Included among these were samples from animals infected with the Omatjenne agent, some apparently healthy and others which had died apparently suffering from heartwater.

We obtained a total of 279 non-chimeric sequences and compared them with the RDP databank. Apart from one sequence, which was very different from any in the databank, the remaining 278 sequences could be divided into 3 groups: Rickettsiales (193 clones, 69%), Pseudomonadaceae (62 clones, 22%) and other miscellaneous sequences (23 clones, 8%). The sequences were aligned and genetic distance (Fitch) phylogenetic trees were inferred (Figure 1) using the Phylip phylogeny package (Felsenstein, 1989, 1993).

Among the Rickettsiales sequences a cluster of 5 different sequences were considered to be *Cowdria* genotypes. These comprised 141 clones (50% of the total). Two of the 5 were identical to those already published (Crystal Springs and Senegal) and 3 were new. These 3 were named Mara 87, Ball 3 and Omatjenne. The Omatjenne genotype was that of the *Ehrlichia*-like Omatjenne agent isolated by Du Plessis from *H. fruncafum* (1993) and the Ball 3 genotype was that of the vaccine isolate. These 5 *Cowdria* genotypes differ among themselves at 1-4 positions in the V1 loop. The question of whether they should be considered to be different species is not settled, but they are as closely (or distantly) related as some of the differently named *Ehrlichia* species to be found in the databank. For example, the sequences of *E. equi* and *E. phagocytophila* differ by only one base over the whole 1432bp of the gene, this difference being in helix 3.
Other Rickettsiales sequences we obtained were related to *E. platys* (89–99% sequence identity, 10 clones), *E. equi* (97% sequence identity, 2 clones), *E. canis* (89–97% sequence identity, 6 clones), *R. rickettsii* (95–97% sequence identity, 11 clones), *Coxiella burnetii* (83–90% sequence identity, 16 clones), *Rochalimaea vinsonii* (87% sequence identity, one clone) and *A. marginale* (94–97% sequence identity, six clones). The other numerically important group was that of 62 sequences related to various *Pseudomonadaceae*, with a further 23 sequences related to disparate groups. The only sequences identical to those already known from the database were the two *Cowdria* sequences, Crystal Springs and Senegal.

We designed 5 oligonucleotide probes to detect each of the *Cowdria* genotypes, and a further 3 probes to detect organisms in the groups which we labelled (for convenience) *Ehrlichia*, *Anaplasma* and *Pseudomonas*. We obtained 44 goats from heartwater-free areas of South Africa, they were held in quarantine and appeared to be free from any disease. We purified DNA from blood samples from these animals and conducted PCR with the rickettsia-specific primers mentioned above. The 8 newly designed probes were then hybridized to the PCR products. We also collected serum samples from the goats and performed ELISA and mouse macrophage IFA tests for

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**Figure 1** Fitch tree showing some heartwater-associated sequences

[Diagram of Fitch tree showing some heartwater-associated sequences]

Omatjenne  
Senegal  
Mara 87  
Crystal Springs  
Ball 3  
*Ehrlichia ewingii*  
*Ehrlichia canis*  
*Ehrlichia chaffeensis*  
*Ehrlichia equi*  
*Ehrlichia platys*  
*Ehrlichia bovis*  
*Anaplasma marginale*  
*Pseudomonas mendocina*  
*Flavobacterium lutescens*  
*Pseudomonas aeruginosa*
Figure 2. Examination of goat samples from heartwater free areas. Number of animals positive by serology and by oligonucleotide probing for Cowdria, Pseudomonas and Anaplasma-related sequences.

Fifteen animals were serologically positive either by the ELISA test or by the IFA test. Forty three samples hybridized with the *Pseudomonas* group probe, 31 with the *Anaplasma* group probe and 9 with the *Cowdria* Omatjenne probe. No samples hybridized with the *Ehrlichia* group probe or with any of the other four *Cowdria* genotype probes. Only three samples were positive by both heartwater serology and with the *Cowdria* Omatjenne probe. All these animals were from areas where heartwater is never seen and none of them had any history of heartwater-like infection. It is therefore evident that the presence of the Omatjenne agent, closely related though it is to *Cowdria ruminantium*, is not responsible for the positive serological reactions, nor for any obvious signs of disease. We recall that after 3 passages through sheep and *A. hebraeum* the original Omatjenne agent caused a clinical syndrome which was not distinguishable from heartwater. The sequences which we obtained from the original mouse which had reacted to the *H. truncatum* injection were all of the *Cowdria* Omatjenne type, but blood from the sheep which subsequently died from a heartwater-like infection yielded sequences related to *E. platys* and to *Pseudomonas*, as well as *Cowdria* Omatjenne. We cannot now say whether these additional organisms were present in the original *H. truncatum*, but at a level sufficiently low that we did not detect them, or whether they entered later from apparently clean ticks or sheep. What we can say is that it seems unlikely that the Omatjenne agent on its own is responsible for any form of heartwater. There were other instances of heartwater blood failing to yield any *Cowdria*-type sequence. In one case this was probably a quantitative effect, since tissue cultured material established from the same original isolate contained *Cowdria* of the Mara 87 genotype. In the other instance the blood stabilate yielded sequences related to *E. canis* and to *Pseudomonas*.

The next step is to determine the relative clinical importance of the multitude of organisms which appear to be present in animals suffering
from heartwater. Some of them could be opportunistic infections which arise as a result of immunosuppression but it would be unwise to assume that they all fall into this category. We now recognize that clinical heartwater can be caused by at least 4 different genotypes of *Cowdria*, and possibly also by one or more closely related species of *Ehrlichia* either with or without attendant *Pseudomonas*. We also recognize that traditional heartwater serology frequently gives positive reactions as a result of immunity to unknown organisms which do not appear to cause any disease. Molecular biology appears to offer the only methods with which this extraordinary jigsaw is likely to be assembled.

**References**


HEARTWATER DNA TECHNOLOGY ON THE TRAIL OF THE REAL HEARTWATER DISEASE AGENT


The meeting of the Foreign Animal Diseases Committee opened with remarks by Gale Wagner about the change in format for the committee deliberations, a 5-year program designed to raise awareness about 5 important topics: Ticks, Biocontainment, Emerging Diseases, Risk Assessment in International Trade, and FAD and Pest Diagnostic and Research Needs. The presentations each year will be organized into one of the topic areas. A subcommittee for each area (with an appointed subcommittee chair) will spend the next few years identifying needs, preparing a discussion paper, then producing a final briefing document that describes a unified approach to meet the needs. The papers presented included:

**Topic Area - Ticks**

**Status of Tick Control Efforts in the Caribbean.** Alex Thiermann, USDA/APHIS. Dr. Thiermann reported on the regional Tropical Bont Tick
FOREIGN ANIMAL DISEASES

program in the CARICOM islands of the Caribbean. Field activities are due to begin January, 1995. USDA-APHIS has supported these efforts through a Memorandum of Understanding with IICA. Key participants currently are FAO, IICA, CARICOM and APHIS. The initial phase of the program will focus on tick surveillance starting with the northern islands. This will be followed by eradication efforts in those islands affected with heartwater. Eradication efforts in the French Caribbean islands began in 1994 and are funded and managed by the French Government.

New Tick Control Methodologies. John George, USDA/ARS. Formulation of ivermectin into copolymers of polylactic and polyglycolic acids to produce injectable microspheres provides a method, through a single subcutaneous injection of cattle, to control Boophilus microplus for 11 weeks. The distribution of ivermectin-medicated whole kernal corn to white-tailed deer or other ungulate wildlife species can be used as a method for controlling tick species for which the wildlife hosts are critical to the maintenance of a tick population. The technique has promise for eradicating B. microplus or B. annulatus in situations where a pasture vacation approach is unsuccessful because dense populations of white-tailed deer maintain ticks in the absence of cattle. Dr. George also reported that problems of toxicosis in cattle resulting from the accumulation of potosan in dipping vats can be minimized by lowering the pH of vats to 5.5. The low pH of the vat contents inhibits the activity of the anaerobic bacteria responsible for degrading coumaphos to potosan. Coumaphos remains in normal suspension and does not lose tickicidal activity in a vat with a pH as low as 4.5 nor does dipping in these conditions have an overt adverse effect on cattle.

Topic Area - FAD and Pest Diagnosis and Research

A Vaccine Prepared from Genetically Engineered, Adsorption-Defective Foot-and-Mouth Disease Virus (FMDV) Protects Cattle from FMD. T. McKenna, E. Rieder, J. Lubroth, B. Baxt and P. Mason, USAD/ARS. Dr. Baxt presented the first 4 papers on studies with FMDV. Using an infectious cDNA clone from type A FMDV, a virus has been engineered that lacks the RGD peptide found on the VP1. The virus is unable to bind to cell surface receptors, rendering it non-infectious, and cattle immunized with uninactive preparations of this virus did not show any clinical signs of FMD and were fully protected from FMD challenge with live virus.

The Cell Binding Site on FMDV Includes Sequences Outside of the RGD and Receptor Specificity Can Select Antigenic Variants From Animal-Derived Virus Populations. E. Rieder, B. Baxt and P. Mason, USDA/ARS. Virus isolated from bovine tongue tissue was found to have antigenic and cell binding characteristics differing from virus grown in tissue culture. The differences were attributed to mutations in 2 amino acid residues near the RGD cell binding site. These characteristics have been engineered in an infectious cDNA clone from FMDV to produce a virus that is similar to that found in infected animals.
Antibodies Against the Vibronectin Receptor (Integrin alphaV beta3) Inhibit Binding and Infection of FMDV to Cultured Cells. A. Berenstein, M. Roivainen, T. Hovi. P. Mason and B. Baxt, USDA/ARS. FMDV binds to cultured cells via an RGD tripeptide sequence on the G-H loop of VP1 in a manner similar to extracellular matrix proteins. Using a series of polyclonal and monoclonal antibodies, the vibronectin receptor has been shown to be the cell surface receptor for type A FMDV on cultured cells.

Mutational Analysis of the Leader protease of FMDV. M. Piccone, M. Zellner, P. Mason and M. Grubman, USDA/ARS. The leader (L) protease of FMDV is a nonstructural protein that has an essential role in the viral life cycle. Amino acid residues that are required for L biological activity have been identified. This, plus previous information on the life cycle is being used to design antiviral compounds that are effective against FMD.

Nonstructural Protein 2C Differentiates Infected and Vaccinated Animals in Foot and Mouth Disease. Juan Lubroth and Fred Brown, USDA/ARS. Cattle and swine that have been vaccinated against FMD can be distinguished from convalescent animals by radioimmunoprecipitation and SDS-PAGE analysis of the virus-induced proteins reacting with the respective sera. Protein 2C is precipitated only by serum from convalescent animals. Dr. Breese presented the paper.

The S10 Gene Segment is a Virulence Determinant of African Horsesickness Virus. T. Burrage, A. Carville, M. Stone-Marshat, A. Skowronke and W. Laegreid, USDA/ARS. Dr. Laegreid reported the the pathogenesis of African Horse Sickness (AHS) varies between virus isolates. A nonstructural protein, AHS/4SP, has been found to be associate with viremia and thus, correlated with pathogenesis. The AHS/4SP may funcin in the membrane budding stage of the viral life cycle and subsequent release of virus from the infected cell.

African Swine Fever Gene 23-NL Encodes a 23 kDa Nuclear Protein That Delays Viral-Induced Apoptosis. C. Alfonso, A. Brun, S. Dhume, J. Neilan, T. Burrage, G. Kutish and D. Rock, USDA/ARS.


A Bcl-2-Related Protein of African Swine Fever Virus Enhances Apoptotic Cell Death. J. Neilan, L. Zsak, E. Caler, G. Kutish, T. Burrage and D. Rock, USDA/ARS. These 3 papers, presented by Dr. Zsak, described work on African Swine Fever (ASF), a highly significant lethal disease of domestic swine, which remains poorly understood because little is known about how the virus actually causes disease in the infected animal. Recently, 2 highly novel putative virulence/host range-associated genes of ASF virus have been identified. The ASFV gene 23-NL that shares significant similarity in a 56-amino acid carboxy terminal domain to a myeloid differentiation primary response gene and a neurovirulence-associated gene of HSV has recently been shown to encode a 21 kDa nuclear protein that
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promotes cell survival when expressed in recombinant vaccinia virus and baculovirus systems. ASFV gene 5Hb shares highly significant similarity to the bcl-2 family of proteins and surprisingly has been shown to enhance apoptosis in insect cells when expressed in a recombinant baculovirus. This gene is the first bcl-2-like viral gene described whose function is induction or promotion of apoptosis in virus-infected cells. Most significantly, the presence of both 23NL and 5HL genes in ASFV indicate for the first time that careful modulation of cell survival during ASFV infection may be critical to some aspect of virulence, host range and/or persistent infection.

Overview of Animal Health Activities in Israel. Aron Shimshony, VSAH. Dr. Shimshoney went over the use of sequence analysis of FMDV-VP1 to evaluate several outbreaks of FMD that occurred in Israel last year, in contrast to several preceding years when FMD outbreaks were few. The results indicated that FMD outbreaks in Israel are seasonal, non-endemic, mostly confined to northern parts of the country. It appears that wild boars are a natural reservoir of infection, and that these animals may act as disseminators of the virus. The intraseasonal reservoir is not known.

Topic Area - Biocontainment

IVI - A List “A” Laboratory in a Dairy Area. Ulrich Kihm and Peter Mani, IVI. This paper was discussed as presented in the General Session. The full text is included elsewhere in these proceedings.

The New U.S. Biocontainment Labs for Foreign Animal Disease Research and Diagnosis at Plum Island. Roger Breese, USDA/ARS. Dr. Breese reviewed the facilities consolidation, renovation and modernization program at Plum Island that has taken about 10 years to complete, with an investment thus far of about $40 million. The result is a completely modern laboratory and administrative facility that integrates ARS research andAPHIS diagnostic functions into a single building, with nearby offices, library and seminar rooms. The facility includes a 6000 square foot laboratory solely for the use of collaborating institutions. Although the renovation has met almost all of EPA requirements, an additional $60 million is projected to complete modernization of equipment and auxiliary facilities.

Biocontainment - How much is enough? Bill Sterritt, AgCanada. The lack of biocontainment standards directed specifically at animal pathogen laboratories has served as a source of confusion and uncertainty to laboratory designers, administrators and scientists alike. Such a set of generally-agreed guidelines would reap benefits for the safety of the livestock industry; international acceptance of animal health systems; potentially reducing litigation; as well as design facilitation. Dr. Sterritt urged the informal “Veterinary Biosafety Workshop” group, which meets annually, to complete study and draft a set of veterinary biosafety criteria that can be discussed and adopted.

Status of the Development of the Southwest Regional Animal Biocontainment Facility. Garry Adams, Texas A&M. Dr. Adams pre-
REPORT OF THE COMMITTEE

Presented details of a proposed facility to be built at Texas A&M University that will meet the needs of the U.S. and Mexico. The facility is being designed to provide biocontainment space for research and training on food animal diseases of regional importance such as bovine TB. It will be operated as a bilingual facility with an advisory board representing the university communities, state and federal agencies in both countries and private enterprise. Planning, design and construction is expected to require 4 to 5 years and to cost about $35 million.

Topic Area - Risk Assessment in International Trade

Risk Assessment - A Guide for Epidemiologists. Tari Kindred, USDA/FSIS. Dr. Kindred discussed the elements of risk assessment in terms of the utility of constructing scenarios that estimate risk without complete epidemiologic information. The scenario, or risk assessment "tree," sets up a system that is responsive to new information, and gives the epidemiologist a clear indication of the kinds of information about a disease that are needed in order to calculate accurate estimates of risk. Concepts of risk assessment, risk management and risk communication were also presented, as were the differences in qualitative and quantitative concepts of disease incidence, relative risk and attributable risk.

Risk Assessment in International Animal Trade. Hugh Metcalf, USDA/APHIS. Risk assessments in international livestock trade must first discard the old paradigm regarding areas as either "free" or "not free" of FAD. Dr. Metcalf suggests that a new concept of degrees of risk will result in a new paradigm of "acceptable risk" vs "non-acceptable risk." A series of proposed categories or classifications of risk were discussed. Such classifications should be easily codified into the existing regulations to provide the livestock industry the maximum protection it wants and deserves but still providing for market access.

Quantitative Assessment of the Risk of FMD Introduction by the Importation of Meat from Selected Areas in the Rio Plato Regional FMD Eradication Project. Paul Sutmoller, PAHO. Dr. Sutmoller discussed the details of a preliminary quantitative risk assessment for Uruguay and the Argentine Mesopotamia since that region offers animal health conditions that may qualify for "regionalization" and the subsequent export of frozen meat to the CARICOM countries. The preliminary results of the quantitative risk assessment indicate that the probability is exceedingly small that deboned frozen meat will contain FMD virus.

Risk Assessment Following Screwworm Eradication in Central America. John Wyss, APHIS. The risk management of future screwworm outbreaks in the U.S. (and, in fact, in Mexico and Central America where screwworm has been or soon will be eradicated) is based on the establishment and maintenance of various barrier locations. Dr. Wyss discussed the elements used to identify and define the sources of risk, how barrier maintenance reduces those risks, and how risk management leads to mutual benefit.
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**Topic Area - Emerging Diseases**

The two standing reports of the FAD committee were presented by Dr. Grow and Dr. Preston.

**VS-Emergency Programs: Foreign Animal Disease Surveillance Activities in FY 1994.** Adam Grow, USDA/APHIS.

**Suspected FAD Field Investigations:** During fiscal year (FY) 1994 (October 1, 1993, through September 30, 1994) veterinarians from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) and the States conducted 283 investigations for suspected foreign animal diseases (FAD). These actions are part of the Emergency Programs approach to foreign animal disease surveillance to insure that exotic diseases do not become established in the United States or its territories. The investigations included 93 vesicular conditions, 58 encephalitic conditions, 37 avian diseases, 36 swine septicemic conditions, 13 mucosal disease conditions, 11 due to excessive death loss, 8 myiasis/acariasis, 7 pox like conditions, 3 unusual respiratory conditions, and 2 abortion cases.

**Hog Cholera/African Swine Fever Surveillance:** The VS surveillance program for classical swine fever (hog cholera) and African swine fever was continued in 1994. Swine blood specimens are regularly collected at slaughter from abattoirs located in Maine, Massachusetts, New Hampshire, New Jersey, Arizona, Texas, and Puerto Rico. Additional samples were sent from California, Hawaii, Maine, and the Virgin Islands. The National Veterinary Services Laboratories (NVSL) in Ames, Iowa tested 12,556 samples and all were determined to be negative for the two diseases.

**Velogenic Viscerotropic Newcastle Disease (VVND) Surveillance:** There were no outbreaks of VVND in caged pet birds or domestic poultry in the U.S. in FY 1994.

**Avian Influenza Surveillance:** The avian influenza surveillance program for live-bird markets was continued for Pennsylvania, New York City, New York, New Jersey and the New England area. Serological evidence of the several non-pathogenic avian influenza viruses was identified in live-bird markets in the northeast states and in south Florida. Surveillance indicated the virus was confined solely to small backyard flocks and live-bird markets. Trace-backs to numerous associated premises showed negative results. Evidence indicates that this nonpathogenic H5N2 AI strain may be endemic in migrating waterfowl and shore birds in the Chesapeake Bay area and can be readily identified wherever routine surveillance is carried out. This large reservoir of virus may be responsible for the maintenance of nonpathogenic H5N2 AI in nature.

There is always a potential for any AI virus to change its degree of pathogenicity. Therefore, extensive surveillance and research efforts with regards to AI are precautionary. The USDA, APHIS will continue to conduct surveillance measures in order to prevent the potential spread of any avian
diseases and to maintain a healthy and prosperous poultry-export market. There has been no highly pathogenic AI in the United States since 1984.

**Bovine Spongiform Encephalopathy (BSE) Surveillance:** The BSE surveillance program which started in May, 1990 is continuing. Pathologists at NVSL and Iowa State University are continuing to examine bovine brains submitted to NVSL from the following sources: 1.) foreign animal disease investigations where suspected encephalitic conditions in cattle are reported, 2.) bovine cases confirmed negative for rabies by the Centers for Disease Control in Atlanta, Georgia, 3.) brain specimens collected at slaughter from abattoirs in selected potential high risk States, and 4.) brain tissues submitted by veterinary diagnostic laboratories in the United States.

To enhance the surveillance process, visits have been made by State or USDA personnel to state public health and university diagnostic laboratories to arrange for the submission of suspicious specimens NVSL in Ames, Iowa. Contacts with practicing veterinarians have increased reporting and the submission of brains from suspicious cattle. As of September 30, 1994, a total of 1,903 bovine brains have been examined; none of these specimens contained characteristic lesions for BSE. Additionally, none of the traced cattle, (434 out of 499), that were imported from the United Kingdom since 1981, have showed any clinical signs of BSE. There have been no cases of BSE diagnosed in the United States.

The first documented case of BSE in North America was made in Canada in 1993. This single BSE case was confirmed in a cow brain submitted to Alberta's Airdrie Laboratory in November. The index case, a 6-year-old Salers cow, was imported in 1987 as one of eight calves that originated from a single herd in the UK. It was later verified that the source herd in England had experienced an earlier case of BSE in another animal which was sold after the export to Alberta, Canada. BSE has not been diagnosed in any of the adult cattle maintained in the source herd.

Agriculture Canada established an enhanced BSE surveillance program in 1990 for all cattle imported from the UK between 1982-1989, when the importation of live ruminants was discontinued. It was through this program that BSE became a reportable disease in Canada and also is how the index case was first discovered. All cattle imported into Canada for Great Britain were destroyed by the Canadian Government.

**Salmonella enteritidis** phage type 4 (SEPT4) Surveillance: On May 2, 1994, the California State Veterinary Diagnostic Laboratory at San Bernardino received a submission of dead chickens from an egg ranch that reported an increase in mortality and a reduction in egg production. The commercial egg ranch in southern California had submitted chickens to the laboratory as part of a veterinary surveillance program in California established with poultry producers to monitor causes of death loss on a weekly basis. The flock history indicated increased mortality (0.5 to 1.6 percent per month) and veterinary diagnosticians noted unusual enteritis lesions that made them suspicious of an atypical infection. Group D *Salmonella*
was cultured from five of six chickens. The California Department of Agriculture / Animal Health Branch (CDA/AHB) was notified of these results on May 5. The owner of the flock gave permission to proceed with serotyping after consultation with the California Veterinary Diagnostic Laboratory System (CVDLS) and the CDA/AHB personnel. Serotyping confirmed the existence of group D Salmonella in all isolates. The State Laboratory submitted these isolates to the National Veterinary Services Laboratories (NVSL), Ames, IA, where a final diagnosis of Salmonella enteritidis phage type 4 was confirmed on May 19, 1994. California placed a State Hold Order on the effected flock on May 11, effectively suspending all poultry and egg movement from the premises.

Salmonella enteritidis phage type 4 was previously classified as an exotic disease to poultry in the United States (U.S.) by USDA. According to USDA regulations, if it were found in commercial poultry it could affect the interstate and international movement of poultry products. As a result of the SEPT4 diagnosis, California Veterinary Officials initiated a foreign animal disease investigation.

Also, on May 11, surveillance of the flock and premises began and additional chickens were submitted to CVDLS by State personnel to determine the prevalence of SE in the flock and to confirm the original diagnosis. Cultures from these birds were confirmed by NVSL as positive for SEPT4 on May 19. California placed the premises and flocks under a State Quarantine and the eggs from the affected houses were diverted to breaker plants for pasteurization. The ranch owner voluntarily stopped all egg sales on May 17, 1994.

Further epidemiologic investigations revealed that only one premises was affected. This egg ranch is in an isolated area and posses little potential for disease spread. The operation produces both table eggs and fertile eggs that are sold to health food markets for consumption. Chickens and eggs from each house were submitted to CDVSL for evaluation. Environmental sampling and trapping of rodents around the houses was also conducted to determine the extent of infection in the flock. Stool samples from the owner and employees were submitted to the county health services for culture. Also, the hatchery and pullet operation that supplied the chickens to this egg ranch were evaluated. This extensive investigation and testing revealed no conclusive evidence to suggest that the SEPT4 organism had originated from either of these premises or from people working in the houses. A creek flows through the premises close to the chicken houses. Upstream there is sewage treatment plant that feeds into the creek and occasionally has raw sewage spill over. This may be the most likely source of the bacteria but all water samples have tested out negative.

Investigation of the flock has continued to verify the presence of SEPT4 in eggs from two of the houses on the egg ranch. The state of California requested that Salmonella enteritidis phage type 4 not be considered to be an exotic animal disease because SEPT4 is widely prevalent in the human
population in southern California. Many in the scientific community suggested that infection in this flock should be managed as an endemic disease and the flock continue to be monitored. Successful eradication of SEPT4 was not likely because SEPT4 was in the environment, it was also isolated from cats, mice, and skunks on the premise. There were no public health problems associated with the infection in this flock.

The current USDA SE program only provides control actions for flocks found to be associated with human disease. California had no authority to take regulatory action if SE is isolated in routine flock surveillance. The State and the California egg industry have now instituted a voluntary quality assurance program to control salmonella. The USDA has elected to take no action on the SEPT4 infected flock at this time, and it is being managed as an endemic disease. All disease control efforts are being directed by the California Department of Agriculture and the poultry industry. A quality assurance protocol was drafted for the egg ranch to reduce the level of disease in the flock and prevent any potential public health problems.

World Status of Foreign Animal Diseases. Kelly Preston, USDA/APHIS.

Vesicular Diseases - Foot-and-Mouth Disease: Foot-and-Mouth Disease outbreaks continued in South America, the Middle East, Africa, and Asia with nearly the same prevalence as in past years. Spread of type O FMD from Turkey occurs almost annually. In 1993 it spread to Bulgaria. In 1994 FMD was detected in Greece and confirmed at Pirbright July 24, 1994. The probable origin of the disease was traced to the island of Lesbos. Illegal movement of sheep from Turkey is suspected. Quarantine measures have been implemented and stamping out procedures are being utilized.

The Middle East continues to be the major endemic area. Tunisia, Israel, and Jordan reported outbreaks of Foot-and-Mouth disease. Quarantine and vaccination are being used for control. Southeast Asia continues to have FMD spread by the legal and illegal movements of both animals and products. Malaysia reported an outbreak during 1994.

South America reports advances of FMD eradication in the southern tip of the continent in national and regional programs in Uruguay, Argentina, several southern states of Brazil, and a portion of Paraguay.

Swine Vesicular Disease: Swine Vesicular Disease occurred in Belgium, Spain, Italy, and the Netherlands. The case in Belgium was an incidental finding on serology and the three Spanish cases were from imported animals. Italy reported an outbreak of Swine Vesicular Disease which is believed to have originated from a shipment of swine from the Netherlands.

Swine Diseases - Hog Cholera (Classic Swine Fever): Initial European hog cholera outbreaks in Germany spread to France, Switzerland, Poland, Estonia and Belgium by feeder pig sales and the feeding of swill or garbage. Currently, only the German state of Lower Saxony continues to experience outbreaks.

Hog Cholera outbreaks in the Americas occurred in hog cholera-free
areas of Costa Rica and Mexico. Both started in small rustic backyard operations. The Mexican outbreak was successfully eradicated.

Mexico has accelerated their national hog cholera control and eradication campaign by placing nine Mexican states in an “intensive control phase.” At the present time, the Mexican states of Baja California (South and North), Sonora, Sinaloa, and Chihuahua are considered to be hog cholera-free by Mexican Animal Health Officials.

Germany, Costa Rica and Cuba are still attempting to control outbreaks which started in 1993 and 1994.

**African Swine Fever:** There have been no new reports of outbreaks of African Swine Fever outside the endemic area for 1994. ASF is still confined to southwestern Spain and the island of Sardinia, Italy.

The Republic of South Africa and Zambia reported cases this past year.

**Porcine Reproductive and Respiratory Syndrome:** PRRS continues to be detected throughout the major swine producing regions of the world. In August 1993, Japan reported the finding for the first time. A preliminary analysis of sera in their serum bank indicated at least 15% of the swine imported into Japan from 1987 to 1989 were positive. A viral isolation was made in Central America during 1994 from a Guatemalan swine producer experiencing abortions and mummies in his farrowing operation.

**Equine diseases - Venezuelan Equine Encephalomyelitis:** Suspect equine encephalitis cases in Latin America have continued since the single December 1992 outbreak of Venezuelan Equine Encephalomyelitis. To date, only Guatemala and Mexico have had confirmed cases of equine encephalitis. The clinical cases in Chiapas, Mexico and the serology from non-clinical cases in Guatemala were both confirmed to be enzootic virus type I-E. No new outbreaks were reported during 1994.

**African Horse Sickness:** There have been no reported outbreaks of AHS outside the endemic area since the Moroccan outbreak of 1991.

**Acute Equine Respiratory Syndrome:** Australia reported the appearance of a disease affecting equine and man. Of 21 affected horses, 14 died and 7 recovered. One human death occurred and 2 other persons have developed antibodies. Preliminary studies show this virus to be a member of the Paramyxoviridae family and most likely to be a morbillivirus.

A survey of over 600 horses in the vicinity has established that the only positive horses have been those located on or originating from the index stable. Movement controls were in place until 10/11/94. An individual quarantine remains on premises where there was evidence of presence of the virus.

**Bluetongue:** Biotype 16, which had not been isolated since 1975, was identified in the Middle East this past year.

**Contagious Bovine Pleuropneumonia:** Contagious Bovine Pleuropneumonia affected almost every African country south of the Sahara. In Europe, there was a considerable decrease in the occurrence in
Italy in 1993. Outbreaks have been reported in North Western Colombia. Affected herds have been placed under quarantine.

**Rift Valley Fever:** After a (12) year absence, Rift Valley Fever reappeared in Egypt in June 1993. It was confined to Aswan Governate, where it was responsible for cases of abortion among cows, buffaloes and ewes. A vaccination campaign was undertaken and over 4 million ruminants were vaccinated.

The World Health Organization is closely monitoring the situation in southern Egypt where 55 cases of ocular dysfunction have occurred.

No new outbreaks reported outside the endemic areas.

**Rinderpest:** In Africa Ethiopia, Uganda, and Iran reported the disease this last year. In Asia, India, Pakistan, and Sri Lanka reported Rinderpest. The outbreak in Iran was attributed to the illicit importation of animals.

Turkey experienced an outbreak in mid-April. The control measures include vaccination and control of animal movements.

**Bovine Spongiform Encephalopathy:** After thousands of cases of BSE in the United Kingdom, BSE has now decreased its incidence. Some new cases are still being reported. To date, almost 130,000 head of cattle in 30,000 herds have been sacrificed. France, Ireland and Switzerland reported cases this past year. The countries of Canada, Portugal and Germany had cases in imported cattle during 1993. During 1994 Portugal reported a case of BSE.

**Screwworm:** The USDA, APHIS Regional Central American Screwworm Eradication Program has advanced the eradication zone to Nicaragua. This eradication zone is also the sterile fly biological barrier between infested and screwworm-free countries. It is expected that Nicaragua will be eradicated by the end of 1995. This is a milestone in screwworm eradication as it is the largest country in Central America and has the largest overall livestock population, and has been the main source of reinfestations into screwworm-free areas to the north.

A strategic planning need for APHIS continues to be the construction of a new sterile fly production plant proposed for Panama.

During 1994, a single case of screwworm was detected in the Miami Animal Import Center on polo ponies from Argentina. Immediate control measures were implemented and proved successful.

**Avian Influenza:** In the United States nonpathogenic AIV serotypes H7N2 and H7N3 were isolated during the live bird markets sampling program.

In Mexico the Ministry of Agriculture continues to monitor the avian influenza outbreak which was confirmed May 25, 1994. Avian Influenza virus type A, subtype H5, nonvirulent strain, has been isolated. An ongoing serological monitoring program is being established by the Ministry of Agriculture.

The Netherlands reported an outbreak in ratites in April 1994.

**Newcastle Disease:** Germany has recorded 128 outbreaks of Newcastle Disease. A stamping out policy is being utilized. Zimbabwe, Swaziland and South Africa have also reported outbreaks.

**Viral Hemorrhagic Disease of Rabbits:** An outbreak of VHDR oc-
curred in Cuba during 1994. The outbreak was controlled by depopulation and movement restrictions.

Isolates of Non-pathogenic Avian Influenza Viruses in Mexico. Eduardo Rivera Cruz, SARH. Avian Influenza was diagnosed for the first time in Mexico in May, 1994. Dr. Rivera Cruz reported that a total of 77 viral isolates have proven to be H5N2 serotype, a non-pathogenic strain. Mexico is conducting a national serologic survey to determine free areas for this disease and establishing a program for certification of free flocks.

Risk of Screwworm Re-introduction into Mexico and the U.S. Armando Mateos, SARH. Dr. Mateos described the recent outbreaks of screwworm in Mexico (92-94) as examples of the particular importance Mexico places on surveillance activities, and of the urgent need to relocate the screwworm production plant to Panama. The plant in Chiapa de Corzo, Chiapas is due to be closed by the end of 1996. In evaluating the specimens submitted for identification during the 92-94 outbreaks, Mexican authorities noted that Dermatobia seems to be taking over the niche previously occupied by screwworm.

NNVD in Ostrich in Zimbabwe. Joan Jeffrey, Texas A&M. Dr. Jeffrey reviewed the current situation in Zimbabwe with NNVD in commercial chicken flocks, a concern for the many commercial ostrich producers who export to the U.S. The regulatory program in Zimbabwe relies on surveillance, vaccination and biosecurity to contain VVND in commercial poultry. However, ostrich producers, in cooperation with the Zimbabwe Ministry of Agriculture, are keeping ostrich flocks un-vaccinated and disease free to meet USDA import regulations.

A Molecular Approach to Studies of Heartwater - DNA Technology on the Trail of the Real Heartwater Disease Agent. Basil Allsopp, OVI. This paper was discussed as presented in the General Session. The full text is included elsewhere in these proceedings.

Aliens in Aliens. Tom Craig, Texas A&M. Dr. Craig expressed concern that exotic wildlife have been imported without looking for exotic internal parasites. Several Ostertagia-like intestinal nematodes of exotic wildlife have been isolated and identified. Some of these parasites are now cross-infecting indigenous wild species and domestic livestock, and many of the new parasites have been shown to be resistant to ivermectin. Imported ratites have been another source of exotic parasites.

Emerging Diseases and International Livestock Development - Nepal and the Pacific. David Ligda, UNDP. Dr. Ligda related experiences with UNDP-financed livestock programs in countries such as Nepal. Concern was expressed about the lack of attention to livestock production and development projects have received in recent years in less developed countries.

Committee Deliberations: The members discussed and voted to support the resolution that the USDA urgently identify the resources necessary to assure the building of a new sterile screwworm production facility in Panama.
THE NEW US LABORATORIES FOR FOREIGN ANIMAL DISEASE RESEARCH AND DIAGNOSIS AT PLUM ISLAND

Roger G. Breeze, BVMS, PhD, MRCVS
Center Director

July 1994 marked the 40th anniversary of the establishments of the USDA foreign animal disease research and diagnostic Center on Plum Island. The Center was created by the Congress in response to the outbreaks of foot and mouth disease (FMD) in Mexico (1946-54) and Canada (1951/52). Before the Center opened, there was no capacity for laboratory diagnosis of FMD in North America - to confirm that the vesicular disease outbreak that began in Mexico in 1946 was in fact FMD, samples had to be shipped to Great Britain for examination at the Pirbright laboratory. Delay in diagnosis allowed the epidemic to become established over a wide geographic area.

Diagnosis of FMD in the US depended until 1955 on differential animal inoculation on the farm where the disease outbreak occurred. Pigs, cattle and horses from a clean area were brought to premises with a vesicular disease outbreak and inoculated intradermally with vesicular fluid/tissue. If all three species developed vesicles on the tongue, the diagnosis was vesicular stomatitis. If only pigs and cattle were infected, the diagnosis was FMD. This method had been used successfully since the early 1900s - except when vesicular exanthema occurred for the first time in swine in the 1930s and was mistakenly thought to be FMD.

One important attraction of Plum Island as a site for the new Center was that a biocontainment laboratory for infectious diseases (Building 257) had recently been constructed on the island by the US Army Chemical Corps. When USDA took control of Plum Island from the Army in 1954, diagnostic work began immediately in Building 257, the first priority being to establish diagnostic competency for vesicular diseases. Initial work with vesicular stomatitis was quickly extended to vesicular exanthema. FMD diagnosis began in July 1955. Research did not get underway to any great extent until a newly constructed research laboratory (Building 101) was opened in 1956.

Plum Island is some 840 acres and about three miles long by a mile wide at the widest point. It is situated about two miles off the eastern tip of the North Fork of Long Island, New York, and about nine miles off the Connecticut shoreline. USDA is the only occupant, with research programs of the Agricultural Research Service (ARS) and the Animal and Plant Health Inspection Service's (APHIS) Foreign Animal Disease Diagnostic Laboratory. The island is self-contained, like a small city, with its own emergency fire and medical services, power plant, sewage decontamination plant and marine transportation.
Wherever situated, maintenance and operation of any biocontainment laboratory are very expensive since mechanical, electrical and other systems essential to sustain biological safety must operate 24 hours/day, 365 days/year - and there is twice the normal amount of machinery because back up systems and personnel must be constantly available in the event of critical unit failure. All these costs are necessarily higher at this Center because of the island location and general expense of the Northeast region. From 1954, in addition, there was considerable inherent inefficiency and additional operating and capital expense at Plum Island because USDA had to use existing Army facilities scattered over most of the island. The research laboratory (Building 101), the Administration area and the Foreign Animal Disease Diagnostic Laboratory (Building 257) were each about one mile from one another (Figure 1). Many miles of roads, sewer, water, gas, electrical and telecommunications lines had to be maintained and transportation between various buildings was a major cost factor in terms of lost work time, vehicle and fuel expense. There were substantial additional costs of heating and operating numerous small, old-fashioned buildings that did not meet modern building and safety codes.

In 1983, Edward Diamond, Chief Engineer at the Center, proposed that diagnostic, administrative and facilities support operations be consolidated in one place at Building 101 to provide about $2 million/year in operations savings and avoid $20 million or more in urgent facilities repair/renovation costs of old Army buildings. By Spring 1995, his proposal will have become a reality.
NEW US LABORATORIES AT PLUM ISLAND

Facilities Consolidation

About 40% of the research building (Building 101) has been renovated to provide modern laboratory space for both ARS and APHIS scientists. These renovations were needed to provide the infrastructure to support modern scientific equipment and techniques. In addition, a 55,000 square foot Science Support Building was constructed immediately adjacent to Building 101. ARS and APHIS programs will use these facilities as described below (Figure 2).

Diagnostic Laboratory: The West Animal Wing (10,000 square feet) and Laboratories A and B (12,000 square feet) in Building 101 have been given to APHIS' Foreign Animal Disease Diagnostic Laboratory (FADDL), which will move from Building 257 in early 1995 (Building 257 will then be closed).

The West Animal Wing has 18 self-contained animal rooms of either 150 or 200 square feet arranged on a three-corridor system. There is an adjacent necropsy area with large animal carcass incinerator. The air filtration system in the West Animal Wing is being upgraded to provide high efficiency particulate air (HEPA) filtration of supply and exhaust in two of the four quadrants and HEPA exhaust on the other two.

Laboratories A and B, vacated by ARS, were gutted and completely rebuilt to provide space for reagents and vaccine production and for diagnostic and pathology services of FADDL. There are five separate air systems (HEPA supply and exhaust) in the FADDL diagnostic area to provide biological separation of various work functions and to protect against accidental contamination.

The FADDL laboratory and animal space in Building 101 are wholly separate biologically from research space occupied by ARS. APHIS staff enter and leave through separate entry/change facilities and internal access corridors from those used by ARS. The few common walls between ARS and FADDL laboratories are sealed with a cocooning material. Air and mechanical systems are separate. Animals for FADDL are delivered directly to the West Animal Wing: those for ARS go directly into the East or Orient Animal Wings. Supplies and equipment for FADDL pass into the laboratory through an autoclave or through airlocks. Laundry and glassware from FADDL are sent via an autoclave or airlock into facilities shared with ARS in the East Wing. Clean laundry and glassware return to APHIS through an autoclave.

Research Laboratories: Research laboratories are in the West Wing and Labs C and D: all have HEPA filtration of air exhausts. Six new laboratory modules (total 6000 square feet) have been constructed for ARS researchers in the West Wing. These modules have HEPA filtration of exhaust air. Half of laboratory D was renovated to accommodate an x-ray crystallography unit and electronmicroscopy - the remaining portion of Lab D is being modernized for FMD research.
Laboratory C (6000 square feet) is vacant. This laboratory, formerly used for FMD research, may be utilized by external researchers from academia, industry or government who need BL3 Ag level research space.
NEW US LABORATORIES AT PLUM ISLAND

The East Animal Wing has 18 self-contained animal rooms of either 150 or 200 square feet and arranged on a three-corridor system. The Orient Animal Wing has six self-contained animal rooms of 400 square feet and two of 900 square feet: this wing is a single corridor system. Both wings have individual necropsy and carcass incineration facilities. Both wings have HEPA filtration of all exhaust air systems. The Orient Animal Wing can be operated in isolation from the rest of the ARS facilities. It has been used for large animal challenge with zoonotic agents, such as Rift Valley fever and Venezuelan Equine Encephalitis viruses - including recombinant Class 5 organisms. A small laboratory is also available in the Orient Wing and is used to work with specimens containing zoonotic agents when the wing is isolated from the rest of the building. This wing can hold about 80 adult cattle indefinitely.

Science Support Building: A 55,000 square foot Science Support Building was constructed in 1993 immediately adjacent to Building 101. This new facility provides space for scientific and administrative offices, Safety, Photography, Library, auditorium and cafeteria. In addition, there is BL1/BL2 laboratory space (2,000 square feet) outside biocontainment for work that does not involve infectious foreign animal disease agents.

Animal Holding Facilities: From 1954 until 1991, horses, cattle, swine and other animals purchased for research or diagnostic use were held for a period of weeks or months in open pens in an isolated part of the island in order to receive preventive veterinary care or be pre-conditioned before entering the ARS or APHIS biocontainment laboratories. These animals were held in strict quarantine by a dedicated cadre of animal caretakers who had no contact with other animals and who did not enter biocontainment laboratories where infectious organisms were present.

Over the years since the Center was established, local animal agriculture has disappeared - indeed, today the State of New York recognizes no commercial herds of cattle, sheep or swine on Long Island. In Connecticut, cattle, sheep and swine farms are dwindling yearly. In 1991, the only susceptible domestic animals within a 20 mile radius that might amplify FMD virus accidentally released from the biocontainment laboratories were here on Plum Island. An amplification of FMD virus could potentially contaminate people, vehicles and materials leaving the island, presenting a risk to the US.

To remove the biological safety risk and to consolidate facilities and reduce operations costs, the animal holding facilities were closed in 1991. We now issue strict specifications and prerequisites to vendors and use "just in time" delivery whereby animals go directly into the biocontainment labs when they arrive. There is no possibility of on-site amplification of FMD virus by domestic animals because there are no domestic animals outside biocontainment. Occasionally, one or two deer will swim onto the island. Each spring, any deer are culled.

As a result of removing the potential for contamination by virus ampli-
fication in FMD-susceptible animals, it is no longer necessary to continue
the rigorous disinfection, quarantine and restriction of vehicles and materi-
als entering and leaving the island - which has also greatly enhanced effi-
ciency and cut costs. Of course, any equipment or materials that have
been in a biocontainment building are fully disinfected before removal from
biocontainment to ensure no viable infectious organisms remain. Person-
nel entering biocontainment are still subject to seven day personal quaran-
tine and showering/clothes change procedures and a three day personal
quarantine is in force for all visitors to the island.

   Facility Closures: By Spring 1995, research and diagnostic work will
take place entirely in Building 101 - Building 257 will be closed. A dozen
other buildings in the Administrative area and two in the animal quarantine
compound at the east end of the island have already been closed as staff
and functions have moved to the Science Support Building. From some 56
buildings scattered over 840 acres, we have consolidated into less than 20
(only five of which are occupied) on about 100 acres between the research/
diagnostic building and the dock (Figure 1).

   This Facilities Consolidation construction/renovation cost about $20
million. However, the considerable operations and capital savings projected
by Edward Diamond have been achieved. Operations support staff have
been reduced by 15%, oil and electricity use are greatly diminished and
many inefficiencies have been eliminated. In addition, USDA has avoided
major renovations that would have been needed to bring old Army facilities
to current safety and environmental standards.

Facilities Modernization

   Over the past 40 years, the harsh environment and continuous opera-
tions have exacted a heavy toll on major equipment and facilities at the
Center. Boilers and electrical motors are worn out, other equipment has
been rendered obsolete by technological advances and/or inability to ob-
tain spare parts. Beginning in 1989, therefore, USDA began what is in-
tended to be a 10 year Facilities Modernization program to sustain the in-
frastructure needed for Center operations over the next 25 years. Three
new ferry boats were purchased and many major renovation projects have
been completed or are underway, including: replacement of deep bed fil-
ters by HEPA filters in laboratories and animal wings; new chiller and waste-
water treatment plants; upgraded electrical and motor control systems; new
medical and fire emergency vehicles; removal and replacement of under-
ground oil storage tanks. In the past 5 years, about $20 million have been
spent on modernization. We project another $60 million will be needed. A
significant portion of these funds will be required to bring island facilities up
to environmental standards in utilities, water, fuel and waste handling, and
to meet occupational safety and health laws.
NEW US LABORATORIES AT PLUM ISLAND

The Scientific Base

In 1983, a National Research Council study group was generally critical of scientific programs at this location and recommended that the Center be relocated to the mainland, preferably adjacent to a major university. Among the reasons for such a move were: scientific recruitment problems; scientific isolation; difficulties in interacting with other scientific institutions; facilities deficiencies; and the high cost of island support operations. In 1994, science at the Center is meeting the nation's highest priorities and setting the standard worldwide for quality and originality.

Scientific recruitment is not a problem. We have exceedingly strong research programs providing exciting opportunities in cutting-edge science. Positions here are sought after by first rate scientists.

Scientific isolation is no longer an issue. High-speed ferry service has boosted communication with Long Island and Connecticut - about one third of Center staff now live in the latter state. ARS has research labs at Yale University and the University of Connecticut (no infectious foreign animal disease agents are in these labs) and recent/current collaborations with many scientific institutions in the US and abroad, including: University of California, Purdue University, University of Nebraska, Tufts and Harvard Universities, SUNY Stonybrook, University of London and government research labs in Great Britain, Spain, Holland, Germany, Australia, Argentina, Africa and Brazil. Modern telecommunications and genetic engineering techniques permitting non-infectious materials to be shared with scientists outside Plum Island have overcome geographical isolation. Rather than dreaming of a move to the mainland to be near one university, we see ourselves as already neighbors of any university or potential cooperator that can further the interests of the US and American agriculture, wherever these might be in the world. We already interact with a dozen or more institutions worldwide where there are staff or resources that can further US interests. Other US and/or foreign institutions and companies or governments regularly approach the Center with collaborative projects which require BL3 Ag level biocontainment. These projects may range from: evaluating curing methods for Parma or Serrano hams in terms of the ability to kill foreign animal disease viruses; developing and testing a genetically engineered vaccine for rinderpest; evaluating a vaccine for African horsesickness; validating a commercial bluetongue diagnostic kit; or studies with exotic animal germplasm without the expense and time of importing this into the mainland US. These projects may be from a day to months in duration. In the past 7 years, none of the wide and varied requests for collaboration or cooperation in use of ARS laboratory or animal facilities has been refused, to my knowledge. To make access even easier for external scientists than it has been in the past, as part of Facilities Consolidation 6,000 square feet of BL3 Ag laboratory space (Lab C) and 1,000 square feet of non-biocontainment BL1/BL2 space are available and dedicated to other outside users. We will also work with potential external users to make
animal rooms available in the East and Orient Animal Wings. This requires more coordination because there is no dedicated external user space. Of course, we expect to charge for the cost of providing space - just as the home institution of the user would charge. But we do not necessarily expect to recover 100% of all costs. No potential user or cooperator should be discouraged from approaching the Center to use facilities because of perceived cost problems.

Facilities deficiencies are inevitable at a time of tight budgets, especially when standards are changing (usually upward), scientific methodology and equipment grow more sophisticated everyday and there is hard wear from the environment and continual use. Very significant improvements have been made in the past 5 years at Plum Island through Consolidation and Modernization and we have a systematic plan to correct remaining problems. It is important not to lose sight of the fact that an institution is defined only by the quality of its scientific staff and programs - not by its facilities, good or bad as they may be. Plum Island, I can confidently tell you, has a depth and breadth of research on the nation's priorities unmatched in this country or abroad.

Costs and Center Location

Plum Island is an expensive facility to operate. Biocontainment buildings to BL3 Ag standards are costly to run and maintain - irrespective of where they are located. We are situated in one of the nation's most expensive regions for wages, electricity and services, which make costs higher. In addition, there are further expenses associated with the island location. Consolidation and Modernization together with rigorous attention to smarter ways of providing services have cut operating expenses and greatly increased efficiency in the last few years. Unfortunately, inflation and costs of meeting new laws, regulations and standards at the federal, state and local levels have swallowed many of these savings. Further investment in automation and energy efficiency can trim costs further - but the biggest reductions have already been achieved. In short, despite the high bottom line, the basic costs of operating the Center are almost as low as they can be in this part of the country, given the facilities.

The most obvious additional costs associated with the island location are: marine operations; work time lost because of bad weather interrupting marine services; employee travel time (one way) to the island; and power plant operations 24 hours/day (on the mainland a dedicated Center power plant might not be necessary). These costs would not be incurred if the Center were not on an island. We do not regard these extra costs as inefficiencies but as additional safety costs. When Congress chose an island site for the Center in 1954, this was with the knowledge that further operations expenses would be incurred compared to a mainland site. The Congress accepted these additional costs because of the additional level of biological safety the location provided. This foresight paid off in 1977 when FMD virus accidentally released from Building 101 infected animals in holding
NEW US LABORATORIES AT PLUM ISLAND

facilities on the island. These animals developed clinical FMD and amplified the initial release. Internationally, this was defined as an intra-laboratory infection that did not involve the continental USA. As a result, there was no embargo or restriction on US animal or animal product exports.

A frequently asked question is whether the Center still needs to be on an island given biocontainment technology available in 1994 that was not available in 1954. The answer is no (but the island location is critical to international issues and opinion as outlined above). Incidentally, it is worth noting that the US is not the only country with such a Center on an island - the German lab on the Island of Reims was built in 1909 and the Danish lab at Lindholm in 1925.

Clearly, a laboratory and animal facility can be constructed anywhere in the country to meet the highest standards for biocontainment at the BL3 Ag level. But construction is the most straightforward part - the challenges are: to operate the facility without a biocontainment breakdown 24 hours/day, 365 days/year for 40 years or more until the plant is closed down and disinfected; to continually upgrade biological safety equipment processes and procedures as technology changes over the years; and to avoid human error by laboratory and support staff at work and at home. Of these, human error is the hardest to overcome.

All the major foreign animal disease labs around the world have had one or more accidents breaching biocontainment at some stage in their history. The risk of human error or a facility accident is the same irrespective of laboratory location. But the consequences of a biocontainment breach are not independent of location, the most important consideration being the numbers of susceptible domestic and wild species and competent insect vectors in the area.

The worst scenario for a laboratory release of FMD virus would be one that led to infection of swine on local farms and massive amplification of the virus - one infected pig can release 100 million infectious doses of FMD virus per day. Under suitable meteorological conditions, aerosolized virus can travel over long distances in concentrations high enough to infect recipient animals - for example, the 150 mile spread across the English Channel from Brittany, France to the Isle of Wight, Great Britain.

For the US, the advantage of a coastal site such as Plum Island (whether on an island or not) is that half the periphery is ocean, over which accidentally released virus would be harmlessly dispersed before it could reach the nearest susceptible animals three thousand or more miles away. Another plus at Plum Island is that animal agriculture, particularly swine, is absent or diminishing locally, making it much less likely that personnel would accidentally breach personal quarantine rules and come in contact with susceptible species outside work. To put these location issues in perspective, Switzerland has an area of 15,935 square miles and no coastline. A circle with a radius of 71 miles centered on Plum Island would enclose about 7,000 square miles of the Atlantic Ocean and much of Connecticut, Rhode Island and Long Island, New York - in an area equal to all of Switzerland.
The same radius around the new Swiss biocontainment laboratory encloses most of Switzerland and parts of three other countries - France, Germany and Italy.

Future Needs

For the foreseeable future, the US will need a BL3 Ag level (or higher) biocontainment facility for laboratory research and large animal challenge studies with foreign animal and zoonotic disease agents. This lab will be needed not just for foot and mouth disease but for all diseases whose unintentional release from the laboratory to the environment could cause public concern and serious economic damage to our agricultural industries. Old disease problems are still with us and free trade, easier international travel and changing public opinion present new challenges in regulation and control. New diseases emerge constantly - bovine spongiform encephalopathy, porcine respiratory and reproductive syndrome, the new and lethal viral infection of horses (and perhaps humans) in Australia, for example. Contrary to popular expectations, genetic engineering techniques do not remove or reduce the need for live animal studies - if anything, these will be increased as better understanding of microorganism virulence mechanisms and vaccine protection is obtained. It is true that there are many laboratories around the country in which research with foreign animal disease agents could be performed - and that there are biocontainment animal facilities in which a handful of large animals could be exposed to certain less contagious foreign animal disease agents. However, the major foreign animal disease threats to the US demand research with significant groups and numbers of large animals held for weeks or months in strict biocontainment from each other and from the environment, and exposed to field strain and genetically engineered live viruses with attendant amplification of the infection. The only place this research can be done is at Plum Island.

One example is the recent work presented to the USAHA Foreign Animal Diseases Committee at this meeting by Drs. Lubroth and Brown demonstrating that foot and mouth disease virus non-structural protein 2C can be used as a serological indicator to differentiate animals previously vaccinated against this disease from animals that have recovered from infection and which may be inapparent carriers of the virus. This study involved single or paired cattle or swine that were maintained in the strictest isolation from each other to avoid any possibility of accidental cross-infection for a period of a year or more while a series of vaccination and/or live virus infections with different viral serotypes was conducted. Any breakdown of biocontainment permitting unintended infection could have negated this expensive, long, yet highly significant study. Another is our research showing that swine recovered from African Swine fever virus infection are persistently infected for life - work that required swine to be held in biocontainment for two years. A third is the research on collection of embryos from cattle infected with foot and mouth disease virus that led to new regulations permitting germplasm importation - the experiments lasted years and involved scores of cattle bred and allowed to calve in biocontainment.
REPORT OF THE COMMITTEE
ON GOVERNMENT RELATIONS

COLLEGE PARK, MARYLAND
FEBRUARY 28 - MARCH 4, 1994

Chairman: Dr. H. Wesley Towers, Dover, DE

Dr. Jones W. Bryan, SC; Mr. Joe B. Finley, TX; Dr. Thomas J. Hagerty, MN; Dr. John P. Huntley, NY; Dr. Maxwell Lea, Jr., LA; Dr. Michael R. Marshall, UT; Dr. J. C. Shook, PA; Dr. Marion T. Szatalowicz, WI; Dr. Robert J. Vellure, ND; Dr. Richard D. Willer, AZ; Dr. Larry L. Williams, NE.

The Government Relations Committee of the United States Animal Health Association met on February 28 and March 1, 1994, in the United States Department of Agriculture Building in Washington, DC, with representatives of the United States Department of Agriculture and the Food and Drug Administration, Center for Veterinary Medicine. It also met with representatives of the Allied Industries at the national Cattlemen's Association Office, 1301 Pennsylvania Avenue, Washington, DC.

It was a pleasure to have Acting Assistant Secretary of Agriculture, Pat Jensen, and Acting Administrator of APHIS, Dr. Lonnie King, at the meetings and to hear of the new initiatives which are being planned as well as their interest in programs we feel important to USAHA.

The efforts of Dr. Don Luchsinger and his staff in making this meeting a very open and informative one were greatly appreciated. The continued opportunity to keep this meeting as a direct and open line of communication between APHIS and USAHA is viewed by our organization as being of the utmost importance.

USDA-APHIS-VS

Dr. Don Luchsinger, Acting Deputy Administrator, Veterinary Services, welcomed the USAHA Governmental Relations Committee to Washington, DC and introduced the following members of his staff:

CATTLE DISEASES SURVEILLANCE STAFF - Dr. Granville Frye

Brucellosis - There was a review of brucellosis statistics for infected herds on a nationwide basis. Dr. Frye discussed the brucellosis problem in bison in Yellowstone National Park. He presented seven proposed alternatives for handling the situation non of which have been adopted at this time.

Tuberculosis - There are presently 41 TB Free States in the country. There are seven cattle herds and eight cervidae herds under quarantine for TB. Dr. Frye also stated that there are proposed changes in TB
regulations for the movement of cattle from Mexico into the U.S.

IMPORT-EXPORT STAFF - Dr. Bob Kahrs

A concise risk analysis needs to be completed for implementation of the NAFTA treaty in light of the regionalization concept. User fees are now in place for import/export operations.

EMERGENCY PROGRAMS - Dr. Chris Groocock

BSE - One cow which originated from the United Kingdom has been diagnosed with BSE in Canada. The herd in which the disease was diagnosed has been depopulated. While 459 cattle were imported into the U.S. from the U.K. from 1981 to 1989, there are no indications of any clinical cases in the U.S. Brains from 1410 animals were submitted for histopathology to NVSL from 1990 to January 1, 1994.

Hog Cholera - The single suspect case of Hog Cholera in Texas proved to be negative.

Avian Influenza - Sample testing in live bird markets in New York. Three markets have revealed positive results for the H7N2 strain. Viruses isolated thus far have not been pathogenic for poultry.

SWINE DISEASES - Dr. Joe Annelli

Swine Brucellosis - Dr. Annelli discussed the swine brucellosis infection in employees in a packing house in the eastern U.S. Thirty-four swine herds at that time were under quarantine in the U.S. at the present time. APHIS is attempting to acquire authority to pay indemnity for depopulation of exposed swine in infected herds.

Pseudorabies - The eradication program is moving forward at a satisfactory rate. Nationally the number of quarantined herds has been reduced significantly.

MISCELLANEOUS PROGRAMS - Dr. Gary Colgrove

Scrapie - Sixty sheep flocks are now enrolled in the volunteer certification program with no diagnosis of the disease in any of these flocks. There are 73 known infected flocks and eight source flocks.

Salmonella enteritidis - The traceback program is continuing and progressing well.

Aquaculture - APHIS is seeking authority to develop certification programs for export products.

INTERNATIONAL SERVICES

Dr. Alex Thiermann stated that the mission of International Services is to monitor and assist eradication of major animal diseases in foreign countries. This aids in worldwide knowledge of problems, containment, and disease spread, and safety to the U.S. animal and poultry populations.
REPORT OF THE COMMITTEE

This process involves 100 employees overseas of which 25 are animal health professionals. The heaviest concentration of IS employees are located in Mexico and Central America.

The major concerns were screwworm eradication, foot and mouth disease, tuberculosis in Mexico, international movement of ratites and camelids, and hog cholera in Cuba and Germany. Increased emphasis is being placed for individual countries to bear their own financial budget responsibilities if they wish to participate in U.S. trade. There is concern about the infrastructure of regulatory systems in animal disease control in these foreign countries. Consideration is being given to establishment of offices in Central Europe (Budapest or Prague), the former Soviet Union, and in Beijing, China.

TRADE SUPPORT TEAM

Dr. Dan Sheesley stated that his office of five professionals tracks trade issues and assesses joint agreements between countries around the world. Worldwide FSIS trade systems are also included. Decisions should not be made in a "vacuum". NAFTA was ratified by Congress in 1993 and involves 360 million people, and $6 trillion; thus becoming the world's largest trade agreement. It was stressed that animal health, disease control, and food safety standards will not be lowered. Regionalization of specific world areas is recognized under NAFTA and GATT. APHIS is working through the Office of International Epizootics (OIE) for:

1. Standardization of rules
2. Risk assessments

There are four major committees within OIE, and the countries must provide the following prerequisites:

1. Information systems to generate data.
2. Infrastructure must be in place and functional.
3. Systems of risk analysis or assessment.
4. Must provide a logical, coherent, and sound document.

APHIS UPDATE - Dr. Lonnie King, Mrs. Patricia Jensen

The Governmental Relations Committee expressed a concern that APHIS not approve the IDEXX Laboratories gamma interferon test for TB without first consulting the USAHA TB Committee and upon advice of the scientific advisory TB subcommittee.

The Committee discussed the importance of implementing a standardized reliable slaughter identification system due to the increased emphasis on traceback capability.

Dr. King discussed reorganization of the USDA and the proposed establishment of a Food Safety Division. This will impact directly on APHIS and FSIS. The Committee expressed the importance that APHIS objectives and programs maintain their identity and emphasized the need to maintain adequate funding to complete the current disease programs.
Dr. King reviewed the Administration's proposed budget for FY 95.
Dr. King reported that APHIS is proposing to broaden the definition of the term "disease" to include residues and human pathogens.

Mrs. Jensen discussed the proposed reorganization plans for USDA with emphasis on the development of Food Safety Division and its impact on APHIS.

Secretary Espy has established a Pathogen Reduction Task Force chaired by Mrs. Jensen and made up of representatives from USDA, FDA, and CDC. This task force will address food safety in its entirety from farm to consumer. Mrs. Jensen requested that the USAHA provide a list of problematic areas for producers, processors, and consumers. She invited the USAHA to have a representative appear before the task force to discuss these problem areas.

BBEP - BIOTECHNOLOGY, BIOLOGICS, AND ENVIRONMENTAL PROTECTION

Dr. John Payne and Dr. David Espeseth provided an overview of BBEP activities that include responsibilities in the areas of biometrics, bacteriology, virology, diagnostics, and biotechnology. Recent expansion of ELISA test applications and gene probe technology represents the major portion of new diagnostic products being developed. Applications for products destined for further manufacture have also markedly increased. Nine major areas of emphasis were discussed by Dr. Espeseth.

The Committee commended BBEP for their recent efforts to streamline the approval process for diagnostic tests and products. The efforts to establish scientifically valid equivalent diagnostic procedures among potential trade partners is viewed as an essential component of the process of evaluating the health risk posed by imported livestock. The Committee offers strong support for this initiative.

AGRICULTURE RESEARCH SERVICE (ARS)

Dr. Bob Oltjen and members of the staff reported on their activities during the past year.

Dr. Oltjen reported that the facility at Plum Island is outdated. To address this concern, a task force was established; consisting of ARE, APHIS, and industry representatives. These task force was divided into four groups; technology assessment, status of USDA biocontainment facilities, status of University and other facilities, and industry needs for facilities. Several recommendations were made by the task force with emphasis to stay on Plum Island.

The Governmental Relations Committee commends ARS's commitment to maintaining the current status of U.S. animal health and supports their initiative to continue foreign animal disease research.

ARS is conducting ongoing research projects at various facilities lo-
cated throughout the U.S. (122 locations). Some of the areas of research include; identity of animal genome and transgenic animal studies to generate or manufacture precious proteins.

Dr. Robens addressed food safety; an area that presently is receiving major emphasis. Other areas of research activity include residues, microbiology, mycotoxins, and poisonous plants.

Because of human health concerns, Salmonella and E. coli continue to receive major emphasis.

Dr. Walton reported that the screwworm eradication program is going well in Central America.

Dr. Graham presented research information on several internal and external parasites of livestock and poultry.

The Governmental Relations Committee feels that strong emphasis in basic and applied research is essential in maintaining and protecting a productive and viable livestock industry.

FOOD SAFETY INSPECTION SERVICE (FSIS)

Dr. Donald White, Acting Administrator of FSIS addressed the Committee on the changes in emphasis from the traditional methods of inspection that his agency is anticipating. He pointed to the significant reduction in violative residue samples as a successful project and said the agency would now turn its attention to developing a pathogen reduction approach to inspection that would reach from the farm to the table. Baseline information and data are being collected as the agency completes studies on the levels of pathogens on steer and heifer carcasses as well as a future one involving cow and bull carcasses. Future microbial baseline data will be collected on poultry and ground beef as well as preoperation meat handling equipment. In addition, studies are underway that will determine the best way to handle fecal, ingesta and milk contamination of carcasses. The Committee commends the cooperative approach between FSIS and ARS in developing rapid in-plant tests that can be used for the detection of specific pathogen levels on carcasses.

Dr. White believed that a major reorganization of FSIS and APHIS would take place in the near future with emphasis being placed on food safety. He also proposed a greater involvement with state meat inspection, animal health and public health personnel to give a greater comprehensive approach to the food safety issue. The Committee supports the combination of FSIS with APHIS rather than another Federal agency to take advantage of the already existing infrastructure within APHIS which will allow for the implementation of animal identification and on-farm visits as necessary components of a farm to table food safety approach.

COOPERATIVE STATE RESEARCH SERVICE (CSRS)

Dr. Bill Wagner, CSRS Principal Veterinary Scientist, introduced Dr. Peter Johnson who presented an overview of National Research Initiative
GOVERNMENT RELATIONS

Competitive Grants Program. Fifty-eight grants totalling nearly $10 million were issued in FY 93 for proposals submitted for research in such areas as virology, bacteriology and parasitology. Dr. Wagner cited the TB working group's recommendation for a 5 year $20 million research program to be split between CSRS and ARS. At this time, there is no money allocated in the FY 95 budget for this proposal.

FOOD AND DRUG ADMINISTRATION (FDA)

The Committee appreciated the opportunity to talk with Dr. Richard Teske, acting Director of FDA's Center for Veterinary Medicine concerning the Center's current activities and concerns. The Committee commends the Center's initiative in identifying the reasons for reduced numbers of product approvals because of the problems in the submission and approval processes. Correction of the identified problems will hopefully expedite the handling of what seems to be an ever decreasing number of new animal drug submissions and supports the Center's goal of "submit it once - review it once".

The Center takes the position that all new generation antimicrobial products be available by prescription only.

There is a concerted effort from the human medical community to not allow the use of fluquinolones in animal health due to the perception that this would allow the development of resistant strains of pathogenic organisms to this class of drugs.

There is a concern that this precedent would severely limit or exclude access to future drug developments that would be necessary to protect animal health.

ALLIED INDUSTRY

The Committee met with representatives of the Washington, D.C. based allied industries. Some of the topics discussed included TB, M branding and the use of the blue CNG eartag in Mexican imports, individual identification of Mexican cattle for interstate transport, the status of the IDEXX gamma interferon test and the issue of pathogen reduction/preharvest food safety.

An item of particular concern was the proposed reduction of the FY 95 budget for APHIS. The need for third party documentation/certification of quality assurance programs to meet ISO standards for future world trade was also mentioned. The problems created by a recent change in the requirements for the issuance of certificates of veterinary inspection were shared with the group. The Committee complimented the NCA for taking the leadership role in chairing and directing the activities of the TB working group.

The Committee also appreciated the opportunity to meet and discuss pertinent animal and poultry health issues with Veterinary Services staff and regional directors on Wednesday afternoon.
ANAPLASMOSIS IN OKLAHOMA AND THE SOUTH CENTRAL UNITED STATES

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Anaplasmosis is the only major tick-borne disease of cattle endemic in the United States. The causative agent, *Anaplasma marginale*, invades and multiplies in erythrocytes of cattle causing mild to severe anemia. Annual mortality and morbidity due to anaplasmosis among U.S. beef cattle has been estimated at 50,000 to 100,000 head and a cost of $300 million. The average weight loss during acute infections is 86 kg, with increased veterinary costs estimated at $52/head (Palmer 1989). The resultant loss of cattle for the export market has been estimated at $45 million annually (McCallon 1973). Anaplasmosis is prevalent in Oklahoma, especially in the central and eastern parts of the state where arthropod vectors are most abundant (Logan et al. 1985; Wright et al. 1985, Rodgers et al. 1994).

Transmission of *Anaplasma marginale*: Anaplasmosis can be transmitted biologically by several species of hard ticks (Dikmans 1950; Ewing 1981) or mechanically by blood-contaminated mouthparts of biting flies or fomites. Within Oklahoma, transmission has been thought to occur primarily by biting flies and thus the disease has been called “fly fever.” However, ticks most likely play a major role in transmission of this disease. Within Oklahoma, tick transmission is probably effected during summer months by *Dermacentor variabilis* of which the nymphs and adults are active from March to October. Transmission of anaplasmosis during winter months is probably effected by the one-host tick, *D. albipictus* (the winter tick), which is frequently found on cattle in November, December and January. The most abundant tick associated with cattle in Oklahoma, *Amblyomma americanum* (the Lone Star tick), does not vector *A. marginale*. Male ticks are believed to contribute markedly to transmission of *A. marginale* (Coan & Stiller 1987; Coan et al. 1987; Coan et al. 1989; Kocan et al. 1992a; Kocan et al. 1992b; Stiller & Coan 1994). Male ticks can acquire infection as adults by feeding for short periods on infected cattle. Because male ticks are intermittent feeders, they readily detach and can reattach to many cattle during their life. Interhost transfer of male *Dermacentor* ticks among cattle has been clearly demonstrated. These male ticks have been shown to be persistently infected with *A. marginale* and are able to transmit the rickettsia repeatedly to susceptible cattle (Kocan et al. 1992b). Transfer of infected male ticks from dams to their suckling calves could contribute to enzootic stability in herds by infecting young animals that are resistant to clinical disease (Stiller & Coan 1994). These
calves will become asymptomatic carriers and develop solid immunity. Transmission of *A. marginale* by male ticks is suspected to be especially important with one host ticks, such as *D. albipictus*, in which all tick stages would be associated with cattle. With one-host ticks, males would be the stage to transfer most readily among cattle.

**Distribution of anaplasmosis:** The distribution of anaplasmosis has changed over the past 50 years. A distribution map published by the USDA in the 1960's described the disease as being endemic and limited to the south central Gulf states and the northwestern states including Washington, Oregon, Idaho, Wyoming, Colorado, and California. The distribution of anaplasmosis now includes all states except the northeastern ones (Unpublished data, American Cyanimide, Inc.), although the most endemic areas have remained the same.

The distribution of anaplasmosis in Oklahoma has markedly changed, increasing from 35 counties in 1977 to 48 counties in 1991 (Rodgers et al. 1994). In a survey done in Oklahoma in 1972 over 13% of beef cattle producers listed anaplasmosis as their primary cause of economic loss. In a second survey done in 1985, anaplasmosis was reported on an average of 18% of farms. Highest rates (30%) were reported in southeastern Oklahoma. A serologic survey done in 1985, using fluorescent FIA test, reported that 55% of bovine sera tested positive, with 16% having high titers suggesting recent infection. A serological survey conducted by the Oklahoma Animal Disease Diagnostic Laboratory from 1976-1987, using the anaplasmosis complement fixation test, reported an average of 12.8% bovine sera positive for anaplasmosis, and positive serologies ranged from 9% to 19% in various herds (Goodwin 1988). Although the highest number of reactor cattle were found in the central to south central part of Oklahoma, the counties most affected varied markedly over the sampling period of 14 years. In the most recent serologic survey using the complement fixation test, seropositivity rates ranged from 4.7% to 17.6% in adult cows tested. Lowest seropositivity occurred in 1983-84. This period followed a severe period of hot dry weather which greatly reduced arthropod populations. The higher seropositivity rate of the 1976-1987 survey was probably due to a greater sensitivity of the FIA test, although comparative studies between the FIA and complement fixation test were not reported. Results of surveys using the complement fixation test probably represent cattle with higher titers and may not include those whose titers were below the sensitivity of the test.

The widening distribution of anaplasmosis is probably contributed to by increased movement of cattle and the lack of sensitivity of the complement-fixation test. The complement-fixation test for anaplasmosis has been shown to lack sensitivity, especially in calves, carrier cattle and in cattle that have been treated with tetracyclines which lowers antibody titers to often undetectable levels (Goff et al. 1990; Kocan, Unpublished data, 1994). Thus, cattle may be shipped as serologically negative when they are active
ANAPLASMOSIS IN OKLAHOMA AND THE SOUTH CENTRAL UNITED STATES

carriers of the organism. These carrier cattle are then likely to serve as reservoirs of infection for mechanical transmission by blood-contaminated fomites, biting flies or biologically by ticks. Clearly, development of a new diagnostic test for anaplasmosis is needed and would aid in the control of anaplasmosis.

Although anaplasmosis has been most prevalent in the summer months, increasing numbers of serologically positive cattle have been detected in Oklahoma during the winter months. Since biting flies are not present during this part of the year, transmission is probably due either to biological transmission by the winter tick, *D. ablipictus* or to mechanical transmission by use of common needles or blood-contaminated veterinary equipment.

**Diagnosis of Anaplasmosis:** Serologic diagnosis of anaplasmosis is predominately accomplished by the complement-fixation test (USDA, 1968). Many other tests have been developed to measure *Anaplasma*-specific antibodies, including the capillary-tube agglutination test (Ristic 1962), card test (Amerault & Roby 1968), latex agglutination (Montenegro-James et al. 1981), indirect fluorescent antibody test (IFAT) (Goff et al. 1990), conventional ELISA (Shkap et al. 1990), antigen capture enzyme-linked immunosorbent assay (Trueblood et al. 1991) and radioimmunoassay (Schunter & Leatch 1988). The IFA test and antigen-capture enzyme linked ELISA has been shown to have promise for development as a new diagnostic test for anaplasmosis (Goff et al. 1990; Trueblood et al. 991). The sensitivity of the IFA was similar to that of a DNA probe (Goff et al. 1990). Growth of *A. marginale* in cell culture may provide an inexpensive and more consistent antigen for use in serologic tests. We have recently propagated *A. marginale* in tick cell lines (Blouin et al. 1994). Organisms from bovine erythrocytes transformed into large colonies of organisms similar to those found in infected tick cells. If the antigenic composition of the cultured organisms is similar to that of the erythrocytic stage, they may be suitable to use as antigen for development of a serologic test for anaplasmosis. Such a test may be more specific because bovine erythrocyte stroma would not be present in culture-derived antigen. A disadvantage of serologic tests is that they do not differentiate infected from vaccinated cattle.

Nucleic acid hybridization has also been developed for diagnosis of *A. marginale* infections (Goff et al. 1988; Visser & Ambrosio 1987; Eriks et al. 1993a; Eriks et al. 1993b). Using DNA probes, *A. marginale* DNA has been detected in individual tick salivary glands and in erythrocytes at a level of 100-1000 infected cells. Many of these probes utilize radioactive markers which make them unpractical for routine use. Recently, we have developed a PCR-mediated digoxigenen-labeled DNA probe (Ge et al. 1994). This probe was found to be *A. marginale*-specific when tested with 17 species of microorganisms and was able to detect infected erythrocytes 14 days prior to detection of parasites in stained blood smears. The probe
also detected microscopically-inapparent infections in carrier cattle. Because the probe detects DNA rather than antibodies, cattle infected with *A. marginale* could be differentiated from vaccinated ones.

**Control:** Control strategies for anaplasmosis in Oklahoma vary owing to the complexity of the disease and mode of transmission, and may include vector control, administration of tetracyclines, vaccination and maintenance of uninfected cattle. The marked reduction of anaplasmosis in Oklahoma after the extremely hot summers of 1980 and 1981 when arthropod populations were greatly reduced suggests that arthropods (ticks and biting flies) play a major role in transmission of anaplasmosis in Oklahoma (Rodgers et al. 1994). However, vector control is not intensively practiced in Oklahoma.

Tetracycline antibiotics have been shown effective for prevention of clinical anaplasmosis (Brock et al. 1959; Kuttler 1980). Small doses administered as feed supplements or incorporated into mineral and feed blocks have been shown to be effective in reducing parasitemia and preventing clinical anaplasmosis. These feed supplements are widely used in Oklahoma. In areas of Oklahoma where anaplasmosis occurs frequently during the winter, we recommend extended use of tetracyclines. Although some have claimed that tetracycline treatment will clear cattle of *A. marginale* infection, our experience has been that these treated cattle remain carriers even though they may become serologically negative. Despite the widespread and continued use of tetracyclines, resistance of *A. marginale* to this drug has not been reported.

Current vaccines marketed in the U.S. are killed vaccines made from infected bovine blood. These vaccines reduce clinical symptoms but do not prevent infection. Vaccinated cattle that are challenge-exposed thereafter become carriers and their blood can serve as an infective source for ticks and or mechanical transmission via biting flies and blood-contaminated instruments. Vaccination has been used widely in Oklahoma (Rodgers et al. 1994). An important factor in successful control programs is the identification and removal of infected cattle from susceptible ones. Maintenance of a “clean” herd eliminates the possibility of mechanical or biological transmission among individuals. Identification of carrier cattle may be difficult when tetracyclines have been administered because antibody titers may have lowered below detectable limits, especially when using the complement-fixation test. We have tested the effect of tetracyclines and vaccine-induced antibodies on *A. marginale* infections in ticks in hopes that either treatment of cattle would subsequently reduce infections in ticks (Kocan et al. 1991, 1992). Both tetracycline and antibodies are taken into ticks as they feed for extended periods on treated or immunized cattle. The results of our studies have demonstrated that, while both control measures reduce infection in cattle, neither tetracycline nor vaccine-induced antibodies reduce *A. marginale* infections in ticks. Therefore, despite use of these mea-
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sures for control of anaplasmosis in cattle, ticks that have fed on treated cattle will remain persistently infected and capable of transmitting *A. marginale*.

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**References**


ANAPLASMOSIS IN OKLAHOMA AND THE SOUTH CENTRAL UNITED STATES


To be sure those individuals who contracted Babesia microti believe that wildlife babesias are important. However, throughout the world more human cases of babesiosis which were identified are caused by species which infect domesticated animals such as Babesia bovis or B. divergens. A few human cases are presently unidentified and as there are more than 70 species of Babesia recognized throughout the world. It is likely the unidentified Babesia of humans are in fact Babesia of wildlife (Thomford et al 1994).

During the past several years clinical cases of babesiosis have been described in bighorn sheep, Ovis canadensis, woodland caribou, Rangifer tarandus, and wapiti, Cervus elaphus canadensis. The organisms resemble a common parasite of white-tailed deer, Babesia odocoilei, but have characteristics that indicate they are not the same parasite.

The emphasis of this paper is to direct speculation concerning Babesia sp which occur in wild, domestic and exotic ungulates. The literature is replete with observations in which babesias occur in animal species with which it did not evolve, in the environment in which they acquired the infection (Findley et al 1977, Adam et al 1976). In all likelihood the incidence of such interactions will increase, especially with the cavalier attitude that has developed toward the movement of wild and exotic species of ungulates in North America.

Not only is there movement of mammals, there also appears to be movement of some tick species. This may be a temporary aberration or it may be an adaptation to a formerly unsuitable environment that has been altered in such a way to make the off host environment suitable for the ticks. Conversely the spread of the imported fire ant, Solenopsis invicta, may render areas unsuitable for the survival of certain tick species. The lessening of numbers of ticks in a region by fire ants may have the effect of changing the exposure patterns to various Babesia species in such a way that only a small portion of the at risk population becomes infected at an early age. This may lead to an epidemiologically unstable situation in which older animals coming in contact with the organisms are adversely affected.

There are still not good criteria for the speciation of many babesias so when an organism is detected in an unusual host it may be difficult, if not impossible, to determine exactly which parasite you are dealing with. Without this knowledge it is impossible to attempt to control the disease, as the
vector(s) will be unknown as are factors which may lead to clinical disease.

Studies comparing various isolates with defined species are still not definitive. The work of Goff et al (1993) and Thomford et al (1993) indicated that the small Babesia isolated from desert bighorn sheep (Ovis canadensis nelsoni) was distinct from Babesia odocoilei based on cross protection experiments in splenectomized white-tailed deer and culture characteristics. The data definitely indicate the organisms were different but are not compelling enough to say they are different species. They are different isolates separated geographically and temporally. However, the large babesias isolated by Thomford et al (1993) were not subjected to cross infection studies in animals, only cultured in heterologous erythrocytes of bighorn sheep and mule deer (Odocoileus hemionus). The large babesias are clearly different species than the small babesias but there is insufficient data to prove the two isolates of large babesias to be different species.

None of the mule deer, black-tailed or white-tailed deer infected by the small or large Babesia sp showed any clinical signs of disease but had a transient drop in packed erythrocyte volume (PCV) (Goff et al 1993, Thomford et al 1993). However, one wild caught bighorn showed a 70% reduction in PCV and subsequently died. The death of the animal was not directly attributable to babesiosis but was thought to have severely compromised the sheep. Splenectomized white-tailed deer also showed severe signs of disease and were euthanitized. Attempts to transmit the agents to splenectomized domestic sheep (Ovis ares) and cattle (Bos taurus) were unsuccessful.

European reindeer infected by the nominally cattle parasite, Babesia divergens, caused severe disease and death (Nilsson et al 1965). An American woodland caribou confined in a Minnesota zoological garden developed signs of clinical babesiosis. A Babesia with morphological characters of B. divergens and B. odocoilei was cultured from the blood of the caribou. The organism also grew readily in white-tailed deer erythrocytes and was morphologically indistinguishable from B. odocoilei (Holman et al 1994).

Three wapiti died with clinical signs resembling babesiosis. Blood from one of the wapiti was cultured and a Babesia similar in morphology to B. odocoilei was isolated (Holman et al 1994b). There was also serological cross reactivity with B. odocoilei but not with B. bovis. The Babesia isolated from the wapiti was cultured in white-tailed deer and red deer (Cervus elaphus elaphus) erythrocytes.

Babesia from wapiti, caribou and white-tailed deer were inoculated into non-splenectomized red deer. None of the infected red deer developed clinical signs of disease although there was a transient drop in PCV in all the infected deer. The organisms were recoverable by culture for up to 8 months following infection. In addition, Dermacentor albipictus ticks were fed to repletion on the red deer. Numerous D. albipictus had been recov-
ered from one of the fatally infected wapiti. No evidence of infection was detected in the ticks recovered from the three infected red deer.

All of the above mentioned naturally infected animals come from areas known to be within the range of *Ixodes* sp ticks, the vector of *B. odocoilei* (Waldrup et al 1990, Waldrup et al 1992). *Ixodes ricinus* is probably the vector of *B. capreoli* in Europe (Adam et al 1976). Studies comparing *B. capreoli* and *B. odocoilei* in splenectomized sika deer (*Cervus nippon*) indicated that immunologically impaired deer (splenectomized and treated with dexamethasone) can be severely affected by *B. capreoli* whereas only mild disease was seen in *B. odocoilei* infection (Gray et al 1991).

These studies further confused the understanding of babesias infecting various species of ungulates as *B. odocoilei*, *B. capreoli*, and the isolates from mule deer, wapiti, caribou, and bighorn sheep may be strains of a single complex species. The status of *B. divergens* is very much open to question as it parasitizes gerbils (*Meriones unguiculates*), humans (*Homo sapiens*) (Gorenflot et al 1991), reindeer (Nilsson et al 1965), fallow deer (*Dama dama*), red deer, roe deer (*Capreolus capreolus*), mouflon (*Ovis aries musimon*) (Enigk and Friedhoff 1962). It appears to be different from the complex of other cervid babesias but its relationship to its hosts as far as causing disease is uncertain.

From the observations on *Babesia* in cervids it appears that disease can occur, but there is no evidence that any of the recently isolated *Babesia* spp can infect domesticated mammals. Disease has only been seen under crowded, stressful conditions. Is the stress of captivity necessary to cause disease? Or do wild populations die of disease or become easy prey for predators?

Several species of *Babesia* have been described in antelope such as Roan (*Hippotragus equinus*), Sable (*H. niger*), Waterbuck (*Kobus defassa*), Blue wildebeest (*Connochaetes taurinus*), and Bushbuck (*Tragelaphus scriptis*). Many of these species are found in intensive or extensive management situations in the United States. None of the animals imported from outside of North America are retained in quarantine at zoological parks. However, their offspring can and do leave these facilities. If suitable vectors are present many carry hemoparasites as they do gastrointestinal nematodes which they have introduced into North America (Craig 1993).

The importance of this is that until one really looks infections are going to be missed. In free-ranging populations unless animals are really distressed and in a place where they can be observed losses due to disease such as babesiosis will be completely missed.

The cultivation of *Babesia* spp in erythrocyte culture has revitalized the study of *Babesia*. It is apparent that with optimal conditions various *Babesia* can be cultured in erythrocytes or sera of hosts in which they cannot survive in even splenectomized animals. If an organism cannot propagate in the erythrocytes or serum of a host then it cannot serve as host. How-

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ever, just because it is able to grow in a host's cells or serum it does not mean it can survive in that host in nature. A study of populations of wild or exotic ungulates to determine the prevalence of infection, not just serologic activity (Waldrup et al 1992), but by culturing or by new techniques such as polymerase chain reaction (PCR), will give further insight to the dynamics of transmission of these parasites.

Likewise the culturing of exotic hosts especially imported ungulates may save grief in the future under zoo conditions, there is little likelihood that epidemic disease could establish even if the organisms are adaptable to native wild or domesticated species. I do not believe that wildlife babesias are an immediate threat to the well being of human or animal populations. Constant vigilance and a better understanding of the interactions among hosts and the environment may prevent disease caused by Babesia sp from becoming important.

References


REPORT OF THE COMMITTEE ON
HEMOPARASITIC DISEASES

Chairman: Dr. Rube Harrington, Arlington, TX
Vice Chairman: Dr. Robert L. Hartin, Murfreesboro, TN

Dr. J. Lee Alley, AL; Dr. Ronald D. Anderson, NV; Dr. Gerald M. Buening, MO; Mr. Dan B. Childs, FL; Dr. A. A. Cuthbertson, NV; Dr. William C. Davis, WA; Dr. Chester A. Gipson, FL; Dr. Will L. Goff, ID; Dr. Thomas J. Holt, NY; Dr. Owen James, MT; Dr. Maxwell Lea, Jr., LA; Dr. Donald L. Notter, KY; Mr. J. O. Pearce, Jr., FL; Dr. James P. Quigley, GA; Dr. Miodrag Ristic, IL; Dr. George P. Shibley, KS; Dr. Charles E. Starkey, AR; Dr. James E. Strickland, GA; Dr. G. Gale Wagner, TX; Dr. James M. Williams, CO.

The Hemoparasitic Diseases Committee met on Thursday, November 3, 1994, at 1:30 pm in the Kendall Room. There were 7 members and 9 guests present. Four presentations were made to the committee.

Dr. G. M. Buening, University of Missouri, Columbia, Missouri, reported on a proposed project that would determine by flow cytometry the effect of *Anaplasma marginale* infection on the distribution of T-cell subsets populations and the expression of selected cytokines in isolated T cells. The study describes the use of a unique *in vivo* sampling technique which will allow the monitoring of selected cytokine expression in T cells isolated from the peripheral circulation and the spleen. The base levels of T-cell subpopulation distribution and the expression of mRNA for cytokines will be measured prior to inoculation, and thereafter, in paired control and *Anaplasma marginale* inoculated calves. Sampling periods will be preinoculation, prior to and after peak rickettsemias in acute anaplasmosis. The sampling will be continued during recovery and early carrier phase. Enriched bovine T cells will be isolated by a planning technique. Specific subpopulations will be isolated by using specific monoclonal antibodies. The nitrite/nitrate concentration in the plasma of experimental calves will be measured at sampling intervals throughout the experimental period. This assay will be used as an indirect measure of macrophage activation in vivo. The level of rickettsemias will be monitored by a polymerase chain reaction (PCR)/nonradioactive DNA probe assay at sampling intervals throughout the experimental period.

Dr. Tom Craig, Department of Veterinary Pathology, College of Veterinary Medicine, Texas A & M University, College Station, Texas, presented a paper on Wildlife Babesiosis--Just How Important Is It? This paper will be published in the proceedings.

Dr. Katherine M. Kocan, Department of Veterinary Pathology, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma, presented a paper on Anaplasmosis in Oklahoma and the South Central...
United States. This paper will be published in the proceedings.

Dr. G.G. Wagner, Texas A&M University, reported one a vaccine study in Mexico involving approximately 500 cattle is into the second year. Most recently, 80 8-10 month old calves were added to the vaccinated herd. Vaccinated controls indicated the resistance of field challenges. All vaccinated animals continued to apparently resist challenge. The seroprevalence in the vaccinated herd was 96 percent. In a related experiment conducted in Texas, vaccinated cattle that were not subsequently challenged with Babesia infected ticks, apparently resolved the carrier infection within two years.
ANIMAL DISEASE CONTROL IN SOUTH AFRICA

G.K. Brückner and A. Faul

Introduction

South Africa is by nature of its geographical situation at the bottom tip of the African continent and by nature of its economic stability, very much part of the global trading industry and fully committed to maintaining and improving the status quo.

Through the years, the Veterinary Services of South Africa have established a firm international standing in the control of major animal diseases. The success of instituted control is demonstrated by the eradication of disease like Rinderpest (1904), Bovine Contagious Pleuropneumonia (1924), Glanders (1945), East Coast Fever (1954), Hog Cholera (1918) and scrapie (1972). Many of these diseases are endemic in other parts of Africa.

Also, diseases like Contagious Equine Metritis, Equine Encephalomyelitis, Bovine Spongiform Encephalopathy, Swine vesicular disease, Brucella suis, Aujeszky's disease, Transmissible Gastro-enteritis to name a few, remain exotic to the country because of strict veterinary import control at frontier borders.

The primary aim of the State Veterinary Services, in terms of the Animal Diseases Act, 1984, and the Abattoir Hygiene Act, 1992, is to provide for the prevention and control of animal diseases and parasites, to promote animal health, to provide a public health service and to allow for the international trade and global movements of animals and animal products, and facilitate movement of genetic and biological material, without putting the health of our livestock industry and the public at risk.

The basic organizational structure of the State Veterinary Services in South Africa will be described as well as a brief outline of the actions taken to control the most important controlled animal diseases.

Organizational Structure of Veterinary Services in South Africa

The political changes that took place in South Africa during the past two to three years, culminated into a Government of national unity on 27 April 1994. The existing Government will operate under the jurisdiction of an interim constitution for the next 5 years. It is inevitable that the constitutional changes will have an effect on the final structure for the deliverance of Veterinary Services in South Africa. Yet, the interim constitution does not clearly imply a true federal division of executive authority. The country has been divided into 9 Provinces with a clear distinction between the functions to be carried out by respectively central government and the provincial
executive councils. In respect of Animal Disease Control, executive func-
tions like legislation, import and export control, training and the mainte-
nance of a national disease surveillance system, were reserved by legisla-
tion for execution by the Central veterinary authority. The way in which
State veterinary services are to be restructured on provincial level, are
currently being negotiated. It is estimated that it might take another 12
months or more before finality is reached for these structures to become
operational and until such time, the status quo will be maintained to ensure
continuity of existing services.

The organizational structure currently comprises a centrally controlled
Chief Directorate of Veterinary Services within the Department of Agricul-
ture with Directorates for Animal Health, Veterinary Public Health, Stock
Improvement and Stock Remedies and Foot and Mouth Disease Research.
The Onderstepoort Veterinary Institute now falls under the jurisdiction of
the Agricultural Research Council - a parastatal Government subsidised
institution. However, close links are still maintained with the Department of
Agriculture.
The incidence of animal diseases in South Africa is monitored by the Directorate of Animal Health, by means of a national disease surveillance programme. A total of 45 State Veterinary offices is strategically placed in seven geographical regions, each with its own Regional Director responsible to the Director of Animal Health. State Veterinarians in each region are assisted by trained Animal Health Technicians who are primarily responsible for disease surveillance in their respective areas.

Further support for surveillance is provided by 16 diagnostic veterinary laboratories distributed between the seven veterinary regions. Approval was given for the establishment of a separate Directorate for Information Management and Laboratory Services within the existing Chief Directorate of Veterinary Services to strengthen the disease surveillance and diagnostic capabilities of the national Veterinary Services.

The aims of existing disease control measures

With a cattle population of 9.8m, smallstock population of 28.6m, equids of 0.2m and pigs of 1.1m and a gross value of agricultural production of $4 billion, consideration to protect these industries is important.

Existing legislation in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) distinguishes between controlled and notifiable animal diseases. The former being diseases such as foot and mouth disease where State intervention, active disease surveillance and the instigation of zoosanitary control measures in the event of an outbreak is compulsory while the actions taken with an outbreak of a notifiable disease, is in line with the definition of monitoring (2) in which the gathering of data from the field takes place but no official action is necessarily implied.

The Animal Diseases Act, 1984 makes provision for the:
1. compulsory notification of the incidence or suspected incidence of a controlled or notifiable disease by a stock owner, veterinarian or para-veterinary personnel;
2. isolation, seizure, quarantine or disposal of infected and susceptible animals;
3. compulsory vaccination of animals eg. rabies, anthrax, bovine Brucellosis and Foot and mouth disease in certain areas;
4. the institution of controlled areas in which permanent control measures are applicable, eg. Foot and mouth disease, rabies and African swine fever control areas;
5. the establishment of animal health schemes eg, for Bovine Brucellosis and Tuberculosis
6. Steps to be taken by stock owners to prevent the introduction or spread of certain diseases.

A total of 29 diseases is now listed as controlled animal diseases of which African swine fever, foot and mouth diseases, rabies, sheep scab, brucellosis and tuberculosis are the most important. Diseases that are listed
as notifiable are Blue tongue, African horse sickness, Johne's disease and Lumpy skin disease. Four diseases of salmonids are included under the list of controlled animal diseases.

The status of the major animal diseases in South Africa

Foot and mouth disease

Foot and Mouth disease is endemic in game in the Kruger National Park (KNP) (6). The African Buffalo (*Syncerus caffer*) acts as the major reservoir for the maintenance of SAT types I, II and III infections in the KNP. A well defined control (buffer) area, was declared in the northern and eastern parts of the Transvaal province next to the KNP and bordering Zimbabwe and an area in Natal, bordering Mocambique and a strip in Western Transvaal and the Cape Province, bordering Botswana, and a small area around the Onderstepoort Foot and mouth disease Laboratory. Stock and game proof fences, which are regularly patrolled and maintained, have been erected over a total distance of 2 400 km. The main aim is to create
ANIMAL DISEASE CONTROL IN SOUTH AFRICA

a barrier and to prevent spread of the disease. Programmed weekly, two-weekly or monthly inspections of susceptible stock are carried out in these control areas and movements of stock are controlled by permits. As an additional safeguard, polivalent inactivated Foot and mouth disease vaccines are used biannually in the area adjoining the Kruger National Park the area bordering on Zimbabwe. The last outbreak in domestic animals occurred in 1983 in cattle adjacent to the Kruger National Park and in game in 1993 in the Kruger National Park.

A proposal for the zoning of South Africa into infected and disease-free areas for Foot and mouth disease has already been submitted for approval by the OIE.

FOOT AND MOUTH DISEASE
CONTROL AREA

African swine fever

The infected area in the Northern Transvaal, has been declared a control area as result of an extensive surveillance programme to establish the geographical distribution of the arthropod vector and wild pigs (7). Domestic pigs are not permitted to be moved from this area to the rest of the country, except for a limited number from approved piggeries, who may send pigs to two approved quarantine abattoirs in the Transvaal, from where products may not be exported. The last outbreak of ASF occurred in 1993 within the ASF control area involving a few animals in a backyard operation.
Bovine Tuberculosis and Brucellosis

An official testing scheme for these two diseases was established in 1969 and 1971 respectively. The Scheme makes provision for the compulsory testing of infected premises, surveillance testing of identified areas, maintenance testing of herds declared free of one or both diseases and compulsory slaughter of positive reactors. Therefore the national prevalence of Bovine Tuberculosis was brought down to 0.003% and that of Bovine Brucellosis to 1.3% at the end of 1993 (3).

Rabies

Rabies is endemic to South Africa with an average of 500 - 600 positive cases diagnosed per annum. Dogs (mostly in the Natal Province) represent 44% of all cases, *Cynictus spp.* 19%, cattle 17% and other farm animals and game 20%

Four enzootic forms of the disease exist (8), namely:

- **Canid - virus** in the Natal, Eastern and Northern Transvaal, East- and Western Cape areas where the dog and jackal are the most important disseminators of rabies.
- **Viverrid virus** in the central plateau where viverridae like mongoose and other wild felidae are the primary vectors.
- **Duvenhage bat virus** in localised areas in the central Northern Transvaal.
- **Bat and rabies related virus** in the Natal area and most Northern districts of Northern Transvaal.

Such epidemiological differences are difficult to explain as they cannot be wholly correlated with regional population densities of animal species.
ANIMAL DISEASE CONTROL IN SOUTH AFRICA

Immunological differences in the virus are unknown but it could be suggested that questions of adaptation and invasiveness to species could be considered.

From a disease control and eradication point of view, the distinction is important as it involves the application of the most suitable measures. The extent of area placed under restrictions as well as the most effective use of canine vaccination come into play.

The degree of control varies between endemic areas (eg in the central plateau where rabies are primarily transmitted by viverridae) and the traditional canine rabies areas or epidemic rabies areas (eg. in Natal and the Northern Transvaal)(9). In the latter stricter control measures are applied in respect of frequency of vaccination and movement control.

Those areas where the dog and jackal play the major role in the transmission of rabies, namely Natal and certain districts in the Northern Transvaal, have been declared rabies controlled areas.

Dogs in such an area must be vaccinated with an inactivated rabies vaccine between 3 and 7 months of age and after that at least once every three years. In high risk areas such as Natal, vaccination of all dogs annually is practised as a routine control measure. A movement permit is required for dogs, cats, wild carnivores and ground squirrels within, into and out of the control areas. Where outbreaks occur outside the control area, dogs and cats are vaccinated within a reasonable radius around the outbreak.

Scrapie

South Africa is free from scrapie for the past 22 years. Scrapie has been introduced to South Africa on only one occasion. The first suspected case was found in Natal in 1966 and the diagnosis confirmed by histopathology.(11) Subsequently scrapie was diagnosed in a total of 11 sheep on nine farms in Natal, the Eastern Cape and the Orange Free State. All cases were first or second generation progeny of a group of Hampshire Down sheep imported from England. A strict slaughter policy was applied and no new cases have been identified since 1972, indicating successful eradication of the disease.

Small ruminant imports from countries where scrapie occurs are prohibited. The only countries where live sheep and goats and genetics may be imported from are Australia, New Zealand (embryos only), Germany and Finland.

Continuous active surveillance is maintained by inter alia the screening of brain samples of food animals submitted for rabies diagnosis or other diseases of the nervous system for BSE and other conditions, including scrapie.

Newcastle disease

Newcastle disease was first diagnosed in South Africa in 1944. A slaughter-out policy was followed until 1967. Vaccination of poultry started in 1971 and only isolated outbreaks (an average of 10 outbreaks per year) have
occurred since vaccination started. Approximately 2.5 million doses of Newcastle disease vaccine are manufactured locally per year, and 1.3 million doses are imported per year. Vaccines used include the Hitchner B1 strain, La Sota strain, Komarov strain, as well as several inactivated products. Although vaccination of chickens are routinely carried out by larger producers, back-yard flocks are usually not vaccinated and there is therefore always a danger of Newcastle disease present. (5)

During June 1993 a virulent (velogenic) strain of Newcastle disease was isolated following extensive outbreaks of the disease in broilers. The outbreaks in broilers was followed by subsequent outbreaks in ostriches in the Eastern Cape Province with further outbreaks in the Transvaal. Control measures employed include the general vaccination of ostriches by means of the simultaneous of the inactivated chicken vaccine, with a live La Sota strain vaccine (eye-drop application), registration of producers who wish to supply ostriches for slaughter for export purposes, monitoring of the disease status of these flocks, etc. (5)

"Avian influenza" in ostriches

An outbreak of Avian Influenza in ostriches was reported to the OIE during 1991 after the virus strain H7 N1 was isolated from infected ostriches. It was however essential to know the virus's pathogenic potential in chickens under experimental conditions and if the virus strain isolated in the field from ostriches corresponds with the criteria laid down by the Standards Commission of the OIE to be identified as an outbreak of "fowl plaque." A series of tests were carried out in collaboration with the CVL at Weybridge, UK to confirm that South Africa is free of fowl plaque as defined by the OIE Standards Commission.

Summary of the disease status of the most important animal diseases

The animal diseases which never occurred in South Africa or which were eradicated, as well as those under control and those regarded as endemic are as follows:

1. The following diseases were eradicated in South Africa:
   Rinderpest (1904)
   Contagious bovine pleuropneumonia (1924)
   East Coast fever (1954)
   Hog Cholera (1918)
   Glanders and Farcy (1945)
   Scrapie (1972)
   Equine Infectious Anaemia (1955)

2. South Africa never had the following diseases and regards itself free from them:
   (Disease surveillance done to substantiate this claim)

   OIE List A diseases:
   Vesicular Stomatitis
ANIMAL DISEASE CONTROL IN SOUTH AFRICA

Swine vesicular disease
Peste des petits ruminants
Sheep pox and goat pox
Fowl plague

OIE List B diseases:
Multiple spp: Aujeszky's disease
Screwworm (C. hominivorax)
Cattle: Bovine spongiform encephalopathy
Sheep and goats: Caprine arthritis encephalitis
Contagious agalactia
Contagious caprine pleuropneumonia
Nairobi sheep disease
Salmonella abortus ovis
Horses: Contagious equine metritis
Equine encephalomyelitis
Japanese encephalitis
Salmonella abortus equi
Surra
Venezuelen equine encephalomyelitis
Pigs: Porcine brucellosis (B suis)
Transmissible gastro enteritis
Trichinellosis (sui)
Enterovirus encephalomyelitis
Porcine Respiratory Reproductive Syndrome
Fish: Viral haemorrhagic septicaemia
Spring viraemia of carp
Infectious haemopoietic necrosis
Salmonid herpes virosis (type 2)
Renibacteriosis (R salmoninarum)
Ictalurid herpes virosis (type 1)

3. The following diseases are well controlled and are contained in control areas, regulated in terms of the Animal Disease Act, 1984.
- Foot and Mouth Disease (last outbreak 1993 in impala in the Kruger National Park)
- African Swine Fever (last outbreak 1992 in free running pigs in the control area)
- Nagana (last outbreak 1992)
- Corridor disease (last outbreak 1992)

4. Diseases occurring in South Africa (albeit in controlled areas) that are regarded as a disease risk to importing countries.
   1. Foot and Mouth Disease
   2. Lumpy Skin Disease
   3. Rift Valley Fever
   4. Blue Tongue
   5. African Horse Sickness
6. African Swine Fever
7. Newcastle Disease
8. Ostrich Influenza
9. Heartwater

The favourable disease situation in South Africa, was not only achieved through veterinary movement control and other measures, but also by cooperative actions taken with neighbouring countries, in the southern African region. South Africa entered into bilateral agreements with Botswana, Lesotho, Swaziland and Namibia, ease the international movement of animals, animal products, parasites and infectious things, while maintaining equal standards of animal disease prevention and control and equal animal health standards regarding such importations. As far as intransit movements through South Africa is concerned, it was agreed that such introductions should comply with the minimum veterinary import requirements of the RSA.

Import and export control

In terms of the Animal Diseases Act, 1984, no person may introduce an animal, animal product, infectious or contaminated thing, into South Africa without the authority granted by a permit or differs with conditions as required by the permit. These permits are only issued by the Director of Animal Health. Decisions to allow imports and under what conditions, are continually being revised as disease situations change in the world.

All animals, except for those from our immediate neighbours (in extreme cases), are all quarantined, during which period certain tests are conducted to determine that the animals are not incubating (or carriers of) a disease. These animals are quarantined at one of four official Quarantine Stations viz, Jan Smuts, Cape Town and Durban at the owner's risk and expense.

At the prominent ports of entry there are permanent Quarantine Masters who are on duty 24 hours of the day, where imports are examined and dealt with in terms of the Animal Disease Act, 1984. Unfortunately this is not so at all ports of entry. In this instance the State Veterinarian in control of the area is responsible, but it is impossible for him or his personnel to man the post 24 hours out of every day. In this instance, the preliminary checking of the consignment and the documents, is done by the officials of the Department of Home Affairs and the SA Police, at the borderpost. They inform the State Veterinarian at destination of the consignment for final inspection and release.

Since the poultry industry is mostly privatised, nine private poultry quarantine facilities are approved and run by the private veterinarian of the company and supervised by the State Veterinarian. Only day old chicks are allowed to be imported.

For the importation of game (antelope mostly) from African countries,
ten game quarantine facilities, in the province of Transvaal, are approved. Testing for Foot and Mouth disease is repeated during quarantine. Zooborn game are quarantined in privately owned facilities, approved by this Directorate. This is also the case for zoos and snakeparks (mainly used for reptiles from equine encephalomyelitis countries).

Some products need further inspection and clearance after importation. Fresh meat is always detained for the Directorate of Veterinary Public Health for further inspection, testing and release. Other products, e.g. hides and skins, trophies, feathers, meals, are usually destined for approved facilities for further processing and final release by the State Veterinarian.

Intransit Movements
Whenever an animal or animal product is moved in transit through South Africa, it is regarded as having landed on South African soil and therefore an introduction into South Africa. For each movement, an intransit permit is issued by this office. Similar conditions as for imports would usually apply for intransit movements as well, because of the offloading, storing and reloading of products before departure from South Africa. Offloading, storing and reloading take place under official veterinary supervision and control within SA. If the consignment poses a threat to the SA agricultural industry, intransit is not allowed. The veterinary infrastructure taking care of intransit movements is similar to that for imports.

Export control
No export permits are issued by this Directorate. Importing countries stipulate their conditions of import as contained in their import permit or licence, after which a health certificate is issued by the local private veterinarian practitioner and endorsed by the State Veterinarian. Often this office would negotiate with veterinary authorities of the importing countries, when import requirements are not practical, and a compromise is reached or the product is not exported. If we believe our circumstances and disease control are such that the importation of products or animals can be done with a minimum disease risk, the Veterinary authorities of the importing country would be requested to investigate it for themselves and do an evaluation to reach an agreement. This agreement would then also stipulate the conditions acceptable to the importing country. For instance, two USDA-approved game and ostrich quarantine facilities are operational, another game quarantine facility is approved for Zambia; also an egg powder manufacturing plant for Japan; two petfood manufacturing plants for Israel and two abattoirs for EC.

Summary
The State Veterinary Services in South Africa has through the years sought to set and maintain a high standard of animal disease control. The increased international traffic in animals and animal products require that
we jealously guard against anything that might endanger our high standing in the international veterinary community. This will enable us to set a role model for the rest of the African continent to strive equally for a high standard of animal disease control.

Bibliography
REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: Mr. Dan B. Childs, Lake Placid, FL
Vice Chairman: Mr. Jay C. Lemmermen, Thornton, CO

Mr. W. L. Adams, GA; Mr. Duncan Alexander, IL; Ms. Linda T. Benson, NY; Dr. Bob H. Bokma, PR; Mr. Jess Burner, Jr., TX; Dr. Ronald B. Caffey, MD; Dr. Richard A. Carmichael, IA; Mr. James L. Copper, MA; Dr. Michael David, MD; Dr. Linda A. Detwiler, NJ; Dr. P. M. Eppele, SD; Dr. William Fales, MO; Dr. Robert Fetzner, VA; Dr. Warren C. Foote, UT; Mr. Robert Frost, CA; Mr. Frank H. Harding, IL; Dr. Rube Harrington, TX; Dr. Jack Haslam, DC; Dr. Robert D. Heilman, VA; Dr. Werner P. Heuschele, CA; Dr. G. Reed Holyoak, UT; Dr. James L. Hourrigan, VA; Dr. Thomas H. Howard, WI; Mr. Tom Hunt, MI; Dr. Robert F. Kahrs, MD; Dr. Ralph C. Knowles, DE; Dr. Nels Konnerup, WA; Dr. Donald W. Luchsinger, VA; Mrs. Amy Mann, DC; Dr. Charles A. Mebus, NY; Dr. Andrea Morgan, MD; Dr. Claude J. Nelson, TX; Dr. Patrick Phillips, WI; Dr. Gerardo Quaassdorff, VT; Dr. Glenn B. Rea, OR; Mr. Terry J. Seubert, WI; Dr. D. A. Stringfellow, AL; Dr. Paul Sutmoller, VA; Dr. Paul J. Taylor, MT; Dr. Lynn Anne Tesar, SD; Mr. Shelby V. Timberlake, NY; Mrs. Michele C. Turner, TX; Dr. William Utterback, CA; Dr. Charles D. Vail, CO; Mr. Willard H. Waldo, NE; Dr. Jerry S. Walker, MD; Dr. Gary M. Weber, DC; Dr. William White, NY; Dr. R. D. Whiting, MD; Dr. George O. Winegar, MD; Mr. David Winters, TX;

The Import/Export Committee of USAHA met November 2, 1994 in the Heritage Hill Room of the Grand Plaza Hotel, Grand Rapids, Michigan. Mr. Dan B. Childs presided. Twenty-four members and twenty guests were in attendance. After a welcome and introductory remarks by Mr. Childs, Shelly V. Timberlake, Chairman of the Sub-committee on Embryo Movement, reported on the Sub-committee meeting held Monday, October 31. Mr. Timberlake brought forth a resolution concerning funding research to resolve the discrepancies in the results reported by Utah State University and the Neuro Pathogenesis Unit in Edinburgh, Scotland. (See Resolution #1). A complete copy of his report and the papers presented to the Sub-committee accompany this report.

Dr. Gideon Bruckner Director of Animal Health, Department of Agriculture, South Africa presented an update on "Animal Disease Control in South Africa". What he presented was a broad overview of his paper presented in the General Session. Highlights he pointed out included the climate, which consists of a prolonged dry season, and a rainy season, with extensive flooding. Tick-bourne and insect-bourne diseases are of great concern during the long rainy season.

New government structure consisting of several "provinces" with a certain amount of autonomy present a challenge on the control of disease. They have, however, managed to maintain a central structure for the con-
IMPORT-EXPORT

trol of Import/Export, vet services, and disease programs under a Federal Directorate.

Most of their serious disease control programs seem to be along the northern border and the Kruger National Park. They have established control areas for FMD, Bovine TB, Bovine Brucellosis and African Swine Fever, along the northern border and the KNP. The last outbreak of FMD outside KNP was 1984. In the control areas they use vaccination. Bovine TB scheme was started in 1969, the recent budget crunch has caused them to concentrate on infected herds. Private vets retest certified clean herds. Bovine Brucellosis program started in 1971. There is no compensation for destroyed animals, as they feel the infection rate is too high, and they don't feel they will be able to eradicate the disease. He mentioned many other interesting things but his whole paper was presented in the General Session.

Dr. Robert F. Kahrs, DVM, PhD, USDA, APHIS, VS moderated the USDA presentations. His opening remark was the major challenge in Import/Export for USDA is regionalization, with the re-invention of government, and risk assessment.

Dr. Hugh Metcalf presented "Regionalization and Risk Assessment- USDA's perspective." Adoption of NAFTA introduced the concept of infected vs. free regionalization. GATT will bring on the concept of high or low incidence. Vaccines have been improved. New paradigm is needed. The old one was "free vs. not free". The new one must be what is acceptable risk vs. not acceptable. What must we do to prevent increasing our risks of disease introduction. Factors studied to determine risk assessment include: Source risk, commodity risk and destination risk. Source risk factors include incidence of disease in the country where the shipment originated. Is the disease eradicated, risk equals RN for negligible or is there a high prevalence of the disease and no control, risk equals R4. An unknown disease status of an area would rate RU.

Commodity risk would consider if the commodity is capable of transmitting the disease. Destination risk would consider the disease status of the area of destination. If the area is highly infected the risk would be different than moving into a clean area. A copy of Dr. Metcalf's paper accompanies this report.

Live animal export activities were presented by Dr. Michael David. Highlights included, most exports of livestock are destined for Canada and Mexico. These two countries account for over 90% of our exports of cattle. Mexico alone accounts for 90% of our live sheep and hog exports.

A new item is the data on export movement is computer entered, downloaded to Ft. Collins, and compiled daily from export application papers, eliminating the tedious job of hand compiling and submitting quarterly reports. His complete report is included in the USDA packet.

Live animal import report was presented by Dr. David Vought. High-
light: In response to requests from previous USAHA resolutions and after soliciting comments from industry, the Import Animals Branch initiated regulation changes to allow the importation of sheep and goats from countries that are infected with scrapie. These animals would enter flocks enrolled in the Voluntary Scrapie Control Program. The rest of his report is included with the USDA papers.

Dr. Manolo Garcia presented the import/export report on animal products. Considering the high cost, in personnel and resources they have proposed to deregulate "very low risk" materials. The following countries were recognized free of FMD and Rinderpest in 1994: Austria, Belgium, Germany, Hungary, and S. Korea. His complete report is included with the USDA papers.

For the report on Avian Imports, Dr. Kahrs pinch-hit for Dr. Pierson who could not attend the meeting because of a conflict. The biggest change over the last year was a huge increase in the imports of Ostrich eggs due to the ban on adult Ostriches after ticks capable of carrying heart water disease were found on several birds. Most of these imports are through private quarantine facilities with strict government oversight. Complete report included with USDA papers.

Dr. Ron Caffey, Assistant to Deputy Commissioner for Plant Protection Quarantine presented the PPQ report. Highlights of his report follow. Passenger Baggage, as Customs move out of this area PPQ is using more X-ray and sniffer dog teams. A new type X-ray is being developed featuring 3-D image, rapid movement (120 ft/min) an ability to store the image for later reference. The machine will also bar code the luggage as it goes through. This will allow scanning prior to passenger pick-up and re-direction of both passenger and baggage to secondary inspection area (open and search!). He also noted the increased participation in ACS by airlines and sea carriers. The program is voluntary at this time. Under IBIS, Interagency Border Inspection System, past violators of APHIS programs are electronically listed, and automatically diverted to secondary inspection area (open and search!). In response to a question from the committee, Dr. Caffey indicated that lizards and reptiles imported from the Philippines are visually inspected for ticks. He also reported twenty fewer violations of MARPOL annex V requirements due to greater awareness on the part of boat crews. The rest of his report is included with USDA papers.

Mr. Childs asked Dr. Kahrs to address the Olympic Horse Importation. The major issue regarding the entry of foreign competitive horses concerns equine piroplasmosis. Dr. Kahrs explained the background for the departmental policy. USDA has made exceptions for horses entering the U.S. for competition, setting up a temporary special events quarantine under the following conditions:

1. The special exemption must be in accordance with state laws and local environment.
2. There must be proper facilities to handle the quarantine.
3. The ability of USDA to provide resources and personnel for oversight.

Question: Will USDA then grant exception for these Olympics? No.
1. Georgia has a state law against it.
2. Georgia has an endemic competent tick vector population.
3. The logistical challenge to treat the area for ticks and EPA resistance to allowing use of effective pesticides.

A risk assessment is going on to re-assess the situation.
1. There would be relatively low risk to competitors for the amount of time necessary to complete the competition. However, due to the climate and hot temperatures at that time of year, many of the horses would have to come in early to acclimatize before competition.
2. A relatively high risk of native ticks setting up a permanent infection caused by feeding on a piro-positive horse.
3. Serological survey done in Georgia and Florida found only two confirmed piro-positive horses, both in Florida. No positives were confirmed in Georgia horses. Unlikely the ban will be lifted.

Dr. Roger Breeze, Director of the Plum Island Disease Research Center, apprised the committee of recent events and changes. They have gone through a program of facilities consolidation. The diagnostic lab and the administration offices, which were located a mile apart and each a mile away from the research facility have all been combined at the research facility. While expensive, these moves have combined to save in excess of two million dollars annually in transportation and road upkeep. Claims have been made that there is no bio-contain lab facilities available. With this consolidation ARS now has 10,000 square feet of available empty lab space. There is also plenty of room available for large animal maintenance, also bio-contained.

The biggest danger of a virus escaping the secure lab is the possibility that it will infect an animal population. Spare animals used to be kept on Plum Island for future projects. Now animals are purchased on a "just in time" basis, removing this animal reservoir threat.

Modernization continues as old machines break down and are replaced. However, sixty million dollars will be needed over the next ten years.

It has been proposed that any bio-contained facility should be located close to a university to facilitate collaborative studies. Plum Island currently is collaborating with 12 universities. Question: 1) Do we need Plum Island anymore? FMD is still with us. New diseases are emerging. We are going to need bio-contained labs somewhere. 2) There are lots of labs in the U.S. with bio-contain capability. In order to do large animal disease studies you must be able to work on groups on animals. Plum Island has
the capacity for many groups of many animals. Connie Greig, NCA, addressed the committee regarding NCA import/export policies. NCA supports Plum Island and is concerned about the lack of funding. They have backed resolutions to increase funding for all large animal research labs.

Dr. Don Lien brought a resolution concerning import regulations for bovine semen (See Resolution #2). Discussion followed and resolution was passed.

Dr. Terry Beals, Director, Texas Animal Health Commission, discussed the use of Gamma Interferon TB test in the eradication efforts in the southwest. He compared for us the operational, in the field, pros and cons of the Gamma Interferon versus caudal fold test. After much discussion, the feeling of the committee was that workers in the field should be provided with the necessary tools to complete their work. A resolution supporting approval of the Gamma Interferon test was presented (See Resolution #3). Discussion followed and resolution was passed.

There being no further business, the meeting was adjourned at 5:10 p.m.

Respectfully submitted,

Dan B. Childs, Chair
Jay C. Lemmermen, Vice-Chair

UNITED STATES ANIMAL HEALTH ASSOCIATION - 1994

RESOLUTION NUMBER: 1
SOURCE: IMPORT/EXPORT COMMITTEE
SUBJECT MATTER: EMBRYO TRANSPLANT
DATES: GRAND RAPIDS MEETING, OCT. 29-NOV. 4, 1994

BACKGROUND INFORMATION:

RESOLUTION:

As a result of the disparate findings from published research to date on the potential for transmission of scrapie via embryo transfer, and the profound impact this has had in restricting the international movement of ovine embryos, the U.S.A.H.A. urges the USDA to make available the funds necessary in support of the collaborative research project between Utah State University and the Neuropathogenesis Unit in Edinburgh Scotland with the objective of resolving the discrepancies in the results of both groups.

RESOLUTION NUMBER: 2
SOURCE: IMPORT/EXPORT COMMITTEE
SUBJECT MATTER: MYCOPLASMA, UREAPLASMA, AND
BACKGROUND INFORMATION:

Mycoplasma, Ureaplasma, and Hemophilus somnus (M, U, Hs) can cause infertility, abortion, or mastitis in infected cattle.

Bulls may be potential and subclinical carries of M, U, or Hs by harboring M U or Hs in the preputial cavity, urethra, or more proximal segments of the bovine male urogenital tract.

M, U, and Hs are ubiquitous microorganisms and because of diagnostic limitations are not suitable to a donor bull or herd diagnostic test program.

M, U, and Hs may be controlled by the appropriate antibiotic treatment of the semen product or determined by culture that the semen product is not contaminated with M, U, or Hs.

The United States Artificial Insemination industry, as represented by the members of Certified Semen Services (CSS), a subsidiary of the National Association of Animal Breeders, has voluntarily established an antibiotics and extender semen processing protocol that has been thoroughly evaluated and determined to control the potential for dissemination of M, U, and Hs as well as other bovine venereal diseases.

It is in the best interest of the U.S. cattle industry, both beef and dairy segments, that semen used for AI be processed or culturally evaluated to control the probability that it is contaminated with M, U, Hs, or other bovine venereal diseases.

Cultural evaluation of 201 collection codes of bovine semen recently imported into the U.S. under the present USDA import protocol identified 12 codes to be contaminated with Mycoplasma microorganisms and four additional codes to be contaminated with Ureaplasma microorganisms.

RESOLUTION:

USDA, APHIS, VS, should include in the Pattern Protocol for the Importation of Bovine Semen a statement that imported semen be processed with an antibiotics and extender combination that has been scientifically proven efficacious to control the dissemination of Mycoplasma, Ureaplasma, and Hemophilus somnus, and other bovine venereal diseases - OR - that semen to be exported to the United States be cultured in the country of origin in the a laboratory designated by the national government of the exporting country and shown to be negative for Mycoplasma, Ureaplasma, and Hemophilus somnus, and other bovine venereal diseases.

RESOLUTION NUMBER: 3
SOURCE: IMPORT/EXPORT COMMITTEE
SUBJECT MATTER: OFFICIAL TEST STATUS - GAMMA INTERFERON TEST
DATES: GRAND RAPIDS MEETING, NOVEMBER 2, 1994
BACKGROUND INFORMATION:

The current skin test technology for bovine tuberculosis has served the
program well and its extensive use is expected to continue. However, at this point in the eradication program, it is vital that test procedures with the following attributes be approved for official use:

A. Test results are generated objectively.
B. Test results are quantifiable.
C. Tests can be repeated without the time interval delays dictated by in vivo methods.
D. Test results can be analyzed by creating profiles or trends and patterns to depict rising, stable, or declining levels of response (achievable with A & C above).
E. Test that does not require handling animals twice.

The *M. bovis* Gamma Interferon Test Kit produced by Idexx Laboratories, Inc., has the desired attributes and has repeatedly been validated compared to the current skin test technology, based on sensitivity and specificity and predictive values.

**RESOLUTION:**

RESOLVED the United States Animal Health Association petition the USDA/APHIS/VS to approve the Gamma Interferon test as an official stand alone test for use in the national bovine tuberculosis eradication program.

RESOLVED the test be approved for use in animals/herds of unknown status using the bovis/avium ration of < 1.8 for negative classification. Animals determined to be reactors (B/A ratio of ≥ 1.8) by this method would be confirmed with an approved confirmatory test for final classification. In herds considered at increased risk (infected, epidemiologically traced, adjacent), the designated TB epidemiologist would be empowered to determine the appropriate use and interpretation of results of the Gamma Interferon test and whether it is employed as an official stand alone test or used in conjunction with other recognized skin tests. The designated tuberculosis epidemiologist will classify Gamma Interferon Assay positive animals as suspect or reactors based on the bovis/avium ratio between 1.25 and 1.8 deemed most appropriate for the herd being evaluated. Animals of U.S. origin, classified as reactors by this method, would be eligible for federal indemnification at current rates.
Chairman: Mr. Shelby V. Timberlake, Pelham Manor, NY

Dr. Richard A. Carmichael, IA; Mr. Dan B. Childs, FL; Dr. Jack Haslam, DC; Dr. Nels Konnerup, WA; Dr. Donald W. Luchsinger, VA; Dr. Charles A. Mebus, NY; Dr. Andrea Morgan, MD; Dr. Patrick Phillips, WI; Dr. Paul Sutmoller, VA; Dr. Paul J. Taylor, MT; Dr. Jerry S. Walker, MD; Dr. George O. Winegar, MD.

The meeting was called to order by Chairman Timberlake at 1:45 p.m. on October 31, 1994. There were 22 in attendance including committee members Haslam, Sutmoller, Taylor and Timberlake.

Dr. Michael Thibier, newly elected Director General of the National Veterinary and Food REsearch Center, in France gave an update on the EEC and OIC. His presentation included:

A. Statistics of the ET industry in Europe which will be further detailed in forthcoming IET's newsletter.

B. OIE International Animal Health Code - update on appendices and new changes:
   1) Bovine embryo - appendix changes 4.2.3.1
   2) IVF Bovine embryos - appendix (new)
   3) New official publications of EEC 4.2.3.4

B1. Appendix - Bovine Embryos/OVA
   - Basic strategic point "The Officially Approved Embryo Collection Team"
   - The herd of origin must be free of clinical signs of FMD, Rinderpest, CBR and must not be in an infected zone for 30 days unless the disease is listed in category one by IETS. This is a great achievement.

B2. Appendix - IVF Bovine Embryos
   Key = Reference to "Embryo Production Team"
   Key = When oocyte collection is from live animals - donor animals must meet requirements of Appendix 4.2.3.1 for donor cows.

C. European Communities
Commission decision 94/113 - allows IVF and Zona invaded embryos to move between countries (not including cloned embryos) - with special conditions placed on processing.

Commission Decision - 94/280
Import of Embryos authorized from third countries provided:
MINUTES EMBRYO MOVEMENT SUBCOMMITTEE

1) An authorized list of countries (including U.S./Canada)
2) From officially approved teams for collection or production
3) Accompanied by certificate ??????
   - approved teams
   - bluetongue, EHO free zone
   - from herds free of T.B. Brucellosis, leukosis and no signs of IBR
   for last 12 months
   - semen from AI center that approved

List of approved teams
- 72 vets from U.S. approved
- 50 vets from Canada approved
- 3 vets from U.S. approved for production of IVF teams

Dr. Tony Wrathhall, Central Vet Lab Ministry of Agriculture - Fisheries and Food, Weybridge, England Gave His U.K. Update BSE
- As of July 10, 1994 (Bovine Spongiforme Encephalopathy)
- 143,026 confirmed cases
- 32,000 affected herds
- over 50% of Dairy herds affected
- 14% of cow/calf facilities
- 1/3 breeding beef herds
- Annual incidence - seven cases/1,000 adult animals
- Embryo Transfer Project - object was to collect embryos from infected (BSE) cows and Transfer to clean recipients with proper handling (i.e. using IETS washing) - final results will be available by year 2000 (allow seven years for disease in offspring to develop). No signs so far but a long time to observe yet. All results from mice inoculations have been negative.

General Comments by Dr. Wrathall
1. Increased emphasis on welfare of transported animals
2. Can now bring in embryos sired by bulls that are blue tongue positive
3. Para vet technicians are approved to do epidurals and embryo collections
4. Embryos - must be certified to be born naturally by vet - difficulty for vets transferring IVF embryos.

Canada Update
Dr. Brian Evans, Chief, Artificial Insemination and Embryo Transfer, AG Canada, gave his update
1. Camelids, sheep and goats etc. have posed regulatory challenges regarding embryo imports.
2. Protocols for embryo exports negotiated and in place. 80% of protocols are based on embryo washing.
3. 50 teams accredited for export and used for imports as well.
MINUTES EMBRYO MOVEMENT SUBCOMMITTEE

4. Import protocols in place developing for embryo team for camelids, caprin, equine, ovine, and porcine.
5. Indication that is more emphasis on imports of embryos to Canada than before.
6. Gatt will have an impact on what countries can require in way of health requirements on imported embryos.

Dr. Reed Holyoak discussed Scrapie in Sheep and Goats
1. Research update on project at Utah State University - provided description of research protocol as was presented in past. 72 surviving offspring after embryo transfer with no signs of scrapie transmission.
*Emphasized that this is ongoing research and results are preliminary.
2. Dr. Holyoak also presented a summary of research (pilot worst case scenario) performed in Edinburgh at Neuropathogenesis Unit Scotland. No washing etc. - 26 offspring born -scrapie was said to be transmitted.
- A cooperative study has been proposed involving naturally infected sheep in Scotland as embryo donors and scrapie free recipients at Utah State University’s SPF flocks. Dr. Wrathall and McKelvey have made progress toward funding by the U.K. Ministry of Agriculture and tentative approval has been given to proceed.
- A resolution from the Embryo Movement Committee has been forwarded to the Sheep and Goat Committee to request support from USDA-APHIS to resolve the discrepancy between the U.S. and Edinburgh scrape ET Results.

Update on Import/Export of Horse Embryos - Dr. Reed Holyoak
Dr. Holyoak has prepared a first draft of a set of recommendations for sanitary control of movement of embryos.
Since essentially no basic research done on equine embryo - pathogens interactions the draft was modelled after what has been done on other species (primarily cattle) based on limited information. Appendix will have to depend on traditional testing of donors.

Llama Update - Dr. Paul Taylor
Dr. Taylor explained that he and a partner in Chile have a selective Llama breeding project in Chile and they hope to import embryos to the U.S. eventually.
- Dr. Taylor provided an update on progress on "invivo" and "invitro" Llama embryo production by others.
Dr. Taylor's own research has resulted:
1) in apparent pregnancy from fresh transfers
2) in achieving frozen and thawed embryos without apparent damage
MINUTES EMBRYO MOVEMENT SUBCOMMITTEE

Dr. Taylor concluded with a description of Llama markets.

**USDA/APHIS Update - Dr. David Vogt**

The number of imports of embryos to U.S. increased three fold (many caprin embryos):
- Everyone waiting on part 98 changes to include ruminants. Draft to include permission to import embryos from Scrapie infected countries.
- Draft will include allowance of IVF embryo imports.
- Waiting on protocol for sheep, camelid embryos imports from several countries.

**International Embryo Transfer - Dr. A. T. Wrathhall, Chairman Import/Export Committee**

**Society Report**

Reported for the Regulatory, Research, Forms, and Manual Sub-Committees.

Dr. Wrathall briefly discussed plans for expanding committee membership, proposed horse regulations, current research (especially IVF - embryos), proposed lab animal regulations and a new edition of the IETS manual possibly by end of 1996.

Dr. Wrathall said there would be an open session on Sunday, January 8, 1995 and special scientific session on Monday evening January 9, 1995 at the IET's annual meeting in Calgary Canada which is scheduled for January 8 - 10, 1995.

**Update on Quantitative Risk Assessment for International Movement of Embryos - Dr. Paul Sutmoller**

Dr. Sutmoller itemized risk perimeters that were considered stated that the weakest link in the chain defines the greatest risk. Dr. Sutmoller will be publishing his monograph on this subject in the near future.

**Ethical Concerns in Embryo Transfer - Dr. Brian Evans & Dr. A.T. Wrathhall**

A discussion lead by Dr. Evans:
- Proposed that ethics of certification be considered as a separate consideration from ethics in embryo transfer in general.
- Also commented that was conflict in Canada.
- As of now veterinary people can collect transfer embryos domestically but not for international movement.
- Would it be ethical for approved vets to refuse to certify work of non-veterinary personnel.

These were preliminary thoughts aired for future discussion and consideration. Prior to the close of the meeting the following resolution passed
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unanimously by the members of the Embryo Movement Committee was forwarded to Michelle Turner, Chair of Sheep and Goats Committee for forwarding with the Sheep and Goats Committee resolution to the Import-Export Committee of the U.S.A.H.A. (See Attached).

Resesarch Update on Embryo Transfer and Scrapie Transmission

Utah State University

Work was initiated in 1988 using naturally scrapie infected donors of the Suffolk breed and scrapie free recipients of Targhee-type (white face). Embryos were transferred from scrapie infected or exposed donors to scrapie free recipients. All embryos were washed 3 times in dilution volumes of medium greater than 100. All viable embryos, including those with damaged zonas, and those hatching, were transferred. Donor animals are kept at the USDA/APHIS/VS facilities at Mission, Texas, and embryo transfer offspring and their recipients are kept in specific pathogen free facilities at Utah State University, Logan, Utah. There are 72 surviving offspring, none of which have developed clinical scrapie. Of these 38 are from donor dams that were confirmed scrapie positive on histopathologic examination following necropsy or that were lymph node PrP
\(_{\text{res}}\) positive in ante-mortem sampling. An additional 26 of the 70 offspring are from dams that are still alive, clinically negative but could develop scrapie. Years of birth of the 72 embryo transfer offspring are as follows: 1988 - 8 lambs; 1991 - 7 lambs; 1992 - 22 lambs; 1993 - 23 lambs; 1994 - 12 lambs. The 8 lambs born in 1988 all tested PrP
\(_{\text{sc}}\) negative at 30 months of age and none were positive on PrP
\(_{\text{sc}}\) western blot nor on histopathologic analysis following necropsy at 60 months of age; 4 were from scrapie positive donor dams. Other tests on these 8 offspring are pending. None of the recipient ewes have developed clinical scrapie and none of those that have died have been positive on histopathologic analysis. It must be recognized that this research is ongoing and these are preliminary findings.

A Cooperative study has been proposed involving naturally infected Suffolk sheep in Scotland as embryo donors and scrapie free recipients of Targhee-type (White face) at Utah State University's SPF facilities.

Drs. Wrathall and McKelvey have made major inroads in securing funding by the UK Ministry of Agriculture and tentative approval has been given to proceed.

Neuropathogenesis Unit, Edinburgh, Scotland

* It was a 'preliminary' study designed to test a 'worst case scenario.'

* Unwashed embryos from scrapie-inoculated Cheviot donors of homozygous short incubation genotype were transferred to mostly (15/16) homozygous long incubation genotype recipient ewes.

* 26 offspring were born
  - 6 died prior to 12 mos of age
- 3 were homozygous long incubation
- 11 were heterozygous
- 6 were homozygous short incubation
* All six of the homozygous short incubation offspring developed scrapie and were confirmed scrapie positive on histopathology or other diagnostic testing. (Foster et al, 1992)
* Of the 11 heterozygous offspring:
  - 2 have developed scrapie
  - 6 died due to other problems, of these 2 were diagnosed as scrapie positive on post-mortem testing
  - 3 have remained clinically normal

Testing continues at the NPU in Edinburgh
During FY 1994, the staff of USDA, APHIS, National Center for Import and Export faced the challenge of addition of aquaculture products, and incorporation of regionalization and risk assessment. We are also incorporating directives for liberalization of import requirements without compromising biosecurity into the review, analysis, interpretation development, and promulgation of regulations for import-export activities, especially as regards foot-and-mouth disease and rinderpest.

Animal Import Activities

Five hundred and two llamas and alpacas were imported from Peru through the Harry S Truman Animal Import Center (HSTAIC). Peru was having an active outbreak of foot-and-mouth disease (FMD) but the animals tested negative and were released in June 1994.

The Animal and Plant Health Inspection Service (APHIS) sent a team of veterinarians to South Africa in September 1994 to approve an embarkation quarantine facility and begin the quarantine of 684 Boer goats. These goats are expected to enter HSTAIC on November 20, 1994.

The regulations governing the lottery to determine opportunities to use the HSTAIC facility were amended in 1994. The tier system was eliminated and there is now a $32,000 application fee to enter the lottery. Veterinary Services (VS) received 595 applications for the 1995 calendar year. The deposits are due November 29, 1994, for the December 6, 1994, lottery drawing.

In response to requests from previous USAHA resolutions and after soliciting comments from industry, the Import Animals Branch initiated regulation changes to allow the importation of sheep and goats from countries that are affected with scrapie. These animals would enter flocks enrolled in the Voluntary Scrapie Control Program.

A regulation change is going forward for the purpose of allowing the embryos of species in addition to cattle to be collected and imported from countries affected with FMD and rinderpest.
Part 98 of 9 CFR is undergoing cyclic review with the intention of forming a proposed regulation which is less restrictive to importers, and is more closely in line with current technology and science.

The import staff initiated a regulation to recognize Mexico free of Venezuelan equine encephalomyelitis (VEE). This change drops the requirement for a 7-day quarantine in vector free facilities, and other restrictions imposed due to the 1993 outbreak of VEE in Chiapas.

VS supervised a 90-day FMD quarantine for approximately 40 South African Angora and Boer goats in Poland. After clearing the FMD quarantine in Poland the animals were imported through the New York Animal Import Center (NYAIC). These animals are currently in a 5-year quarantine research project in Texas.

There is a proposed rule to change identification requirements for steers and spayed heifers from Mexico. The proposed rule will require that steers from Mexico be permanently identified with an "M" high on the right tail head, and that spayed heifers be permanently identified with a slightly modified "M" high on the right tail head.

APHIS has proposed to add all countries of the European Union to the list of countries where contagious equine metritis exists and to add the countries of the Arabian peninsula to the African Horse Sickness list.

The 1996 Olympic Games Equine Committee is requesting that USDA approve a private quarantine for horses entering the United States to compete in the Olympics. They have also requested that horses entering for the Olympic Games be granted a waiver for piroplasmosis. USDA has agreed that a private quarantine can be done if the facility is inspected, approved, and supervised by USDA officials. USDA is not granting the waiver for piroplasmosis because of the risk of introducing piroplasmosis into Georgia. The decision has the support of the equine industry and the State Veterinarian’s office in Georgia.

During 1994 the Import Staff developed a protocol for importing porcine semen from France and approved an artificial insemination center. Semen for import is collected under USDA Veterinary supervision. This restriction is necessary because swine vesicular disease and hog cholera exist in France.

There were 2 importations of Wagyu cattle from Japan in 1994.

APHIS published an interim rule which prohibits the import of hedgehogs and possums from New Zealand because of the high incidence of Mycobacterium bovis found in the animals.

The Import Animals Branch completed negotiations on the following revised or new germ plasm protocols during Fiscal Year 1994:

1. Poland and The Netherlands - Protocols were finalized for bovine semen and embryos.

2. Canada and Brazil - Protocols were revised and completed for bo-
vine embryos.
3. Germany - The protocols for bovine semen and embryos are being negotiated.
4. Argentina - The protocols for bovine semen and embryos are under negotiation pending final approval.
5. South Africa - The protocol for goat semen is being negotiated pending final approval. The embryo protocol is drafted and will be available when regulations are published allowing the importation of goat and sheep embryos from FMD countries.
6. Namibia, Botswana, and Zimbabwe - Protocols for sheep and goat semen and embryos are in draft.
7. United Kingdom - Draft protocols for cervidae semen and embryos have been presented to the MAFF. Import Animals Staff is working on incorporating regionalization and risk assessment into import regulations.

Avian Import Activities

A. Poultry and Hatching Eggs
   There were 2,725,542 poultry, including day old chicks, and 10,048,120 hatching eggs imported into the United States during fiscal year (FY) 1994.

B. Commercial Birds
   As in recent years, the importation of commercial birds continues to be at much lower levels than in the mid 1980's. Fiscal year 1993 was subjected to importation quotas on many species of birds by the Department of Interior. This year the quota is no longer in effect and the importation of most species of birds is prohibited or restricted. This resulted in the importation of many of the non-prohibited species such as finches and other song birds.
   There were 131,184 commercial birds quarantined with a total of 110,570 birds released at the end of the quarantine period. Disposition of a shipment containing 10,503 birds is pending due to the isolation of avian influenza.

C. Pet Bird Program
   Pet birds continue to be imported and quarantined at Rock Tavern, New York; Miami, Florida; Los Angeles, California; Honolulu, Hawaii; and Mission, Texas. There were 1,520 birds imported and quarantined during FY 1994. All pet birds are tested for VVND, and no virus was isolated.

D. Smuggled Birds
   A total of 973 birds were smuggled, confiscated or seized at U.S. borders. Such birds are quarantined at USDA quarantine facilities. After com-
NATIONAL CENTER FOR IMPORT AND EXPORT

Completing the minimum 45-day quarantine, they are sold at public auctions as permitted by a Memorandum of Understanding between the Departments of Agriculture, Interior, Justice, and Treasury.

E. Ratite Importation

There are 108 foreign ostrich farms in 11 countries approved by USDA to export ratites and ratite hatching eggs to the United States. Namibia in southern Africa continues to be the largest supplier. So far, the only ratite hatching eggs to be imported have been ostrich eggs.

A total of 55,439 ostrich eggs were placed in privately owned bird quarantine facilities. Of these eggs imported, 16,509 (29.8 percent) were released at the end of quarantine. One shipment of ostrich eggs was refused entry in Miami, Florida because they arrived without the required permits.

During FY 1994, 7 rheas were imported, quarantined, and released. A total of 692 ostrich chicks were quarantined at the New York Animal Import Center (NYAIC) and the Miami Animal Import Center. Of these, 643 (92.9 percent) were released from quarantine. In addition, 1,145 emu were placed in quarantine. Of these, two lots at the Hawaii Animal Import Center totaling 315 were refused entry due to the isolation of Salmonella enteritidis (SE) phage type 4; and 775 were released from quarantine. Another lot at NYAIC containing 26 emus was refused entry because they were also found to be infected with SE phage type 4. One additional lot at the NYAIC containing 12 cassowaries and 18 emus was refused entry after an avian influenza virus was isolated.

The total number of ratites and ratite hatching eggs imported into the United States has declined slightly. However, the percentage of live chicks released after hatching increased from 22 percent in FY 1993 to 29.8 percent in FY 1994. The decline of live exotics imported could be related to the drastic decline in the value of these birds more than any other factor.

Animal Export Activities

During FY 1994, APHIS continued negotiations with various countries to update current animal health protocols or establish new ones. New protocols were established or were updated for exporting bovine semen to Argentina, and Ireland; bovine embryos to Sweden, Australia, and Japan; cattle to China, Brazil and Colombia; horses to Argentina; equine semen to Australia; swine to Barbados, Guatemala, Chile, Japan, New Zealand, and Malta; porcine semen to China, New Zealand, Chile, and Japan; poultry to Nicaragua; and camelids to Australia. These and many other import health requirements for various other countries and species are reflected in the computerized International Regulations Retrieval System.

The European Union (EU) revised its health requirements for bovine embryos exported from the United States. They will now accept micromanipulated and in vitro fertilized embryos. A post-collection test of
the donor cow for bluetongue is no longer necessary, and bovine semen, either imported into the United States from the EU or collected from donors resident in Certified Semen Services collection centers, may be used to inseminate donor cows.

In addition, APHIS has asked the EU to consider certain changes to the conditions for importing horses regarding the equine viral arteritis and has discussed requirements with the Standing Veterinary Committee. APHIS hopes to resolve this issue within the next few months.

A United States-Canada border port meeting was held in November 1993 to establish uniform import and export procedures. APHIS also continues to hold the Canada-United States Trade Agreement Technical Working Group meetings with Agriculture Canada.

The United States-Mexico Animal Health Working Group continues to meet about every 8 months to discuss changes to import requirements in each country. Mexico's health requirements for importing poultry and poultry products, sheep, and cattle were recently clarified.

One additional port of embarkation with export inspection facilities that satisfied the necessary requirements was added to Title 9, Part 91 of the Code of Federal Regulations.

The occurrence of porcine respiratory and reproductive syndrome (PRRS) in the United States continues to affect our exports of live swine, porcine semen, and pork. Other diseases, such as avian influenza and salmonella, are periodically used by many countries to limit the entry of our poultry and poultry products.

Animal Product and By-Product Activities

A total of 6,557 permits were issued in FY 1994 by the Import-Export Products (IEP) Staff authorizing the importation of organisms, vectors, biological materials, and animal products and byproducts. There were 187 more permits issued in FY 1994 than in FY 1993.

User’s fees for animal products, organisms and vector permits and approved establishments were implemented on January 21, 1994. For FY 1994, $140,240 was collected for permit user fees (excludes fees for approved establishments which are handled at the area offices). During this same period, a total of 325 approved establishments (mostly taxidermy facilities) asked to be deleted or refused to renew their agreement citing the new user fee as the reason.

The IEP Staff is changing importation procedures for very low risk materials from countries free of diseases of concern to the USDA. These include specimens from non-livestock species not susceptible to epizootic livestock and poultry diseases. The veterinary services of the exporting country or the exporting facility (depending on the country and product) will be required to certify the species and country of origin of the material in lieu of a VS permit.
The regulations allowing the importation of beef cooked using new methods from FMD-affected countries are being finalized.

The EU delayed until December 1994, the implementation of EU directives regulating the export of animals and products to the EU from third countries. Some of the EU requirements are impossible for United States industries to comply with. Negotiations to modify some of the EU directives are now underway for rendered products and dog food from the United States.

**ACTION TAKEN ON REQUESTS BY FOREIGN GOVERNMENTS TO BE RECOGNIZED FREE OF SPECIFIED DISEASES**

The following countries were recognized free of foot-and-mouth disease and rinderpest in 1994: Austria, Belgium, Germany, Hungary, and South Korea. Portugal was placed on the bovine spongiform encephalopathy list when a case in native cattle was reported. Portugal was also recognized as being free of African horsesickness.

**Plant Protection and Quarantine -- Port Operations**

**X-RAY Baggage Inspection**

Plant Protection and Quarantine (PPQ) continues to expand the use of "x ray" as a screening tool in passenger baggage clearance at major international airports. There are x-ray scanning machines located at all foreign-arrival and predeparture sites. Such machines for predeparture clearance are at Ponce, Roosevelt Roads, Aguadilla, and San Juan, Puerto Rico, and four islands of Hawaii, where passengers bound for the U.S. mainland are inspected because of plant pest concerns, such as the Mediterranean fruit fly. The international airports are San Juan, Miami, Honolulu, Chicago, Kennedy (New York), Houston, Dallas, Boston, Atlanta, Dulles (Washington, DC), Los Angeles, San Francisco, Seattle-Tacoma, Philadelphia, Orlando, and Newark. San Ysidro, on the Mexican border, is a land border port with such a device. It is used to screen the items carried by the average one million pedestrians that cross at that port each month. X-ray Machines are used in two postal facilities.

PPQ in partnership with the U.S. Army, Picatinny Arsenal, New Jersey, are developing a prototype x-ray system. This system will:

- Detect quantities weighing 10 grams.
- Throughput of 120 ft/min as required for "check-in" baggage inspection.
- Accommodate baggage dimensions allowed for check-in items.
- Store scanned images for unlimited length of time; be capable of retrieving and displaying them at will.
- Barcode the baggage having detected product.
- Neural net analysis with continuous learning.
Detector Dog Program

Forty seven trained dog teams at 19 major airports are used in clearing passenger baggage. The airports are: Atlanta, Orlando, Miami, Houston, Dulles, Dallas, Charlotte, Philadelphia, San Juan, Newark, Kennedy, Boston, Chicago, Los Angeles, San Francisco, Seattle-Tacoma, Detroit, Bangor, Honolulu. Los Angeles, Honolulu, and San Francisco (Oakland mail facility) have dog teams in their post offices.

The program will expand in the next 5 years to 108 teams.

Automated Commercial Systems (ACS)

As of October 25, 1994, there are 368 sea carriers operating in 38 ports and 82 airlines operating in 26 airports on the Automated Commercial System (ACS). In 1993, 1,324 brokers and importers provided 93 percent of the data entered into ACS.

To facilitate trade and reduce the paperwork burden on industry, the National Performance Review (NPR) has tasked the U.S. Government to establish an integrated database for the collection and dissemination of all international trade data through the expansion and redesign of the U.S. Customs Service Automated Commercial System. The following enhancements will be provided through an International Trade Data System:

a) more accurate and complete trade statistics and data
b) standardization of both import and export data
c) reduction of government and trade community processing time and costs
d) knowledge to promote informed compliance with trade statutes
e) elimination of duplication and unnecessary reporting
f) enhanced fraud detection capabilities
g) improved financial controls

Regulated Garbage, Marpol Annex V

The U.S. Coast Guard is the enforcement Agency for Annex V of the International Convention to Prevent Pollution of the Seas (MARPOL 73/78). This Annex prohibits discharge into the sea of “all plastics including, but not limited to, synthetic ropes, fishing nets, and plastic garbage bags.” It also prohibits discharge of food wastes and other floating materials within specified distances of land. These regulations became effective December 31, 1988. USDA regulated garbage handling requirements have not changed. All food or food-contaminated materials, such as plastics contaminated by galley waste, must be retained aboard the vessel in covered, leakproof containers. If offloaded, such garbage must be incinerated or heated to an internal temperature of 212 °F for 30 minutes. There continue to be increasing interest in the strict enforcement of MARPOL Annex V requirements. APHIS PPQ continues to play a major role because of their vessel inspection and garbage control requirements.

During the period of October 1993 through October 1994, APHIS gen-
erated 38 percent (34 reports) of the MARPOL V violations.

Airport 1990's

Processing the increasing number of international travelers continues to present many challenges to all Federal clearance agencies. As reported last year Customs has adopted a new plan, "Airport 1990's," which calls for Customs to be more selective and examine reduced numbers of passengers and bags. In response, APHIS is using "rovers" and "choke points" to control passenger movement.

IBIS, Interagency Border Inspection System, is used by approximately 20 agencies to focus on the individual person and any past violation history. Thus, past violators of APHIS programs can be "electronically" listed. Because of the numbers, PPQ has limited this to $100 violators, persons who flagrantly did not declare prohibited items and attempted to conceal those items. IBIS is being expanded to more effectively identify passengers requiring personal inspection by the FIS agencies including APHIS. This system designated Advance Passenger Inspection System (APIS) is being implemented to expedite passenger clearance but more effectively protect American Agriculture.


<table>
<thead>
<tr>
<th>ANIMALS</th>
<th>FY 1992</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>1,962,375</td>
<td>2,528,537</td>
<td>2,248,842</td>
</tr>
<tr>
<td>Swine</td>
<td>705,414</td>
<td>801,162</td>
<td>965,001</td>
</tr>
<tr>
<td>Horses</td>
<td>24,867</td>
<td>32,489</td>
<td>29,866</td>
</tr>
<tr>
<td>Sheep</td>
<td>20,651</td>
<td>24,306</td>
<td>26,622</td>
</tr>
<tr>
<td>Goat</td>
<td>125</td>
<td>961</td>
<td>2,184</td>
</tr>
<tr>
<td>*Other</td>
<td>6,316</td>
<td>13,256</td>
<td>1,508</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>2,719,748</strong></td>
<td><strong>3,400,711</strong></td>
<td><strong>3,280,620</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>FY 1992</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semen (doses)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine</td>
<td>338,302</td>
<td>320,981</td>
<td>588,668</td>
</tr>
<tr>
<td>Goat</td>
<td>56</td>
<td>500</td>
<td>3,258</td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>0</td>
<td>775</td>
</tr>
<tr>
<td>Equine</td>
<td>570</td>
<td>2,009</td>
<td>2,594</td>
</tr>
<tr>
<td>Elk</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Swine</td>
<td>0</td>
<td>0</td>
<td>1,458</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>338,928</strong></td>
<td><strong>323,490</strong></td>
<td><strong>596,767</strong></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th><strong>Embryos</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>347</td>
<td>820</td>
<td>940</td>
</tr>
<tr>
<td>Goat</td>
<td>0</td>
<td>1,000</td>
<td>4,390</td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>0</td>
<td>152</td>
</tr>
<tr>
<td>Deer</td>
<td>0</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>347</strong></td>
<td><strong>1,920</strong></td>
<td><strong>5,597</strong></td>
</tr>
</tbody>
</table>

262
KAHRS

<table>
<thead>
<tr>
<th></th>
<th>Canadian Ports</th>
<th>Air / Ocean Ports</th>
<th>Mexican Ports</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE</td>
<td>1,104,555</td>
<td>1,190,675</td>
<td>998,374</td>
<td>2,296,2375</td>
</tr>
<tr>
<td>SWINE</td>
<td>700,766</td>
<td>796,787</td>
<td>963,178</td>
<td>1,955,141</td>
</tr>
<tr>
<td>EQUINE</td>
<td>20,585</td>
<td>2,228</td>
<td>1,364</td>
<td>32,489</td>
</tr>
</tbody>
</table>

Other animals include goats, sheep, or swine; wild ruminants, llamas, alpacas, water buffalo, deer, elephants, hippopotami, rhinoceroses, tapirs, wild birds, or pet birds.


<table>
<thead>
<tr>
<th></th>
<th>FY 1992</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry and day old chicks</td>
<td>6,723,351</td>
<td>6,282,363</td>
<td>2,725,542</td>
</tr>
<tr>
<td>Poultry hatching eggs</td>
<td>20,928,075</td>
<td>17,593,184</td>
<td>10,048,120</td>
</tr>
<tr>
<td>Commercial Birds</td>
<td>271,913</td>
<td>133,435</td>
<td>110,570</td>
</tr>
<tr>
<td>Ratites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ostrich eggs</td>
<td>1,193</td>
<td>15,556</td>
<td>16,509</td>
</tr>
<tr>
<td>ostrich chicks</td>
<td>1,825</td>
<td>1,322</td>
<td>643</td>
</tr>
<tr>
<td>emu</td>
<td>0</td>
<td>1,238</td>
<td>776</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>27,926,357</td>
<td>24,027,098</td>
<td>12,902,160</td>
</tr>
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</table>
### ANIMAL, POULTRY, GERM PLASM EXPORTS - FY 1993 AND 1994

<table>
<thead>
<tr>
<th>Livestock (including slaughter)</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>31,088</td>
<td>86,919</td>
</tr>
<tr>
<td>Equine</td>
<td>18,839</td>
<td>53,654</td>
</tr>
<tr>
<td>Ovine</td>
<td>623,911</td>
<td>793,029</td>
</tr>
<tr>
<td>Caprine</td>
<td>34,769</td>
<td>71,050</td>
</tr>
<tr>
<td>Porcine</td>
<td>34,778</td>
<td>43,992</td>
</tr>
</tbody>
</table>

TOTAL LIVESTOCK: 743,305 1,048,644

<table>
<thead>
<tr>
<th>Poultry</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live (chicks/poults)</td>
<td>9,395,100</td>
<td>54,997,336</td>
</tr>
<tr>
<td>Hatching eggs (dozens)</td>
<td>10,507,602</td>
<td>32,092,223</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Other Avian</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>829</td>
<td>21,567</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Germ Plasm</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine semen</td>
<td>1,382,562</td>
<td>7,042,655</td>
</tr>
<tr>
<td>Equine semen</td>
<td></td>
<td>1,198</td>
</tr>
<tr>
<td>Porcine semen</td>
<td>156</td>
<td>340</td>
</tr>
<tr>
<td>Caprine semen</td>
<td></td>
<td>369</td>
</tr>
<tr>
<td>Bovine embryos</td>
<td>1,725</td>
<td>7,139</td>
</tr>
</tbody>
</table>

Canine/Feline                  | 45,107  |

<table>
<thead>
<tr>
<th>Other Species</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>170,888</td>
</tr>
</tbody>
</table>

### PLANT PROTECTION AND QUARANTINE PORT ACTIVITIES
September 1993—August 31, 1994

**Vessels and Aircraft Arrivals**

- **72,749** Vessels arrived
- **49,850** Vessels boarded
- **11,783** Vessels monitored for garbage violations
- **7,975** Lots consisting of 4,213,312 kilograms of garbage were removed from these vessels
- **437,247** Aircraft arrived from foreign locations
- **25,873,934** Kilograms of garbage removed from these aircraft
### Meat and Other Animal Products confiscated/Refused Entry

<table>
<thead>
<tr>
<th>Category</th>
<th>Lots</th>
<th>Kilograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ship passenger baggage</td>
<td>1,438</td>
<td>3,948</td>
</tr>
<tr>
<td>Aircraft passenger baggage</td>
<td>231,953</td>
<td>566,675</td>
</tr>
<tr>
<td>Border crossing</td>
<td>21,348</td>
<td>39,553</td>
</tr>
<tr>
<td>Post offices</td>
<td>7,997</td>
<td>12,042</td>
</tr>
</tbody>
</table>

### Footwear Cleaned and Disinfected

- **5,689 Pair**

### Maritime Garbage Civil Penalties

- **268** - $51,800

### Baggage Civil Penalties

- **22,164** - $1,186,310

### Notification Violations

- **133** - $51,800

### Predeparture Baggage Violations

- **171** - $7,570
The Committee on Infectious Diseases of Cattle, Bison, and Llama met from 1:30 - 5:30 p.m. on November 1, 1994, and from 1:30 - 3:30 p.m. on November 2, 1994, at the Amway Grand Plaza Hotel, Grand Rapids, Michigan. Chairperson Card and Vice Chairperson Siegfried conducted the meeting.

Committee members present included: Dr. S. Bolin, IA; Mr. Jack Brooks, KY; Dr. Thomas Bunting, IL; Dr. Clyde S. Card, PA; Mr. Donald Christ, OR; Dr. Thomas Conner, IN; Dr. Robert Crandell, TX; Dr. George Crenshaw, CA; Dr. Steve Bolin, NADC, USDA, ARS presented information on BVDV-Type 2, and also discussed the genomic relationships between Type-1 BVDV and the Type-2 strain. Dr. Bolin and subsequent speakers on BVDV pointed out that Type-2 can be both clinically severe or clinically mild, and that genomic studies indicate the presence of the strain for several years. Bovine viral diarrhea virus is now segregated into two genotypes, BVDV type 1 and BDWD type 2. Type 1 BVDV includes vaccine and laboratory reference viruses BVDV-NADL, BVDV-Singer, BVDV-C24V, and BVDV-NY1. Type 2 BVDV are not yet commonly used for laboratory diagnostic purposes or for vaccines. The good news is that the viral genotypes share
some antigenic determinants. Antibody raised against BVDV-1 will neutralize infectivity of BVDV-2; however, the efficiency of viral neutralization is less. This means that current diagnostic reagents can detect BVDV-2 and current vaccines will stimulate cross-reactive antibody that should offer some protection from disease.

Most isolates of BVDV-2 are non-cytopathic in cell culture, but cytopathic BVDV-2 exist. The range of clinical disease associated with BVDV-2 is similar to that of BVDV-1. Some isolates of BVDV-2 have come from herds with subclinical to mild disease. Persistently infected cattle and mucosal disease occur with BVDV-2. Abortions and other signs of reproductive failure occur with BVDV-2. Also, there are some exceptionally virulent BVDV-2 that induce severe disease processes which include thrombocytopenia and hemorrhagic syndrome, high fevers and sudden death (peracute BVD), and enteric disease with lesions similar to those of mucosal disease. Thus, the BVDV-2 appears to be a group of viruses whose members vary in virulence.

The virulent BVDV-2 have cause substantial losses to individuals producers and have spread among neighboring farms to cause substantial losses over geographic areas. At this time, there is need to establish, both the geographic distribution of BVDV-2 and the impact these BVDV have on beef and dairy production. Outbreaks of acute BVD that are usually severe clinically, or are mild clinically but have an unusually high morbidity, are of interest. Currently, serologic based diagnostic tests cannot differentiate viral type; therefore, a viral isolation from these outbreaks is important to type the BVDV involved. The typing procedure is expensive and time consuming, so there is a need to limit use of this procedure. It is best to work closely with state and federal diagnostic laboratories in selection of viruses.

Dr. Susy Carmen (Ontario Ministry of Agriculture) provided committee members with an update of the severe acute outbreak of BVD. In Ontario in 1993 non-cytopathic BVD virus (NCP-BVDV) strains with enhanced virulence caused unprecedented outbreaks of severe acute BVD in 150 dairy, 600 beef and 100 veal herds. Fever, pneumonia, diarrhea were consistent signs in all age groups. Older animals also frequently had oral ulceration. the disease spread slowly through herds, with clinical course lasting hours, days or weeks before death or recovery. Clinical severity varied in herds from a few animals affected to 10-50% overall herd mortality. Calves/heifers were more severely affected. Pregnant animals aborted over two months. Recovered adults returned to milk production and were readily rebred. Outbreaks were associated with recent purchase of cattle and inadequate initial/booster vaccination.

For animals presented for necropsy, a difference in the distribution of lesions was evident in animals <6 months and >6 months of age. Gross lesions of mild to moderate erosions and ulcers in the upper alimentary tract, with necrosis of intestinal mucosa (especially Peyer's patch (PP)), were more prominent in animals >6 months of age. Necrosis of intestinal
cryptal epithelial cells with lymphocytolysis/lymphoid depletion of PP were consistent microscopic lesions in all age groups. Pneumonia occurred in all age groups. BVDV was isolated from 67.5% of all cases diagnosed on the basis of these lesions, with 93.8% being NCP-BVDV.

During the outbreak overall recovery rate of BVDV increased 23% over the mean of the previous four years, with 15.4% of 1993 specimens positive for BVDV by virus isolation. Most isolates were NCP-BVDV (87.5%). Using monoclonal antibodies, cross-neutralization assays with polyclonal antisera (Cornell) and PCR typing (USDA, Ames), the viruses were typed as Group II.

When comparing three month rolling averages over the previous four years, the monthly pattern of proportion of diagnoses associated with BVDV was consistent from 1989 to 1991. A rise in BVDV-associated diagnoses (BVDV-AD) occurred regularly each fall, followed by a cyclic 30% decline in the following Jan-Mar period. In the fall of 1992 the usual rise occurred, but a 32% higher level, BVDV-AD in Jan-Mar 1993 were 91% higher than expected. The epidemic peaked in Jun-Aug of 1993, with total monthly BVDV-AD 4X higher than the mean for the same months for the previous four years. The regular cyclic drop occurred in Jan 1994, but the rate of BVDV-AD was still 2X higher than before the outbreak.

As a result the annual bovine respiratory tract (BRT) histology diagnoses associated with BVDV detection increased 3X to be 19.1% of all BRT histological diagnoses in 1993. Annual bovine gastrointestinal tract (BGIT) histology diagnoses associated with BVDV increased 2.3X to be 34.8% of all BGIT diagnoses. Annual bovine abortions submitted for histology and associated with BVDV increased 2.4X to be 7.4% of all bovine abortion diagnoses for 1993.

Dr. Charles Thoen (ISU) gave a brief update of recent research on Brucellosis testing in llama. Dr. Thoen stated that llamas develop titers to Brucellosis vaccine (St. 19) in about four to ten weeks after vaccination. In another phase of the research, Brucella 2308 was deposited on the eye in two doses. Llamas receiving either level of exposure developed antibodies.

Dr. Ed DuBovi (NY State CVM) described the BVD status in New York. The disease is present throughout the state, and the number of BVDV isolates is significant. New York was not affected by the Ontario outbreak. Dr. DuBovi stressed the current vaccines, if used according to directions, will protect cattle against death, but not against abortions. Therefore vaccines will prevent some economic loss in herds that have followed adequate vaccination protocols.

Dr. DuBovi described a microplate virus isolation procedure that will detect "persistently infected" cattle. The test is based on the presence of virus in the serum of persistently infected cattle. Testing of 10,000-12,000 New York State cattle using the microtiter technique indicates that 1 to 1.5% of cattle may be persistently infected.
A test of bulk tank milk somatic cells, using the PCR technique, may be available as screening test in the near future.

Dr. Tom Drake (PSU/PADLS) reported on a recent outbreak of acute BVD. In April of 1994, the Veterinary Extension Service at Penn State University (PSU) was notified of a disease causing high morbidity and mortality in dairy cattle in several Northwest counties of Pennsylvania. The Animal Diagnostic Laboratory at PSU subsequently received specimens submitted from cattle at these locations by federal, state, private and extension veterinarians. In early May clinicians at the University of Pennsylvania Veterinary Hospital at New Bolton Center (BBC) were involved in a similar high morbidity and mortality disease problem in a large dairy operation in Southeast Pennsylvania (PA).

Investigations following these events were initiated to define the extent, clinical nature, and etiology of the disease as well as the circumstances that may have precipitated the problem. Specifically, were these events linked by a common etiology; was this an existing or a variant of an existing agent; and were there common management factors? To answer these questions, clinicians were interviewed; diagnostic sample results were evaluated; and a survey of affected herds was conducted.

A case definition of peracute disease of cattle with at least one death within 48 hours of onset, morbidity of at least 20%, mortality of at least 10%, and the common signs listed below identified six herds in the Northwest PA survey as severely affected. The first deaths in the outbreak were found, by retrospective evaluation, in January and continued until the end of May 1994. The age of animals that died ranged from 1 to 92 months. Among the six herds the five most common signs in animals that died were diarrhea, a fever of 103°F to 109°F, off feed, a drop in milk production, and runny or red eyes. Among cows in these herds that survived, abortion was also a common sign. The prevailing signs in cattle dying acutely in two of the herds involved the respiratory system, increased respiratory rate and pneumonia of varying severity, with no evidence of diarrhea or mucosal ulceration. Survey data indicated that three of the six herds used a killed BVD vaccine in at least part of the herd during the fall of 1993. The other three herds were unvaccinated during the year before the outbreak. Five of the herds introduced purchased or leased animals within two months of the clinical problems.

Diagnostic samples from the six severely affected herds indicated the BVD virus was a common factor. Isolation of the virus from affected animals in three herds, fluorescent antibody results in one herd, serology in one herd and histopathology plus serology in one herd were considered diagnostic. Two of the viruses isolated from herds affected during this outbreak have been submitted to Dr. Steven Bolin at NADC, Ames, Iowa for further antigenic characterization. During the period of Jan. 1, 1994 until July 31, 1994, the Pennsylvania Animal Diagnostic Laboratory System (PADLS) has made 15 isolations of BVD virus and had 14 cases positive for
antigens of BVD virus by specific immunofluorescence of tissues, representing approximately a four fold increase compared with the same period a year earlier.

Dr. Jim Cullor (UCD, Vet. Med.) discussed the safety of vaccines now being used by veterinarians to protect cattle against disease. Dr. Cullor stated that:

1. Safe or tolerance levels of endotoxin(s) in vaccines are not known;
2. No vaccine pyrogenic threshold for any small animal, food animal or exotic species has been established;
3. Veterinary biologicals have documented endotoxin units per ml. that range from less than 1 EU per ml. to greater than 7 million EU/ml.
4. Clinical observations and experimental results indicate an increased risk of adverse reactions with single component vaccines or multiple component vaccines that contain large amounts of endotoxins;
5. Immunosuppression due to vaccine administration produces affects on animal health, animal well-being, and creates on-farm food safety concerns.

Dr. Donald Lein (NY State, Cornell) presented a resolution concerning the importation of semen that has not been subjected to the "Pattern Protocol" to which U.S. semen must adhere. The resolution was passed unanimously. (Resolution 2)

Dr. C. A. Whetstone (NADC, USDA, ARS) discussed strains of herpes virus that were known to cause lesions in specific organ systems and species specificity of the virus.

1. BHV-1 causes IBR, IPV and encephalitis in cattle. Genes within regions of the BHV-1 often recombine to more efficiently replicate in animals. Affected animals include cattle, llama and sheep and goats.
2. BHV-2 Mammillitis including nodules on the hands of humans.
3. BHV-3 Malignant Calarrheal Fever (MCF). There are probes available to identify the sheep strain of MCF from FADDL and ARS/NADC (with PCR primers). These probes will assist in the differentiation of BVD and MCF.
4. BHV-4 are slow viruses, cytomegalic and present in many animal species. The role of these viruses in disease are still in question.
5. BHV-5 is an encephalitic herpes virus, identified in Argentina, Australia and Texas. The virus is a true encephalitic virus, killing calves when injected intracerebrally.
6. BHV-6 is a caprine herpes virus even though it is closely related to BHV-1.
MODEL REGULATION FOR INTERSTATE TRANSPORT OF LLAMAS

Dr. Murray Fowler (UCD, SVM)

Dr. Murray Fowler (UCD, SVM) presented a proposal "Model Regulation for Interstate Transport of Llamas" and led a Committee discussion on the proposal.

Preliminary Statements

1. Llamas are a unique group of the New World animals and warrant a separate category in state regulations. Justification for this statement is found in the supporting document for the model regulations.

2. Llamas and alpacas are domestic animals and have been serving humans for over 6,000 years. They should not be categorized with wild animals such as cervids, bison, or other zoo artiodactylids.

3. The model regulations were developed by adapting currently-used state regulations to apply to llamas.

Model Regulations

The model regulations consist of three parts; 1. Definition of terms used in the document, which is consistent with state regulations currently in existence. 2. A general section that states that a standard health certificate is required that vehicles must be clean and sanitized for transporting llamas. 3. Special requirements that a state may choose to include. The general regulations are based on current knowledge of the potential for llamas to transmit infectious agents to livestock, horses or poultry in a state. However, states may exercise their prerogative and impose more stringent regulations, some of which may be as follows:

a. Llamas can enter the state only with an entry permit approved before the llama is moved. The protocol for obtaining such a permit is included.

b. States may require negative testing for selected diseases such as brucellosis, tuberculosis, bluetongue, and anaplasmosis. Clinical brucellosis, bluetongue, and anaplasmosis have never been reported in llamas, however, positive titers (based on cattle cut-offs), do occasionally occur. Statements similar to those used for cattle are included, however, only laboratory tests that have been validated by USDA should be used for testing llamas. Studies are currently being conducted to validate tests for brucellosis and tuberculosis.

Supporting Documentation

Supporting documentation is appended to describe similarities, but much
more, the difference between llamas and livestock ruminants. The differences in disease susceptibility, anatomy, and other factors warrant the establishment of a separate category of llamas, rather than lumping them in with cattle, bison, cervids, or sheep and goats. Tables are presented to indicate how states currently apply testing in llamas. Additional information is supplied relative to brucellosis and tuberculosis in llamas.

References are provided for any who may wish to study further about these unique animals.

**Future Directions**

It is apparent that further research is necessary to evaluate appropriate testing procedures. The USDA has initiated special research on llama brucellosis testing in the United States, and tuberculosis testing in the United States and in Argentina.

Llama and alpaca owners are still greatly concerned about the diversity of regulations and the problems that this may engender. The model regulations and establishment of acceptable testing procedures may alleviate some of the problems.

Anyone wishing to review the entire document is invited to obtain a copy from a llama industry representative. If the model regulations are supported by USAHA, the final draft will be sent to each state veterinarian for them to use as they see fit.

Dr. John Honstead (FDA) discussed a CVM recommendation, proposing the prohibition of using sheep and goat offal in ruminant feed. The proposed recommendation is in 21 CFR Part 589 for review and comment. Copies will be sent to all committee members for perusal and possible recommendation(s).

Dr. John Kopec (USDA, APHIS, VS) completed the discussion initiated by Dr. Charles Thoen on Tuesday, November 1, 1994, concerning a Llama Brucellosis Study supported by the ILA, ISU, and USDA, APHIS, VS.

The study consisted of three phases and was reported in that manner.

**PHASE I**

- 12 male llamas for 12 weeks
  - Stain 19 SQ
  - Heat killed strain 2308
  - Uninoculated Controls
  - 4 llamas 4.9 billion CFU
  - 4 llamas 49.8 billion CFU
  - 4 llamas

**Results**

- High titers to St. 109 and 2308
- Two controls-CF titers from 2 + 1:10 to 4 + 1:20

**PHASE II**

- 12 females llamas for 11 weeks
Challenge was by conjunctional route with live Brucella abortus 2308

Dosage--Phase II

<table>
<thead>
<tr>
<th>Dosage</th>
<th>CFU</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Dose</td>
<td>102 million</td>
<td>4 llamas</td>
</tr>
<tr>
<td>Low Dose</td>
<td>897,000</td>
<td>4 llamas</td>
</tr>
<tr>
<td>Controls--Killed</td>
<td>52308-LD</td>
<td>2 llamas</td>
</tr>
<tr>
<td>Controls--no trt</td>
<td></td>
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</tbody>
</table>

Results
1 pregnant animal aborted 6 weeks after challenge with the HD of 2308 llama. Serological response was similar to cattle with some differences.

Test Recommendations--Phase II

- Standard Tube Test--Very fine agglutination easily dispersed upon shaking--not recommended. Standard Plate Test--diagnostic criteria established for cattle appear accurate for llamas. Card Test and BAPA tests recommended as presumptive tests.

- Card Test also recommended as an official test. Rivanol--Diagnostic criteria for cattle appear accurate for classifying llamas. Compliment fixation--not recommended--48 percent of samples were anticomplementary.

- PCFIA
  - >0.6 Negative
  - >=7.05-<0.6 Suspect
  - <=0.5 or below Reactor

- 1 SU ELISA (Thoen)
  - -0.2-1.0 Negative
  - >0.2-<0.89 Suspect
  - 0.8 or greater Reactor

D-Tec Brucella A, cELISA--use as supplemental test--positive results are significant. Titers not as rapidly detected as rapidly as other tests.

Conclusions:
Llamas are susceptible to infection with B. abortus.
Llamas react serologically to standard brucellosis tests the same as cattle with few exceptions.

Mr. Robert Frost (ILA) discussed progress on diagnostic tuberculosis (M. bovis) that are supported by ILA, APHIS and other agencies.

The International Llama Association has been pro-active in its cooperative efforts with governments, states, and provinces to find a diagnostic test for bovine tuberculosis in llamas and alpacas. These domestic livestock species belong to the camelid family and have blood characteristics, immune system responses, and behavioral patterns different from other North American livestock species. These differences in blood characteristics and immune responses have mandated that species-specific research
be carried out to insure that diagnostic tests are sensitive and specific to llamas and alpacas.

Skin tests studies since 1988 have led to a major study going on now in a cooperative agreement effort between USDA and Argentina's NITA (National Institute of Agriculture Technology) in Argentina. This study's primary objective is to evaluate different intradermal tuberculin testing procedures for the diagnosis of tuberculosis infection in llamas. In addition to exhausting the skin sites, the evaluation of hematological and serological tests for the diagnosis of bovine tuberculosis in llamas and other camelids is being carried out. The Pan American Health Organization and the World Health Organization under Dr. Isabel Kantor will do the first ELISA work, and then USDA, Agriculture Canada, and the United States institutions will continue with various serologic testing procedures. Results of these studies will be forthcoming in 1995. Currently the axillary site for PPD tuberculin skin testing is the USDA recommended site. to date, New York and many other states have used axillary site with no reactors. There is no evidence that any herds of llamas or alpacas in North American have bovine tuberculosis. The "Assessment of Risk Factors for M. bovis in the U.S." published in 1992 by USDA, APHIS, VS states, the "current evidence indicates that camelids have not been a factor in the spread of M. bovis."

The International Llama Association wished to express its appreciation to all the above parties. We especially appreciate APHIS and NVSL for its latest efforts to Ames to utilize llamas used in the recent brucellosis study for an additional study concerning diagnostic test possibilities in bovine tuberculosis. This effort, now underway, will certainly compliment the major cooperative effort being undertaken in Argentina.

Dr. Art Kennel, (ILA) described the administration and policies and research programs of the "Llama Medical Research Group."

Administrative Structure

Since January 1993 almost all of the several llama organizations in North America have joined forces to encourage and support medical research in llamas and alpacas. The International Llama Association has been the largest contributor. In addition the Canadian Llama Association, the Alpaca Owners and Breeders Association, and others have joined in and this spirit of collaboration has been gratifying.

We reported here previously that our research group has established priorities. Infectious disease remains our highest priority of which tuberculosis testing remains our first priority. We are also very interested in brucellosis testing and most recently rabies has been of great concern to us. We know what needs to be done.

Our group also developed longer range goals and a strategic plan for the future. This was done with input from the llama community, from academic scientists, practicing veterinarians, and representatives of USDA,
APHIS, VS. We think we know how to proceed with the tasks ahead of us.

Partnerships
Our research groups has developed fruitful partnerships as part of our strategy.
One of these partnerships is with the Morris Animal Foundation, the largest organization dealing with companion animal research. We rely on their expertise for soliciting proposals, evaluating them for scientific merit, and when approved and funded for administering the project. Our group evaluates proposals for relevancy to the needs of the llama community. We have an opportunity to comment to the Scientific Advisory Panel. I am also on the Board of Directors of the Foundation.

Another fruitful partnership has been with official agencies interested in animal research including Agriculture Canada and the USDA. Some results of these partnerships are being presented herd. We are especially please with the collaboration in tuberculosis testing and brucellosis testing.

Research in Progress
Last year at this meeting Drs. Thoen, Gilsdorf, Fowler, and Kennel developed a proposal for collaboration in evaluating brucellosis tests in llamas. The llama community agreed to provide the animal transportation. A resolution was adopted by this committee and USAHA and VS was responsive and results have been presented at this meeting. We are delighted with the collaboration and the prompt response.

M. bovis
Last year at this meeting the results of collaboration with Agriculture Canada on M. bovis and M. avian testing in llamas were presented. We are pleased that a larger scale project is underway under the auspices of the USDA in Argentina and you will hear more about this from Bob Frost. Dr. R. Ellis, C.S.U. is involved with development of an enzyme immunoassay (EIA or ELISA) for detection of M. bovis antibodies in llama serum

P Tenuis

Epidemiologic Characteristic of Juvenile Llama Immunodeficiency
Choanal Afresia


Drs. Weber, University of Minnesota; Johnson, C.S.U.; Ruth, University of Minnesota; Bowen, University of Minnesota; Wolf, Votmnh, Zhong, University of Michigan; and King, University of Minnesota.

The Minute Chromosome in Llamas: Relative to Embryo Death and Genetic Defects. Three years.

Dr. Murray Fowler discussed the problem of rabies vaccine that can be used in llamas. Presently the Committee does not have a short range plan to the alternative of using a non-label vaccine. Discussion centered on the need to vaccinate llamas with a non-label vaccine and to follow antibody response.
LLAMA PROJECT UPDATE - DIAGNOSTICS/BOVINE TUBERCULOSIS

November 2, 1994

Report by Bob Frost, Research Committee, International Llama Association

The International Llama Association has been pro-active in its cooperative efforts with governments, states, and provinces to find a diagnostic test for bovine tuberculosis in llamas and alpacas. These domestic livestock species belong to the camelid family and have blood characteristics, immune system responses, and behavioral patterns different from other North American livestock species. These differences in blood characteristics and immune responses have mandated that species-specific research be carried out to insure that diagnostic tests are sensitive and specific to llamas and alpacas.

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REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. Ralph C. Knowles, Rehoboth Beach, DE
Vice Chairman: Dr. J. B. Anderson, Nashville, TN

Dr. C. Carter Black, GA; Dr. Joyce W. Bowling, MD; Dr. Jones W. Bryan, SC; Dr. C. L. Campbell, FL; Dr. Leroy Coggins, NC; Dr. Richard H. Fulker, IA; Dr. Chester A. Gipson, FL; Dr. R. D. Hobbs, AZ; Dr. G. Reed Holyoak, UT; Dr. Floyd M. Jones, TX; Dr. Donald H. Lein, NY; Dr. Thomas R. Lenz, KS; Dr. Susan E. Littlefield, RI; Mrs. Amy Mann, DC; Dr. Patrick L. McDonough, NY; Dr. Clifford W. McGinnis, NH; Dr. Robert W. Mead, WA; Dr. Andrea Morgan, MD; Dr. Donald L. Notter, KY; Dr. S. R. Nusbaum, FL; Dr. Roger E. Olson, MD; Dr. William E. Pace, FL; Dr. Linda Schlater, IA; Dr. Charles E. Starkey, AR; Dr. Manuel A. Thomas, Jr., TX; Dr. Susan C. Trock, NY; Dr. Charles D. Vail, CO; Dr. Thomas E. Walton, MD; Dr. Robert H. Whitlock, PA.

Introduction
Horses continue to be an international commodity. Visitors to the committee were the following doctors: Bernard Van Goethem (European Union); Robin Bell (U.K.); Neal Farr (Dubai)

Equine Encephalitis
Appended to this report is Dr. J. E. Pearson's (USDA) "Summary of Equine Encephalitis Surveillance, January 1, 1993 - October 15, 1994."

It is interesting that several emus have been sickened with equine encephalitis.

Equine Viral Arteritis Virus (EVA)
Dr. Tim Cordes (USDA) reported the following on EVA:

EVA ANNUAL TESTS SURVEY
Sixteen laboratories located in thirteen states are approved by USDA to conduct the SN test for EVA. Trace backs are complete. The map represents where horses' blood was drawn, not where the lab testing was done. Our veterinary biologics staff maintains that vaccine production has not varied by more than 1000 doses per year for the last three years. Our equine virologist at NVSL, Ames, Iowa, Dave Alstad, stresses that these numbers reflect export.

Appended to this report is a map entitled, "Equine Viral Arteritis Virus - June 1, 1993 to June 1, 1994," showing 742 positive tests during 21,776 animals tested to give an incidence of 3.41%.
Also appended to this report are, "Guidelines for the Control of Equine Viral Arteritis" (inadvertently omitted from the 1993 committee report).

Equine Infectious Anemia (EIA)

Appended to this report is a map entitled, "Equine Infectious Anemia, Test Reported from October 1, 1993 to September 30, 1994." It should be noted that 1,035,073 animals were tested revealing 1,859 positive tests.

Dr. Tim Cordes reported on a survey that he conducted with the states on EIA, and summarized as follows:

EIA Uniform Policy Program Questionnaire

United States, Puerto Rico and Virgin Islands

1. What Serologic Tests for EIA Do You Use?
   Fifty-two states and territories, or 100%, responded that they used the AGID. Comments basically were that AGID is the standard, or tie-breaker, with increasing interest expressed in both ELISAs.

2. Use of Official Laboratory Form VS 10-11
   Of fifty-two responses, only six had modifications, or "improved" 10-11s, often used with standard VS 10-11.

3. Results Reported to States Department or Director of Agriculture
   Of fifty-two, there were only seven negative responses, but these were to livestock boards or divisions. General comments were that positives were reported immediately and negatives reported periodically and not at all in some private labs.

4. Age to Begin Routine Testing of Foals
   Thirty-eight states have six to twelve month requirements. Others basically test foals at weaning and exempt them when at the side of a negative dam.

5. Frequency of Testing in Various Categories of Movement
   Forty-seven have entry regulations; five do not. Comments indicate increasing concern for reactors slipping through auctions and changes of ownership. Major regulation changes are on-going in New Jersey, Texas, Louisiana, and Wisconsin.

6. Licensing of Horse Sales Operations
   Thirty-four states, or 65% responded that they did require licensing. Fifteen had no plans to do so; generally these were states with small equine populations.

7. Fund On-Site Approved Laboratories for Rapid ELISA-type Testing
   There were fifty-eight responses because six answered both "no funds" and "not feasible," hence there were twelve "not feasible" only. The distribution was a bell curve with hump in the middle of thirty-seven states, or 70%, that responded in part or totally "no money."

8. Require Permanent ID for Routine Testing
REPORT OF THE COMMITTEE

Forty-eight responses were "no" and four responses were "yes". Twelve states commented on attaching the following words to descriptions of better identification: "permanent, electronic, microchip."

9. Description of EIA in Regulations
There was no uniformity here whatsoever. Eighteen states simply did not describe EIA in their rules or regulations.

10. States Definition of Exposed Equine
There were fifty-five responses because nine defined both as the "same premise" and "within 200 yards". Twenty-seven states, or 52%, simply had no definitions with most comments indicating epidemiologic investigation determines exposure (usually per state veterinarian's discretion).

11. Identification of Reactors Following USDA's Code of Federal Regulations
Category "closely" provided for very minor (!) combinations of hot, freeze, tattoo, locations, numerals, etc. Thus, forty-four states, or 84%, closely conformed here.

12. Quarantine of reactors following USDA's CFR
Again, "identically" and "closely" accounted for thirty-nine states, or 79%.

13. Provisions for Quarantine of Farm with the Removal of a Reactor
This provided for an interesting split. "No" responses were generally followed by comments reflecting no specific regulations, but did emphasize authority to quarantine. "Yes" responses averaged 30-60 days with a mean of 45 days.

14. First Priority in Changing Your State's Regulations
Forty-nine excellent comments were provided. A complete copy of the survey is now available to non-members of the committee upon request.

The committee discussed USDA's proposed change in the Code of Federal Regulations to allow EIA test positive animals to be shipped to slaughter in sealed trucks rather than be individually identified by branding. It was the consensus of the committee that individual identification of animals is better than sealed trucks for the movement to slaughter of infected animals.

Dr. Maxwell Lea, Jr. presented the highlights of an EIA control program in Louisiana as follows:

1. A test is current if performed within the last 12 months.
2. A negative EIA serological test is required on the following:
   - All animals entering the state;
   - All animals at concentration points, such as sales or shows;
   - All animals changing ownership.
3. All animals (equidae) exposed within 200 yards are quarantined for
30 days and must test negative.

4. All test-positive animals must be sold to slaughter or euthanized within 20 days, with one exception—pregnant or nursing females. Then disposal is extended to 120 days.

5. All animals tested for EIA are identified by hot or freeze brand or electronic ID.

In calendar year 1993, 33,273 animals were tested revealing 324 positive with an incidence of approximately 1%. In the first 8 months of 1994, 23,268 animals were tested revealing 353 positive animals.

Dr. Lea stated that 2 items were very satisfactory:

1. The quarantine and testing of horses on premises adjacent to EIA-affected premises; and
2. The electronic identification works well in this control program.

USDA has 2 EIA-approved serological test techniques—AGID and Competitive ELISA. The committee discussed a proposal to allow performance of the Competitive ELISA test at horse auction markets. The consensus of the committee was that all official EIA tests should remain in approved laboratories and not be performed at auction markets.

Horse Passports

Dr. Ernest Zirkle presented a progress report on horse passport field trial. Dr. Zirkle has been in contact with the Jockey Club and will pursue a joint field trial in the states of New Jersey and New York.

Equine Piroplasmosis (E.P.)

Dr. William Pace reported on an E.P. survey conducted in Florida, as follows:

**EQUINE PIROPLASMOSIS SURVEY**

Conducted at the request of USDA (Import-Export). Every 15th sample was submitted for EIA testing. 625 samples from a pool of 9,400 were collected from 2/15/94 through 4/15/94.

Results:

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (2+ or &gt; @ 1:5)</td>
<td>9</td>
</tr>
<tr>
<td>B. equi</td>
<td>6</td>
</tr>
<tr>
<td>B. caballi</td>
<td>3</td>
</tr>
<tr>
<td>Suspicious (+1 to 1+ @ 1:5)</td>
<td>10</td>
</tr>
<tr>
<td>Anti-complementary</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>599</td>
</tr>
<tr>
<td>TOTAL</td>
<td>625</td>
</tr>
</tbody>
</table>

Among the 9 horses found to be serologically positive to E.P. A follow
up revealed that: 1) 3 animals had been exported; and 2) the 6 remaining horses were retested and 4 were found negative. The testing disparity for the 4 horses appears to be related to the fact that the first tests were by the micro titre technique, whereas the second tests were performed using the C.F. tube technique.

The committee recommends that USDA review their application of the various E.P. test techniques and take corrective action to bring about testing uniformity and consistency.

In 1993, a Puerto Rican animal health official requested that the committee consider recommendations for more flexibility in the international movements of horses to and from Puerto Rico. After due consideration, the committee recommends that animal health officials approach USDA to discuss possible changes to the international movements of horses to and from Puerto Rico.

Other Disease Matters

Dr. Tim Cordes reported on an Acute Equine Respiratory Syndrome that occurred near Brisbane, Australia from September 7-16, 1994. Three stables were involved. Thirteen horses died. Twenty affected horses recovered and were found serum neutralization test positive for a paramyxovirus of the genus *Morbilli*. A horse trainer who abraded his arms trying to hand feed a sick horse died. The trainer, a stable hand, and the attending veterinarian sero converted to the virus, but 18 other persons associated with sick horses remained sero-negative. Appropriate control actions were taken by Australian and USDA officials to prevent the spread of this disease condition.

**SUMMARY OF EQUINE ENCEPHALITIS SURVEILLANCE**

**JANUARY 1, 1993 - OCTOBER 15, 1994**

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 Services, U.S. Department of Agriculture veterinarians and veterinarians in private practice. The testing is done at the NVSL at no charge.

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 $\geq 1:10$ and hemagglutination inhibition antibody titer of $\geq 1:40$ against only eastern equine encephalitis (EEE) or western equine encephalitis (WEE). Some of the EEE and WEE results have been confirmed by a diagnostic increase in antibody titer or virus isolation, and most positive EEE serology results have been confirmed by EEE IgM capture enzyme-linked immunosorbent assay (ELISA). The results shown in Tables
1 through 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.

January - December 31, 1993

From January 1 through December 31, 1993, there were 454 U.S. and 6 foreign submissions for equine encephalitis at the NVSL. Of the domestic submissions, there were 316 horse submissions, 131 avian submissions (the majority of which were ratites), and 7 other submissions including cattle, cats, dogs, and pigs. The six foreign cases included one avian and five horse submissions.

There were 19 horse and 4 emu eastern equine encephalomyelitis positive cases and 8 horse, 2 emu, and 1 turkey western equine encephalomyelitis positive cases at the NVSL. Venezuelan equine encephalomyelitis was isolated from the foreign submissions of tissues from three horses. These were from Venezuela and Mexico and represented the first isolates of VEE from these countries since 1973 and 1972, respectively. Also, VEE was implicated in Guatemala where unvaccinated horses with nervous signs seroconverted to VEE.

No human cases of WEE have been reported in 1993, but there have been five human EEE cases: one each in Florida, Mississippi, and Rhode Island and two in Michigan. The Rhode Island and one of the Michigan cases were fatal.

January - October 1994

For this period, there were a total of 276 submissions received at the NVSL: 197 equine diagnostic submissions; 72 avian, the majority of which were ratites; 2 bovine; 2 goat; 1 porcine; 1 zebra; and 1 elk. There were 31 positive equine EEE cases and 4 avian (3 emus and 1 duck) and 4 WEE positive equine and 3 avians (2 emus and 1 pigeon). No human cases of WEE have been reported in 1993, but there was one human EEE case in Louisiana.

In 1994, four horses had antibody against VEE. Most horses had stable antibody titers; in all instances vaccination histories were vague or not available, and in most cases there was no apparent explanations for the VEE antibody titers.
Table 1. Eastern equine encephalitis positive cases
January 1 - December 31, 1993

<table>
<thead>
<tr>
<th>State</th>
<th>NVSL Positive Cases</th>
<th>CDC and Other Sources</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>1</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>Georgia</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Indiana</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Louisiana</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Maryland</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Michigan</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Mississippi</td>
<td>3 (1 emu)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>North Carolina</td>
<td>3 (2 emus)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>South Carolina</td>
<td>5 (1 emu)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Virginia</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>23</strong></td>
<td><strong>85</strong></td>
<td><strong>108 (4 emus)</strong></td>
</tr>
</tbody>
</table>

5 human cases - Florida, Michigan (2 cases with 1 death), Mississippi, and Rhode Island (1 death)

Table 2. Western equine encephalitis positive cases
January 1 - December 31, 1993

<table>
<thead>
<tr>
<th>State</th>
<th>NVSL Positive Cases</th>
<th>CDC and Other Sources</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>1 (emu)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>California</td>
<td>3 (1 emu, 1 turkey)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Idaho</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Missouri</td>
<td>0</td>
<td>2 (emus)</td>
<td>2</td>
</tr>
<tr>
<td>Nebraska</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Oregon</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Utah</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>11 (2 emus, 1 turkey)</strong></td>
<td><strong>2 (emu)</strong></td>
<td><strong>8 horses, 4 emus, 1 turkey</strong></td>
</tr>
</tbody>
</table>

Human cases - none reported
## Table 3. Eastern equine encephalitis positive cases
### January 1 - October 15, 1994

<table>
<thead>
<tr>
<th>State</th>
<th>NVSL Positive Cases</th>
<th>CDC and Other Sources</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Florida</td>
<td>1 (duck)</td>
<td>79 (1 deer, 1 emu, 2 geese)</td>
<td>80</td>
</tr>
<tr>
<td>Georgia</td>
<td>0</td>
<td>6 (1 emu, 1 dog)</td>
<td>6</td>
</tr>
<tr>
<td>Indiana</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Louisiana</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Michigan</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>North Carolina</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>New Jersey</td>
<td>3 (all emus)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>South Carolina</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Virginia</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>35</strong></td>
<td><strong>94</strong></td>
<td><strong>129</strong></td>
</tr>
</tbody>
</table>

1 human case in Louisiana

## Table 4. Western equine encephalitis positive cases
### January 1 - October 15, 1994

<table>
<thead>
<tr>
<th>State</th>
<th>NVSL Positive Cases</th>
<th>CDC and Other Sources</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>1 (pigeon)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Colorado</td>
<td>1 (emu)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Idaho</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Nebraska</td>
<td>1 (emu)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Wyoming</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>7</strong></td>
<td><strong>0</strong></td>
<td><strong>7</strong></td>
</tr>
</tbody>
</table>

Human cases - none reported
Equine Viral Arteritis Virus
June 1, 1993 - June 1, 1994

Criteria: Virus neutralization (>1:4 titer) and no history of vaccination or virus isolation (tissue or semen). In limited cases, horses were tested more than once.

742 / 21776 = .03407 = 3.41%
Equine Infectious Anemia

Total Positive 1,974
Total Tested 1,057,377

= 0.18%
GUIDELINES FOR THE CONTROL OF EQUINE VIRAL ARTERITIS (E.V.A.)

Ralph C. Knowles, D.V.M.
U.S.A.H.A. - I.D.O.H.C*

Introduction
Equine Viral Arteritis (E.V.A.) is also known as epizootic cellulitis or Pinkeye. It is a contagious viral disease, caused by equine arteritis virus (E.A.V.) and characterized by all of a combination of the following clinical signs: fever, conjunctivitis, excessive lacrimation, nasal congestion and discharge, depression, loss of appetite, skin rash, edema of the eyelids, conjunctiva, legs, ventral bodywall, scrotum, sheath and udder. Abortions have been recorded from 3 to over 10 months of gestation during the acute phase of the infection. Also, neonatal proliferative pneumonia has been observed in newborn foals. The virus is spread primarily by the respiratory and venereal routes. There is evidence of variation in virulence between viral strains. Very many cases of E.A.V. infections especially in standardbreds are subclinical. An incubation period of 2 - 14 days has been recorded. Infrequently, disease may occur in epizootic form where horses are closely assembled such as at race tracks. A clinical diagnosis of E.V.A. needs to be confirmed by appropriate laboratory tests including virus isolation and serologic examination of paired sera samples.

Objectives
The objective of these guidelines are: to identify occurrences of E.V.A. or any other infectious or contagious disease, to offer direction to veterinarians and others concerned with equine health on how to minimize the introduction of such diseases into horse racetracks and other horse assembly points; and should disease outbreaks occur--then to provide a framework to minimize the further spread and to ultimately control the disease outbreak. To provide for an orderly and safe movement of equids from affected racetracks to other horse assembly points. It is expected that implementation of certain disease prevention and control measures would minimize the need for state and other jurisdictional embargoes and provide uniformity of thinking and actions among officials that deal with equine health.

Control Measures at Horse Race Meets
Since the principle method of spread of E.V.A. is through the movement of subclinically infected animals, efforts directed at disease control need to target preventing and dealing with exposure to E.A.V. as follows:
1. Equids entering racetracks (and other horse assembly points) need to be accompanied by a certificate of veterinary inspection issued within the last 10 days, that includes the recording of the rectal temperature of the
individual animal. Such certificates should declare the non-exposure to animals known to be affected with infectious or contagious diseases. Race-track personnel need to check for certificates at the "check in" gate. Additionally, the official track veterinarian should observe (spot check and prevail on accredited veterinarians to assist in this matter) newly introduced animals and review the certificates of veterinary inspection for validity and completeness within the first 24 hours of arrival at the track.

2. Equids presenting suspicious signs of E.V.A. or any other infectious or contagious disease need to be held in separate isolation from healthy horses. The nature of isolation quarters and the amount of activity, i.e. training and racing allowed should be determined by the official track veterinarian, usually after consultation with other animal health officials. This determination should be made known to track officials and trainers.

3. Confirmation of a provisional clinical diagnosis of E.V.A. or other infectious/contagious disease must be based on appropriate laboratory findings, be they isolation or detection of the causative agent and seroconversion or significant increases in antibody titre by serologic determination. By necessity, any regulatory actions must be based on sound scientific findings. However, it should be emphasized that when the situation warrants, state animal health officials usually have the power and the obligation (to protect the general animal or human populations, i.e., VEE) to issue quarantines on suspicion of disease. This brings everyone to the "table" to define the disease problem.

4. Since the movement of equids is an integral part of racing, provisions need to be outlined to enable a safe (health wise) departure from a racetrack where an E.V.A. outbreak has occurred. After a veterinary examination by a U.S.D.A. accredited veterinarian, a certificate of veterinary examination, including a rectal temperature recording should be issued on apparently healthy individual animals. Such certificates should include a declaration of non-exposure to an infectious or contagious disease during the last 14 days. This non-exposure clause will be contingent on the animal health measures taken at the racetrack to control the spread of E.V.A. and certain elapsed time factors since an outbreak of E.V.A. The receiving farm/racetrack should be notified of the E.V.A. situation and should be willing to accept the horse or horses in question.

5. Vaccination against E.V.A. should be carried out in the face of an outbreak to minimize the further spread of the disease, using a U.S.D.A. licensed product. It is recommended that all horses should be bled prior to vaccination to establish their serological status for the virus. Consideration must be given before vaccinating any animal that has potential for exportation to a foreign country that restricts the entry of seropositive animals, when associated with vaccination.

6. Cleaning and disinfection of stalls and equipment used in conjunction with horses affected with or exposed to E.V.A. should be undertaken.
GUIDELINES FOR THE CONTROL OF EQUINE VIRAL ARTERITIS (E.V.A.)

after the animals are moved into isolation. After cleaning out all manure and other organic matter, apply an antimicrobial (virucide/bactericide/ fungicide) preparation using one approved by the U.S. Department of Agriculture and registered with the U.S. Environmental Protection Agency such as One Stroke environ (but not limited to this one product).

7. Contacts with the news media must be handled through one source at each racetrack to minimize misleading statements and to save valuable time for persons directly involved in handling the disease outbreak.

Control Measures at Horse Breeding Farms

1. All equids on arrival at breeding farms should be isolated for 30 days in separate stables or paddocks at least 90 feet away from resident animals. As part of the daily routine they should be fed and watered last and no cross use of tack, grooming equipment or equipment such as pitch forks should occur. These animals should be accompanied by a certificate of veterinary inspection at the time of arrival.

2. When E.V.A. is confirmed by virus isolation or serologic test results, priority consideration for isolation must be given by the attending veterinarian and the farm management of affected and exposed animals. Additionally, equids that have been exposed or are affected with E.V.A. should be prohibited from leaving the farm. To protect and maintain the business integrity of the farm and the welfare of the horse industry, receivers of equids potentially exposed or affected with E.V.A. must be notified at the earliest opportunity. Vaccination of other equids on an affected farm must be decided by the attending veterinarian with the concurrence of farm management with due consideration being given to the need to bleeding all horses prior to vaccination (to determine EVA negativity). The possible effects on exportation, of any animal vaccinated against E.V.A. must be evaluated.

3. In managing breeding practices, when E.V.A. has been confirmed on a farm; it is necessary that any virus shedding stallions only cover E.V.A. vaccinated (at least 3 weeks prior) or known E.V.A. sero positive mares. Since stallions affected with E.V.A. can become carriers and be semen virus shedders it is necessary to evaluate all seropositive stallions, either by attempting virus isolation from two suitable semen collections or by test breeding such stallions to two seronegative mares, with negative results.

4. The American Association of Equine Practitioners (A.A.E.P.) issued the following series of recommendations in June 1993 (as drawn up by the A.A.E.P. Reproduction Committee and included here as authorized by GC-AAEP):

AAEP Recommendations for Transported Semen as it Relates to Equine Viral Arteritis (EVA) (1992):

Equine Viral Arteritis (EVA) with respect to stallions from which semen is collected and transported from the premises in the fresh cooled or frozen state:
1) Breeding stallions unvaccinated for EVA should be tested for evidence of equine arteritis virus infection using the serum neutralization test. No stallion should be vaccinated for the first time without its pre-vaccination titer first being established.

2) Seronegative Stallions (titers of less that 1:4) should be vaccinated at least 28 days prior to breeding or semen collection and receive an annual booster. Vaccinated stallions should be isolated for 28 days post vaccination.
   a. Seronegative stallions that are vaccinated for EVA should be vaccinated at least 28 days prior to breeding or semen collection and receive an annual booster. Vaccinated stallions should be isolated for 28 days post vaccination.

3) Seropositive stallions (unvaccinated). Shedding status should be determined every 12 months either by:
   a. Attempted virus isolation on semen or
   b. Test breeding to at least 2 seronegative mares and monitoring for seroconversion at 14 and 28 days post breeding.

4) Seropositive stallions (vaccinated) need not be tested for virus shedding if seronegative prior to initial vaccination. (Current available data indicates that it appears not justified to do annual rechecks for E.V.A. on stallions found seropositive for E.V.A. as per P.J.T.).

5) The serologic and shedding status of non-EVA vaccinated seropositive stallions should be made known to mare owners receiving the semen. This information should also be reported to state authorities where so required and to breed associations where so required.

6) Stallions seropositive for EVA from natural exposure need not be vaccinated.

Guidelines pertaining to mares which will be inseminated with transported fresh cooled or frozen semen:
   a) Seronegative mares to be inseminated with semen from an equine arteritis virus shedding stallion for the first time should be vaccinated against EVA at least 21 days prior to insemination. These vaccinated animals should be isolated for 21 days post vaccination.
   b) Mares seropositive for EVA from natural exposure need not be vaccinated.

5. Cleaning and disinfection practices relating to items used in conjunction with E.V.A. exposed or affected horses should be in parallel to those described for horse racetracks.

The Role of Governmental and Other Entities in the Control of EVA

1. Attending practicing veterinarians, breeding farm and racetrack management on an affected premises have the primary obligation to report and undertake control measures to stop the spread of E.V.A.
2. State racing commissions and individual racetrack management through their assigned veterinarians have a responsibility and an obligation to implement whatever measures are required to stop the spread of E.V.A. to help preserve the integrity of racing and to minimize any interruption of racing schedules and horse movements.

3. State veterinarians have the responsibility, obligation and authority to help control the spread of E.V.A. (and other communicable diseases of livestock including equids) within their states and to prevent the entry of such diseases into their states.

4. The United States Department of Agriculture (U.S.D.A.) has a mandate to prevent the interstate and international spread of E.V.A. and other infectious and communicable diseases of equids.

The ultimate solution to the control of E.V.A. is to coordinate the efforts of horse owners, horse farm and racetrack managers, private racetrack and racing commission veterinarians. (Group A) If the efforts of the aforementioned can curtail the spread of E.V.A. and adequately contain any outbreaks of disease, then other governmental entities (state veterinarians and U.S.D.A. (Group B) only need to monitor these control activities. However, if adequate control can not be effected by Group A then Group B personnel have an obligation and responsibility to take affirmative action in the E.V.A. control "picture".

Endorsement for these guidelines is requested from: American Association of Equine Practitioners, American Horse Council, Certain Horse Racing interests, i.e., Racing Commissioners Int'l, Thoroughbred Racing Association and other groups interested in the health of equids, The United States Animal Health Association and the American Veterinary Medical Association.

*Thanks is given to the following doctors who contributed their time and talent to this draft: Peter Timoney, Ronald Jansen, Richard Hull, Kenton Holm, H.A. Virts, David Powell, Kenton Morgan and Andrea Morgan.
PARATUBERCULOSIS AND CROHN'S DISEASE: A RELATIONSHIP?

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School of Veterinary Medicine
University of Wisconsin
Madison, WI 53706-1102

Crohn's disease is one of two similar diseases of the human intestinal tract called inflammatory bowel disease. It is a chronic, incurable, low-grade inflammation of the terminal ileum that afflicts roughly one million person in the U.S. The incidence of Crohn's disease is difficult to estimate because it is not a reportable disease, however, approximately 20,000 to 25,000 new cases are thought to occur in the U.S. each year. The incidence, 4-11/100,000, is similar in most developed countries and records indicate the incidence is increasing. The highest attack rate is in people 15 to 30 years old. Investigators interpret this to imply human exposure to some agent or environmental factor(s) in early childhood that lead to the disease. Patients with Crohn's disease suffer from diarrhea, chronic weight loss, abdominal pain, and general malaise. In Crohn's disease, the wall of the terminal ileum is thickened by a diffuse granulomatous inflammatory response that bears marked resemblance to that induced by a mycobacterial infection. Anti-inflammatory drugs like steroids are usually used to treat the symptoms of Crohn's disease. This treatment is consistent with the prevailing opinion that a major component, if not the cause, of the Crohn's disease is autoimmune-mediated. Treatment temporarily alleviates the symptoms of Crohn's disease but does not result in a cure, and patients must resign themselves to medical management of the condition for the duration of their life. As the degree of intestinal pathology progresses, it is common for Crohn's patients to require surgical removal of a segment of the affected bowel.

Crohn's disease and Johne's disease (bovine paratuberculosis) are remarkably similar in clinical signs and intestinal pathology. However, because *M. paratuberculosis* is readily cultured from animals with Johne's disease but not from humans with Crohn's disease, B.B. Crohn et al. and most gastroenterologists and Crohn's disease researchers since his time, have considered Crohn's disease to have an etiology not related to mycobacteria. In the late 1980s, investigators began reporting isolation of *M. paratuberculosis* from patients with Crohn's disease. They found that the organism was frequently present in these patients in a cell wall-deficient state called a spheroplast. These spheroplasts lack the normal tough cell wall characteristic of mycobacteria and are thus very fragile and easily destroyed by disinfectants. The inability of spheroplasts to survive harsh chemi-
eral treatments typically used to process clinical specimens for isolation of mycobacteria may explain the long history of failure to grow *M. paratuberculosis* from specimens from Crohn's disease patients. Without a cell wall, spheroplasts cannot be stained or therefore seen in tissue sections by histopathology, possibly explaining failure to see mycobacteria in biopsies from affected human bowel tissue.

Genetic probes provided new tools with which to examine the assumption that *M. paratuberculosis* was the cause of Crohn's disease. Results of application of gene probes to tissue samples from patients with Crohn's disease are causing the medical community to reconsider whether mycobacteria, and *M. paratuberculosis* in particular, might not be the cause of this disease.\(^8\)\(^9\)\(^10\)\(^11\)

Data supporting the hypothesis that *M. paratuberculosis* causes Crohn's disease are as follows: Isolation of *M. paratuberculosis* from a patient with Crohn's disease was reported in 1984.\(^5\) This isolate, when orally inoculated into infant goats, caused Johne's disease.\(^15\) Since that time, isolation of *M. paratuberculosis* from Crohn's patients has been reported from almost every developed country.\(^16\) In 1992, using newly developed genetic probes, Sanderson et al.\(^14\) reported that 26 of 40 (65%) of Crohn's patients studied harbored *M. paratuberculosis* in their intestinal tissues. July, 1994, Danish workers, using genetic probes for *M. paratuberculosis*, reported finding evidence of the organism in fresh intestinal tissues in 11 of 24 (46%) Crohn's patients, confirming the findings of the British workers.\(^17\) French investigators reported similar findings; 72% of 18 Crohn's patients tested positive for *M. paratuberculosis* by IS900 PCR amplified probe.\(^12\) Using new tests for serum antibodies to *M. paratuberculosis*, workers in Spain, England, and Italy have found that Crohn's patients have a significantly higher rate of positive results than do control patients.\(^18\)\(^19\)\(^20\)

Data not supporting this hypothesis come from laboratories that have failed to consistently isolate *M. paratuberculosis* from Crohn's patients, or failed to find differences in the frequency of antibody titers to *M. paratuberculosis*, or other mycobacteria, between Crohn's patients and suitable controls.\(^21\)\(^22\) Critics of such reports argue that such studies are heavily dependent on the accuracy of laboratory tests used, the expertise of the technologist performing the assays, and the experience of the investigators in working with fastidious mycobacteria such as *M. paratuberculosis*.

Epidemiological studies have shown no correlation between occupational exposure to animals and the incidence of Crohn's disease. However, recent information suggests that *M. paratuberculosis* may have a wider environmental distribution than previously thought, or be a food-borne microbial pathogen, confounding studies of occupational risk factors for Crohn's disease.

*Mycobacterium paratuberculosis* is found in milk of infected cows.\(^23\)\(^24\) In October, 1993, Chiodini reported data on the thermal tolerance of *M.
paratuberculosis suggesting that it can survive pasteurization far better than other mycobacteria. At the Fourth International Colloquium on Paratuberculosis held in Cambridge, England, July, 1994, a food microbiologist from Ireland also reported that *M. paratuberculosis* can withstand pasteurization conditions. At that meeting, British workers reported that 21 of 336 (6.25%) of cartons or bottles of milk purchased from retail outlets throughout central and southern England tested positive for *M. paratuberculosis* using gene probes. While genetic probes cannot distinguish dead from living organisms, when coupled with other reports, it seems plausible that the pasteurized fluid milk supply could be contaminated with viable *M. paratuberculosis*.

The ability of *M. paratuberculosis* to survive pasteurization may not be required for it to be found in dairy products still viable. Cheese products could contain the organism. Only 38% of milk used in the manufacture of cheeses is subjected to any heat treatment. Furthermore, when milk is heated prior to production of many cheese products, the heating regime (65°C for 15-18 sec) is far less rigorous than used for pasteurization of milk for fluid consumption, and well below that necessary to kill *M. paratuberculosis*.

Dairy products may not be the only means for exposure of humans to *M. paratuberculosis*, however. Studies on the tissue distribution of *M. paratuberculosis* in infected animals indicate that it is present in blood and many organs of the body. Thus, red meat also could contain *M. paratuberculosis*. In addition, surface water contamination by *M. paratuberculosis*, and survival of after treatment of water for domestic consumption, could theoretically expose humans to this organism. This theory is not only plausible, but has precedence. *Mycobacterium avium*, first cousin to *M. paratuberculosis*, occurs in domestic water supplies, and domestic water is considered to be a principle means by which AIDS patients acquire intestinal infections by this mycobacterial pathogen. Lastly, when considering possible mechanisms for exposure of humans to *M. paratuberculosis*, the dairy industry should not be the only focus of attention. Paratuberculosis also occurs in beef cattle, sheep, goats, camels and many exotic ruminants.

While the evidence for a causal relationship between *M. paratuberculosis* and Crohn's disease is not yet overwhelming, it is sufficiently strong that animal industries and the USAHA should take action. If *M. paratuberculosis* becomes established as the cause of Crohn's disease, or even an important complicating infection, and investigations confirm that it is present in foods of animal origin, the magnitude of the paratuberculosis problem as a food safety issue will be profound. Because paratuberculosis is most prevalent in dairy cattle, and because *M. paratuberculosis* is known to be excreted in milk of infected cows, the dairy industry may be first and hardest hit.
PARATUBERCULOSIS AND CROHN'S DISEASE: A RELATIONSHIP?

A general list of actions that USAHA might consider follows:
1. Make paratuberculosis a program disease.
2. Implement the paratuberculosis herd certification program presented by the Johne's disease Committee at the 1993 USAHA meeting.
3. Increase funding for NVSL in support of paratuberculosis diagnostic services and test standardization.
4. Increase funding for research on paratuberculosis both as an animal health problem and as a potential food safety concern.

Research is needed to address many pressing questions such as; Does *M. paratuberculosis* survive after pasteurization using commercial equipment rather than laboratory scale systems? Can gamma irradiation or other milk processing methods kill *M. paratuberculosis*? Do the *M. paratuberculosis* strains isolated from humans originate from dairy cattle, other humans, or other animals? Are present paratuberculosis control recommendations able to effectively eliminate the infection from herds? Could antibiotics be used for chemoprophylaxis of calves to limit the rate of infection? Can more effective vaccines be developed? Do cattle genetics play a role in determining susceptibility of animals to *M. paratuberculosis*?

These and many more questions remain to be answered about paratuberculosis. Bovine paratuberculosis is sufficiently prevalent and costly to warrant increased effort to control the disease for animal production industry profitability reasons alone. If *M. paratuberculosis* is added to the growing list of food-borne microbial pathogens of humans, control of paratuberculosis in animals used for food production may change from a recommendation to a requirement.

References


JOHNE'S DISEASE: THE CURRENT STATUS OF DIAGNOSTIC TESTS

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Introduction

One of the earliest serological diagnostic tests for Johne's disease was the complement fixation test, which is still required for exportation and importation of cattle among different countries. The agar gel immunodiffusion test (AGID) was developed later as a relatively quick test to detect animals showing clinical signs. The enzyme linked immunosorbent assay (ELISA) tests were developed as more sensitive tests to detect infected animals earlier in the disease process. Other antigen preparations have been evaluated in a variety of test formats to detect antibodies or sensitized cells produced as part of the host response to infection with M. paratuberculosis.

At this time, both the AGID and the ELISA tests have high specificity and sensitivity to confirm infection in animals with clinical signs. However, no existing diagnostic test has adequate sensitivity and specificity to detect all infected cattle with subclinical infection. No laboratory test has been devised to detect these animals prior to the onset of detectable fecal shedding. Thus much research must be done to develop tests capable of detecting animals which are in the early stage of infection.

Complement Fixation Test

The complement fixation test is often required for export purposes but is not a good test to detect lightly infected animals. The test is plagued with major faults including false positive results and lack of sensitivity to detect infected cattle. de Lisle gave evidence that the major advantage of the complement fixation test was the ability to detect heavily infected cattle quickly but in that study the test did not detect cattle with low bacterial loads. In a group of 37 lightly infected cattle the CF test detected only 3 animals. The CF antibody response seemed closely correlated to fecal bacterial load when cattle were shedding moderate to heavy numbers of M. paratuberculosis.

Most animals with a CF titer of 1:32 or above are likely to be fecal culture positive. Animals with lower complement test values, i.e. <1:32, are often positive on fecal culture, again suggesting the failure of the test to detect cattle in the early stage of infection. These infected cattle are often detected by sensitive fecal culture techniques. The relatively crude carbohydrate antigen used in the CF test in Canada differs from the antigen...
used in the United States. Most authors agree the CF test as currently employed in the United States has few redeeming features as a reliable diagnostic test for Johne's Disease.

**Agar Gel Immunodiffusion Test**

The Agar Gel Immunodiffusion test (AGID) or Rapid Johne's Test (RJT) (available commercially from Kamar, Eden Prairie, MN) is of significant diagnostic value if the animal tested is showing weight loss and/or diarrhea. A positive test correlates well with clinical signs when the weight loss and/or diarrhea are due to Johne's disease. Lack of sensitivity or failure to detect animals that are fecal culture positive but not showing clinical signs is the major drawback with the AGID test. The reported sensitivity of the AGID test to detect Johne's animals with clinical signs has varied from 54% to nearly 100%. This test is approved for use in many states (an unofficial test) but not in Wisconsin. Some practicing veterinarians use the test, but rarely as a herd screening test to detect non-clinical animals. The test should be reserved for the individual cow with clinical signs compatible with Johne's disease. If the AGID test is positive (laboratory time 72-96 hrs), then the cow has a greater than 95% probability of being infected with the Johne's organism.

The AGID test run by Canadian laboratories employs the protein D antigen and not the crude protoplasmic antigen used in most United States laboratories. Laboratories in several European countries use other antigens, therefore comparison of results between countries may not be directly comparable.

**ELISA Serologic Tests**

ELISA tests to measure serum antibodies directed against *M. paratuberculosis* are currently experimental. Elisa tests are developed and used in research laboratories. Only one ELISA test is USDA approved and commercially available from the Idexx Corporation. Research based ELISA tests are often set up differently from laboratory to laboratory and, thus, the results from one laboratory are not comparable to another. An ELISA test result should not be accepted as a diagnostic test for the individual cow. The Dot blot is a modification of an ELISA test but at this time also remains experimental.

Advantages of ELISA tests are rapid turn around time (2-4 days), low unit cost and the large number of samples that can be processed each day. The test detects and quantitates the host's serum antibody response to an antigen derived from *M. paratuberculosis*. Unfortunately, cattle are exposed to a variety of mycobacterial antigens in the environment and therefore the antibody detected by the ELISA is not fully specific for Johne's infection. Animals with higher ELISA antibody titers are more likely to be infected with *M. paratuberculosis*. The reporting of likelihood ratios in addition to the recorded optical densities provides more information to the
WHITLOCK

veterinarian and the herd owner\textsuperscript{19}. Often investigators must make a decision about the cut-off point for a positive test result from the continuous scale of optical density values obtained from the serum samples. A number of methods exist to determine the optimal cut-off point is optimal. McNabb proposed the use of receiver-operator-curves and the determination of the maximum sum of both sensitivity and specificity to establish the cut-off point\textsuperscript{13}. Alternatively, others use the intersection of sensitivity and specificity plots as the cut-off point.

In addition to different ELISA protocols among different laboratories, the antigens employed among the laboratories differ significantly. A crude protoplasmic antigen derived from strain 18 of \textit{M. paratuberculosis} was one of the first antigens used in the ELISA test\textsuperscript{18}. Thoen (1986) has advocated the use of carbodiimide with the carbonate buffer and a potassium chloride extract of \textit{M. paratuberculosis}\textsuperscript{23}. Other investigators have used different antigens including the purified lipoarabinomannan (LAM) and lipid-free arabinomannan which are common to many mycobacteria and related species including nocardia and corynebacterium\textsuperscript{20,21}. The LAM antigen has nearly 100 fold greater affinity to the polystyrene plate than lipid-free arabinomannan\textsuperscript{20}. The LAM antigen may offer greater specificity but lower specificity than protoplasmic antigens as this antigen has stronger immunogenicity.

Those cattle determined serologically to be at greater risk, ie with the highest ELISA titers, should have fecal samples cultured or have a DNA probe test done to determine their infection status. Thus, the ELISA test would serve to screen cattle prior to fecal culture so only those cattle at highest risk for Johne's disease would be cultured. This would permit a much greater number of cattle to participate in a Johne's control and/or certification program.

\textbf{Dotblot-ELISA or Dot-ELISA}

The Dotblot ELISA has greater sensitivity to detect infected cattle than the conventional ELISA test\textsuperscript{26}. Additional advantages include a very high specificity for cattle with clinical signs, applicability for field use, a visually apparent result requiring minimal equipment, and the short time required to run the test\textsuperscript{26}. Although the Dot ELISA was reported to have a high test sensitivity (85\% for clinical suspects and 64\% for non-clinical infected animals) it should be noted that the gold standard of comparison was a relatively insensitive fecal culture technique, therefore if a more sensitive technique were used to detect infected animals earlier in the course of the disease the sensitivity would be substantially lower.

\textbf{Fecal Culture}

Fecal culture for \textit{M. paratuberculosis} remains the \textit{"Gold Standard"} for routine diagnosis and detection of individual animals in a herd suspected of having cattle with Johne's disease. A fecal culture test, when performed
correctly, is the most specific and sensitive test to detect cattle infected with *Mycobacterium paratuberculosis*. The organism can be detected in fecal samples of some, if not most, infected cattle one to two years before they develop clinical signs of weight loss and diarrhea. The major drawback with fecal culture is the prolonged incubation period of 12-16 weeks before the test results can be reported back to the owner and veterinarian. This fact renders fecal culture a poor test to confirm a clinically suspicious case when results are needed quickly.

**Fecal Sample Acquisition and Culture**

Obtain the fecal sample directly from the rectum using a new plastic sleeve for each animal and do not use lubricants for the plastic sleeve, because they may interfere with the culture process. Place fecal sample in plastic or metal specimen container labeled with the animal's identification number. Ship samples unfrozen and unrefrigerated via overnight delivery to the laboratory. Freezing the samples in a commercial freezer (-20°C) will result in significant loss of organism numbers causing cultures with few organisms to give false negative results. Freezing at -70°C for 2 to 3 weeks results in little loss of organism viability.

Two major advances in the fecal culture test are the centrifugation technique and the double incubation technique (Shin, 1990). Not only are more infected cattle identified with the centrifugation technique but they are detected at a much earlier stage of the infection. The centrifugation technique increased the sensitivity of the fecal culture test three fold enabling earlier detection of an infected animal, perhaps up to two years before the animal would have developed clinical signs. The lower level of detection of the fecal culture test without centrifugation is 50-100 organisms per gram of feces, however with the centrifugation technique, the detection level is substantially increased to 10-50 organisms/gram.

Occasionally, individual samples or most of the samples from a herd will be overgrown with fungus associated with feeding moldy feed. Withholding moldy silage 48 hours prior to collecting the samples will lessen the contamination but often is not practicable. Bacterial overgrowth, especially of *Bacillus cereus* may inhibit *M. paratuberculosis* and necessitate reculturing. The confounding problem of fungal and bacterial contamination is further exacerbated by the centrifugation technique, which concentrates not only *M. paratuberculosis* but also the contaminants. Fortunately, the problem of bacterial and fungal contamination can be easily circumvented by employing the double incubation technique described by Shin. Previously, up to 75% of the samples from some herds were rendered useless by extensive contamination. This relatively inexpensive modification to the culture technique greatly reduces the contamination rate to less than 5% of culture tubes.

In summary, at this time the fecal culture test, the "Gold Standard" to which other tests are compared, is the most sensitive and specific test avail-
able to determine the presence of Johne's disease in cattle on a herd basis. Although the fecal culture is the most sensitive test to detect cattle infected with *M. paratuberculosis* it will detect less than 50% of all the currently infected adult cattle on the farm. However, repeated whole herd annual fecal culturing is recommended for herds known to be infected with *M. paratuberculosis*.

**DNA Probe Test**

DNA probes to identify specific DNA segments present in *M. paratuberculosis* represent state-of-the-art technology to identify this fastidious pathogen. Potentially, the DNA probe approach would have the greatest sensitivity (1 copy of the DNA sequence - IS-900 in the test sample) and exquisite specificity. The greatest obstacles to wide scale acceptance of this approach are the high unit cost per test, and the sophisticated equipment and experienced personnel necessary to run the test. A major advantage is the rapid turnaround time of 48-72 hours and the potential high sensitivity and specificity.

A DNA probe test has been developed by the Idexx Corporation and provides prompt (4 days) detection of *M. paratuberculosis* organisms in the fecal sample when present in large numbers. The major advantage of this test is the short time to detect the organism and the test specificity. However, the disadvantage is the relatively high unit cost, ($25-$30/test), the advanced level of technical expertise and equipment required plus the large number of organisms to be present before a test is positive.

**Histopathology**

For the clinician, histopathological examination of biopsies of the terminal ileum and mesenteric lymph node often offers the most definitive diagnosis within a short period of time (48-72 Hrs). The time required can be further shortened by microscopic examination of smears of the tissues following acid fast staining with either zeihl-neelsen, Kinyoun's method or Auramine-O stain. For a positive finding, acid-fast organisms should be found in clumps within macrophages. A single organism or a few organisms found on the smear should be regarded as saprophytes.

**Summary**

A battery of diagnostic tests are now available for the detection of Johne's disease including the DNA probe, serum AGID and ELISA, gamma interferon response and improved fecal culture techniques. Each test has its advantage and disadvantages in a given situation - individual animals with clinical signs, new addition to the herd or herd survey instrument.

**Acknowledgements:** The Johne's research project at the University of Pennsylvania, School of Veterinary Medicine has been supported primarily by the Pennsylvania Dept. of Agriculture. The author gratefully acknowledges the collaborative support of colleagues at Cornell University (Drs. 303
JOHNE'S DISEASE: CURRENT STATUS OF DIAGNOSTIC TESTS

Shin, Rossiter, Jacobson and Lein) at the Animal Disease Research Institute, Nepean, Ontario, Canada (Drs. Sugden, Duncan, Brooks and Nielson); and at the National Animal Disease Center, Ames, Iowa (Ms. Whipple) among other Institutions.

References
14. Merkal RS. Paratuberculosis: Advances in cultural, serologic, and


REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

Chairman: Dr. Robert H. Whitlock, Kennett Square, PA
Vice Chairman: Dr. Michael T. Collins, Madison, WI

Dr. James L. Alexander, TX; Dr. Robert D. Angus, ID; Dr. Wes Bonner, TX; Dr. H. Michael Chaddock, MI; Dr. Thomas F. Conner, IN; Dr. Robert W. Dellers, WI; Dr. Debbi Donch, MI; Dr. R. J. Eisner, NJ; Dr. Colleen Y. Erbel, TN; Dr. Mitchell A. Essey, MD; Dr. William Fales, MO; Mr. Jim Funk, IL; Dr. William L. Hartmann, MN; Dr. Sharon K. Hietala, CA; Dr. John P. Huntley, NY; Dr. Sarah B. S. Hurley, WI; Dr. Richard Jacobson, NY; Mr. H. M. Lefler, CA; Dr. Donald H. Lein, NY; Dr. Clifford W. McGinnis, NH; Dr. A. R. McLaughlin, WI; Dr. I. Lee McPhail, OH; Dr. Roger E. Olson, MD; Dr. Janet B. Payeur, IA; Dr. Frederic A. Rommel, PA; Dr. Christine Rossiter, NY; Dr. Harvey L. Rubin, FL; Mrs. Sherry Seubert, WI; Dr. Sang Shin, NY; Dr. Shri Singh, KY; Dr. Donald Sackett, WI; Dr. C. O. Thoen, IA; Dr. Max A. Van Buskirk, PA; Dr. Robert J. Velure, ND; Ms. Diana L. Whipple, IA; Dr. Howard W. Whitford, TX.

The committee meeting began at 1:30 p.m. included 7 presentations, vigorous discussions and concluded at 6:00 p.m. Attendance included 25 of the 36 committee members and 41 guests for a registered total of 66 (some attendees did not sign the register).

Dr. Jim Case from California requested the Johne's Committee review the criteria for what constitutes a positive diagnosis for Johne's disease in regards to the National Dx Monitor that reports the prevalence of animal diseases on a National basis. A subcommittee will be appointed to review the issue and then make a report.

Paratuberculosis and Crohn's Disease: A relationship? Dr. Collins presented a brief review of the literature reporting an association between Crohn's disease and paratuberculosis. Investigators are isolating *M. paratuberculosis* from Crohn's patients' tissues with increasing frequency. It is not known if this association causal. It is also not known how humans could become exposed to *M. paratuberculosis*. However, milk from paratuberculosis cattle has been shown to contain *M. paratuberculosis*, and, if it survives pasteurization, it could be consumed by the public. A manuscript briefly reviewing the issue is included in the proceedings. The primary question posed was what if any action on this issue the USAHA Johne's Disease Committee should make.

Following a short presentation by Drs. Detweiller and Rossiter, and intense discussion by the group a motion was passed to further study this issue.

Motion: That the Johne's Disease Committee of the USAHA establish a task force, with balanced representation from animal agricultural indus-
JOHNE'S DISEASE

tries, representatives from regulatory agencies, university researchers, public health organizations and medical experts on Crohn's disease, to 1) evaluate information suggesting *M. paratuberculosis* is a zoonotic pathogen, 2) assess the likelihood that animals serve as the source of infection for man, and 3) determine the potential for this organism to contaminate foods of animal origin. In addition, the task force should recommend to the Committee a policy statement on the public health risks of paratuberculosis in livestock.

**Update on Johne's Programs in Individual States.** Dr. Donch reported experiences in Michigan in establishing a Johne's control program. Her presentation was followed by comments from representatives from Wisconsin, New York, Pennsylvania, Maryland, New Jersey, Ohio and Minnesota on efforts toward formalizing Johne's disease control efforts. There is a general movement toward alignment of state programs with the National Paratuberculosis Certification Program outlined at the 1993 USAHA meeting. Some concern was expressed that the AVMA had not endorsed the National Paratuberculosis Certification Program.

**Code of Federal Regulations regarding Johne's disease (CFR part 80 and 71.3).** Dr. Mitch Essey reviewed the CFR as it pertains to paratuberculosis (Johne's disease) in cattle. This portion of the CFR was added in 1952 and has remained unchanged since that time. Changes to CFR part 80 were proposed in 1985 (Federal Register, Vol. 50, No. 180, September 17, 1985), but the proposed changes were rescinded in 1989 (Federal Register, Vol. 54, No. 196, October 12, 1989). There is no official diagnostic test for Johne's disease in cattle. The CFR as it pertains to Johne's disease was intended to restrict spread of the disease by regulating the interstate movement of paratuberculosis reactors and paratuberculosis exposed cattle.

Committee discussion that followed Dr. Essey's presentation concerned the antiquated nature of the regulations, and whether in their existing form, hamper or support individual state's efforts to control the disease. The discussion led to a motion that a subcommittee be formed to review those portions of CFR part 80 that pertain to issues of concern to the Johne's Disease Committee of the USAHA. The motion passed.

**Johne's disease in a Texas goat herd.** Dr. James Alexander presented a report on Johne's disease a goat herd in Texas that was experiencing a chronic wasting syndrome. While symptoms of disease compatible with Johne's disease and serological evidence of *M. paratuberculosis* infections were demonstrated, Johne's disease was not confirmed by detection of *M. paratuberculosis* by either culture or gene probe.

**Paratuberculosis in a Wisconsin elk herd.** Dr. Don Sockett described detection of *M. paratuberculosis* infections in three elk in a herd of 35 animals in Wisconsin. The elk were in an apparently well managed herd on a 200 acre farm. *M. paratuberculosis* was isolated from fecal samples of the
animals by radiometric culture. Serological studies were not done.

Others in attendance reported occurrence of Johne's disease in farmed cervidae. Currently available serological tests for paratuberculosis in cervidae, other than the AGID, may not be appropriate.

Effect of stress on *M. paratuberculosis* infections in cattle. Dr. Don Sockett reported several occurrences of paratuberculosis in Wisconsin cattle where animals purchased from herds with no history of Johne's disease developed clinical disease shortly (within months) after introduction to a new herd. It was surmised that the stress of transportation and adjustment to a new environment and caused subclinically *M. paratuberculosis* infected cattle to develop clinical Johne's disease. One instance of Johne's disease in cattle following stress associated with a barn fire and animal relocation was also described.

The meeting was concluded at 6:00 p.m.
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

Chairman: Dr. James M. Donahue, Lexington, KY
Vice Chairman: Dr. John R. Cole, Tifton, GA

Dr. Mervyn F. Baker, CAN; Dr. Carole A. Bolin, IA; Mr. John E. Finnell, IL; Dr. Joseph C. Frantz, NE; Dr. James W. Glosser, CA; Dr. Lyle E. Hanson, IL; Dr. Hailu Kinde, CA; Dr. Raymond L. Morter, IN; Dr. Robert M. Nervig, CO; Dr. Harvey L. Rubin, FL; Ms. Barbara Smith, KY; Dr. Herb Smith, ND; Dr. J. Glenn Songer, AZ; Dr. A. B. Thiermann, MD; Dr. Lee Ann Thomas, IA; Dr. Deoki N. Tripathy, IL; Mr. H. E. Vanderslice, DE.

The USAHA Committee on Leptospirosis met on Tuesday, November 1, 1994, with 12 members and guests present.

Dr. Lee Ann Thomas of the diagnostic section of the National Leptospirosis Reference Center (APHIS-NVSL) provided a report on the 1993-1994 activities of the section. *Leptospira* activities at the National Veterinary Services Laboratories (NVSL) have focused on serological testing (microscopic agglutination test [MAT]) and provision of diagnostic reagents. A total of 2,674 MAT was performed, which is an increase of 36% from last year's total. The following diagnostic reagents were provided: fluorescent antibody conjugate (73 vials), flazo orange counterstain (20 vials), reference antisera to 19 serovars (149 vials), and a total of 173 reference cultures for 22 serovars.

In response to concerns expressed at last year's USAHA meeting over diagnostic support for *Leptospira* activities, a survey was distributed to personnel at laboratories that have ordered leptospiral reagents from the NVSL in the last 1-1/2 years and to members of the Leptospirosis Committee. This survey was an attempt to define which services represent the greatest need for diagnostic laboratories. Based on the results of this survey, the NVSL has determined that highest priority should be given to diagnostic reagents. In addition, a diagnostic training course (serology) will be offered in 1995 at the NVSL. Plans will also be initiated for a check test to be distributed to interested laboratories. Before a final decision is made concerning *Leptospira* isolation, laboratories currently performing this service need to be confirmed. A summary of this survey follows.

*Leptospira* Questionnaire Summary

1. Indicate whether your laboratory performs the following tests or submits samples to other laboratories. If samples are submitted to other laboratories, please indicate which laboratory.
## REPORT OF THE COMMITTEE

**Leptospira microscopic agglutination test:**
- Yes - 32 (80%)
- No - 7 (17%)
- No response (NR) - 1 (3%)

Laboratories Named:
- Texas Veterinary Medicine Diagnostic Laboratory
- University of Nebraska Veterinary Diagnostic Center
- Oklahoma Animal Disease Diagnostic Laboratory
- Utah State University Vet Diagnostic Laboratory

**Leptospira fluorescent antibody test:**
- Yes - 28 (70%)
- No - 10 (25%)
- NR - 2 (5%)

Laboratories Named:
- Texas Veterinary Medicine Diagnostic Laboratory
- Centers for Disease Control

**Leptospira isolation:**
- Yes - 20 (50%)
- No - 17 (42%)
- NR - 3 (8%)

Laboratories Named:
- NVSL 4 (10%)
- NADC 1 (3%)

**Leptospira identification**
(by restriction enzyme analysis):
- Yes - 1 (2.5%)
- No - 36 (90%)
- NR - 3 (7.5%)

Laboratories Named:
- NVSL 9 (23%)
- NADC 3 (8%)

Other (specify test):
- Dark field exam done for research purposes primarily
- Only restricted diagnostic samples
- Direct microscopic examination
- MAT for other than the 6 usual serovars are sent to NVSL

2. Does your laboratory anticipate offering any additional *Leptospira* tests not currently available in your laboratory? (Circle those that apply)
   a. *Leptospira* microscopic agglutination test - 3 (8%)
LEPTOSPIROSIS

b. *Leptospira* fluorescent antibody test - 2 (5%)
   Comment: Monospecific FA conjugate testing

c. *Leptospira* isolation - 1 (3%)

d. *Leptospira* identification
   (by restriction enzyme analysis) - 2 (5%)

e. Other (specify test): PCR strain identification
   May look into probe or PCR immunoperoxidase (if reagents are available)
   ELISA Immunoblot

3. Please prioritize your need for the NVSL to provide the following services:

<table>
<thead>
<tr>
<th>Service</th>
<th>High</th>
<th>Med</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leptospira</em> diagnostic reagents</td>
<td>36 (90%)</td>
<td>1 (3%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Comment: FA Lepto serum controls</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Leptospira</em> microscopic agglutination test</td>
<td>7 (18%)</td>
<td>7 (18%)</td>
<td>20 (50%)</td>
</tr>
<tr>
<td>(for diagnostic or export purposes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comment: Confirmation/Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptospira</em> isolation</td>
<td>7 (18%)</td>
<td>13 (33%)</td>
<td>16 (40%)</td>
</tr>
<tr>
<td>(by restriction enzyme analysis)</td>
<td>15 (38%)</td>
<td>9 (23%)</td>
<td>11 (28%)</td>
</tr>
<tr>
<td><em>Leptospira</em> diagnostic training</td>
<td>15 (38%)</td>
<td>10 (25%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td><em>Leptospira</em> serology check test</td>
<td>26 (65%)</td>
<td>7 (18%)</td>
<td>4 (10%)</td>
</tr>
</tbody>
</table>

4. In the services listed in No. 2, which service should the NVSL give the highest priority?

*Leptospira* microagglutination test - 5 (13%)
*Leptospira* fluorescent antibody test - 1 (3%)
*Leptospira* isolation - 3 (8%)
*Leptospira* identification - 3 (8%)
NR - 6 (15%)

Comments: MAT and isolation - 1 (3%)
Diagnostic reagents - 14 (36%)
Check tests to assure standardization of testing between labs - 2 (5%)
Isolation and identification - 2 (5%)
MAT (need uniform standardization among state labs) - 1 (3%)
REPORT OF THE COMMITTEE

5. During the next year, does your laboratory anticipate the need for training in any of the following areas: (Circle those that apply)
   a. *Leptospira* microscopic agglutination testing  12 (31%)
   b. *Leptospira* fluorescent antibody testing  5 (13%)
   c. *Leptospira* isolation  6 (15%)
   d. *Leptospira* identification  
      (by restriction enzyme analysis)  9 (23%)
   No response  6 (15%)
   Comment: No money for travel/training -1 (3%)

6. Would your laboratory participate if a *Leptospira* MAT check test was offered?  (Circle Yes or No)

   Yes - 34 (85%)
   No - 4 (10%)
   Questionable - 1 (3%)
   Comment: If reasonable cost - 1 (3%)

7. Circle the reagents needed and indicate the approximate amount you anticipate ordering (at current scheduled user fee cost) during the next year.

<table>
<thead>
<tr>
<th>Approximate Number Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. <em>Leptospira</em> multivalent fluorescent antibody conjugate (1 ml vial = $19.25) 106</td>
</tr>
<tr>
<td>b. Flazo orange counterstain (3 ml vial = $6.00) 49</td>
</tr>
<tr>
<td>c. <em>Leptospira</em> microscopic agglutination antigen (each antigen = $20.00) 202</td>
</tr>
<tr>
<td>d. Positive control antiserum for microscopic agglutination test (2 ml vial = $4.50) 281</td>
</tr>
<tr>
<td>e. <em>Leptospira</em> transport medium (10 ml vial = $3.00) 63</td>
</tr>
</tbody>
</table>

8. Additional comments: We appreciate the good cooperation from the NVSL. Provision of reagents is vital! I would hope as new techniques/reagents are developed in research labs that they can be made available to diagnostic labs through APHIS.
   The NVSL leptospirosis diagnostic services are essential since it is the only animal leptospirosis reference laboratory available and animal disease laboratory. I further urge a reduction in the price of antigens - high costs will force labs to trade cultures - as a result, improper and unknown serovars may be used to provide export testing.
An accurate, sensitive DNA probe for *Leptospira interrogans* would be in high demand.

My laboratory is involved with leptospirosis on a research basis only. We do not offer any services—those types of services are available at the Oklahoma Animal Disease Diagnostic Laboratory which is directly across the street from us. We rely on the NVSL for conjugates, antisera, and lepto identification. I am not aware of these services provided by the NVSL being available elsewhere and am concerned if these are being considered for discontinuation.

A *Leptospira* MAT check test is needed for QA. This is the single most important service the NVSL could offer for lepto testing. Reagents for the MAT is the second most important assistance needed. In areas of the country where lepto is more prevalent, I am sure that support in other areas of lepto testing would be appreciated.

Our facility is no longer involved with *Leptospira* production (other than an occasional serial of LCI) or research. We offer no diagnostic services and will have no need for the NVSL diagnostic services. We do, however, support the continuation of diagnostic services by the NVSL to whatever extent funding will allow.

We appreciate your efforts and continued support of the leptospirosis work.

We appreciate the expertise of Carole Bolin and the quality of reagents from the NVSL. A check test would be appropriate since it has been a few years since we participated in one.

Research into a latex bead test is needed.

We appreciate the questionnaire. Thanks. We have noticed in the last year that the *bratislava* culture does not QC to the control antisera as it has in the past—there is significant x-reaction to other serovars and also the morphology/growth seems different. Our antisera have not changed and three sequential lots of *Leptospira bratislava* have failed QC that was previously okay. We’ve been told it was variously a antisera problem, a cross-contamination problem, and that the antigen was improperly passaged. Resolution to the problem/concern would be appreciated since all QC was performed from the NVSL antigens and not antigen propagated in the lab. Are we doing something wrong? Is the antigen changing?

A need to develop immunoperoxidase reagents for formalin-fixed tissues when fresh and unfixed tissues are not available. Need to develop monovalent FA conjugate for further speciation from tissues when culture is not possible.

We’ve been very happy with service and help in the past. Hope you are able to keep up the high quality of service. The check test that was offered in 1989 was a helpful indication of how well cultures are doing in individual laboratories and how things are going at other facilities. More standardization would be even more helpful.
A check test is highly desired if the NVSL would offer a training course on MAT and/or other diagnostic techniques. The training would allow the microbiologists who run the test(s) to have a clear standard guideline to read and interpret test results. Since based on the current protocol, to define a 2+ (50% agglutination) or greater reaction as the end point could vary greatly between individuals in the same lab, do not even mention that between labs.

The NVSL needs to continue supplying high quality FA reagents. I believe the cost of antigens for the MAT is too high and that laboratories will start to get other cultures from other sources which will lead to less standardization and possibly errors in the results they provide.

Dr. Carole Bolin of the research section (ARS-NADC) of the National Leptospirosis Reference Center reported on the areas of research conducted during the year. Studies on the efficacy of antibiotics for elimination of chronic leptospiral infection in cattle and swine have been completed. The search for efficacious antibiotic regimens was started because of the removal of dihydrostreptomycin from the U.S. market and introduction of long milk withdrawal times (7 to 14 days) for use of dihydrostreptomycin in the United Kingdom. Three to four weeks after pigs were infected with *Leptospira interrogans* serovar *pomona* type *kennewicki*, urinary shedding was confirmed. Persistently infected pigs were then treated parenterally with a 3- or 5-day regimen of one of several antibiotics. Drugs were administered at the labeled dosage and at an elevated dosage for 3 or 5 days. Antibiotics evaluated in swine included: ceftiofur, oxytetracycline, erythromycin, tylosin, ampicillin, tiamulin, and, as a positive antibiotic control, dihydrostreptomycin. None of the antibiotics cleared the *pomona* infection at the labeled dosage. At elevated dosages, oxytetracycline (40 mg/kg), tylosin (44 mg/kg), erythromycin (25 mg/kg), and dihydrostreptomycin (25 mg/kg) were found to be effective in clearing persistent *pomona* infection in swine. Cattle were infected with *L. borgpetersenii* serovar *hardjo* type *hardjobovis* and were determined to be shedding leptospires in their urine 4 weeks after infection. Groups of cattle were treated with: a single dose of long-acting oxytetracycline (20 mg/kg), oxytetracycline (10 mg/kg) for 3 days, ceftiofur (20 mg/kg) for 3 days, ceftiofur (2.2 mg/kg) for 3 or 5 days, and dihydrostreptomycin (25 mg/kg) as a positive control. Oxytetracycline (20 mg/kg) and ceftiofur (20 mg/kg) were effective in clearing persistent *hardjo* infection in cattle. These new antibiotic regimens need to be tested in larger numbers of animals and doses intermediate to those used here need to be evaluated to find a cost-effective drug regimen which minimizes problems with drug residues in meat or milk.

Studies to compare different PCR assays and different methods of sample preparation for detection of *L. borgpetersenii* serovar *hardjo* in bovine urine were completed. Different PCR assays described in the literature have different sensitivity and specificity for use with cattle urine. The
most sensitive assays were those based on amplification of 16S rRNA genes; however, these assays were not sufficiently specific for routine use in diagnostic laboratories. PCR primers described by Gravekamp et al. (J. Clin. Microbiol. 29:2805-2808), based on a DNA sequence of unknown function, were found to have the best combination of sensitivity and specificity. A method of processing urine based on centrifugation and washing was found to be simpler and more reliable than other methods of sample preparation for PCR assays.

Several studies are ongoing. Preliminary results look promising for the development of a PCR assay for use with urine which can detect and identify (as to serovar) leptospires in clinical samples from cattle and swine. A study to evaluate new candidate vaccines for protection of cattle from hardjo infection is underway. Vaccines under test include new combinations of adjuvant and antigen. Challenge of vaccinates will occur in December. A study is underway to determine if previous infection with serovar hardjo type hardjobovis isolates from various parts of the world will cross-protect cattle from infection with strains of hardjo from the United States. We wanted to know whether it was reasonable to produce hardjo vaccines for the world market or whether inclusion of "local" strains of hardjo in vaccines was necessary.

Dr. Mike Donahue reported on the highlights of some of the papers and posters presented at the VIIIth Meeting of European Leptospira Workers in Anzio, Italy on July 11-13, 1994. There were 73 participants—48 from European countries and 25 from other countries—representing all areas of the world. Forty-nine papers and 16 posters were listed for presentation. Only those papers believed to be of interest to the Leptospirosis committee were highlighted.

Six of the presentations and 4 posters were on Veterinary Epidemiology. Farina and co-workers of the Department of Animal Pathology in Pisa, Italy presented research concerned with Leptospira interrogans serovar hardjo in sheep. They reported that 68 (4.17%) of 1,630 serum samples from sheep had antibodies against L. hardjo and that all 68 sheep were maintained in direct contact with cattle. Four of the seropositive sheep were cultured and leptospires were isolated from 3 urine samples and from 3 kidney samples, but not from samples of the uterus or fallopian tubes. They concluded that sheep were renal carriers and not genital carriers of L. hardjo. Schonberg and co-workers from Germany and The Netherlands reported on the isolation of L. interrogans type hardjobovis from cattle on a large (3,500 milking cows) dairy farm in Germany. An increasing number of abortions occurred on this farm and 13 of 90 cattle tested had titers against leptospirosis. Urine was collected from 3 of the cows, and hardjobovis was recovered from 2. They attributed the increase in abortions to infection with Leptospira interrogans type hardjobovis. A paper was presented by Amaddeo and co-workers from Italy dealing with canine leptospirosis.
They reported that over a 15 year period the percent of serologically positive animals decreased from 33 to 11%. Because of vaccination, cases of leptospirosis due to *canicola* started to decrease in 1985, and by 1990 were no longer observed. However, infection by leptospirae in the Australis and icterohaemorrhagiae groups have progressively increased. Donahue and co-workers from the University of Kentucky reported on their microbiological findings from cases of leptospirosis in aborted horses. Over a 5 year period they diagnosed 121 cases of leptospirosis in equine fetuses. One or more tissues from 97% of the cases were positive using a direct fluorescent antibody test with conjugate supplied by NVSL, Ames, Iowa, and fetal fluids from 76% of the cases were positive against 1 or more leptospira serovars in the microscopic agglutination test. Most (over 90%) of the abortions were caused by infection with serovar *kennewicki* a member of the Pomona serogroup.

There were several presentations concerned with serologic surveys for leptospira. Two of these papers reported on leptospirosis in animals in Portugal. Rocha from the National Veterinary Laboratory in Portugal reported that 15.3% of 9,543 bovine sera, 20.2% of 3,195 swine sera, 3.3% of 5,298 sheep sera, 4.9% of 1,631 goat sera, and 43.4% of 83 horse sera were positive against 1 or more serovars of leptospira. He concluded that leptospira infection in farm animals is widespread in Portugal and that infection in horses needs to be studied in more detail. Collares-Pereira and co-workers from the Institute of Hygiene and Tropical Medicine, Lisbon, Portugal and Royal Tropical Institute, Amsterdam, The Netherlands also reported that there was a wide occurrence of leptospirosis in all livestock species in all geographic regions of Portugal. They also concluded that there is a wide distribution of endemic leptospira infections in dogs and wild mammals in Portugal. These same investigators also reported the first isolations of pathogenic leptospira from animals in the Azores Islands. They demonstrated that there is a wide distribution of leptospira of the Ballum and icterohaemorrhagiae serogroups in different small mammal populations in the Azores Islands.

Vidic and co-workers at the Veterinary Science Institute in Vojvodina, Yugoslavia presented data on a 2-year study during which they tested over 75,000 serum samples for antibodies against 9 serovars of leptospira using the microscopic agglutination test. Approximately 8% of the cattle sera, 7% of the pig sera, 6% of the horse sera, and 1% of the sheep sera were positive against 1 or more serovars of leptospira. The serovar most often positive in cattle was *hardjo*, in pigs *pomona*, and in horses *icterohaemorrhagiae*.

Two papers dealt with antibiotics used for the treatment of leptospirosis in animals. Gerritsen and co-workers from the DLO Institute for Animal Science and Health, The Netherlands, reported that cows experimentally or naturally infected with *L. hardjo* stopped shedding leptospirae in their urine after treatment with streptomycin or dihydrostreptomycin. Alt
and Bolin from Agricultural Research Service, NADC, Iowa, reported on their evaluation of antibiotics for the elimination of leptospirosis in hamsters, swine, and cattle. Antibiotics which demonstrated some efficacy include: oxytetracycline, tylosin, ampicillin, erythromycin, tiamulin, and dihydrostreptomycin/penicillin G in hamsters; oxytetracycline, tylosin, erythromycin, and dihydrostreptomycin/penicillin G in swine; and ceftiofur, oxytetracycline, and dihydrostreptomycin/penicillin G in cattle. Doses exceeding the maximum recommended dose were often required to be effective. Bolin and co-workers also reported on the variation of infections detected when cattle were exposed to l of 6 different strains of harc@6ovis. Three of the strains were from the United States and one each from Portugal, Northern Ireland, and New Zealand. Differences were detected in the immune response, tissue colonization patterns, lesions produced, and urine shedding in heifers inoculated with the different isolates of hardjobovis.

Results of 2 investigations on the use of PCR to detect leptospira DNA in urine were presented. Bal and co-workers from the Royal Tropical Institute reported that PCR was more sensitive than culturing for detecting leptospires in human urine. In contrast, Ellis and co-workers from the Veterinary Sciences Division, Belfast, United Kingdom and the Royal Tropical Institute reported that culturing was more sensitive than PCR for diagnosing the renal carrier state in cattle infected with serovar hardjo.

Four papers were concerned with other aspects of leptospires that might be of interest to workers in veterinary medicine. Bazovska and co-workers from the Department of Epidemiology, Bratislava, Slovak Republic reported that there were no cross-reactions between leptospira and some other bacterial antigens, especially borrelia. Lipcsey and co-workers from the National Institute of Hygiene, Budapest, Hungary reported that it was not possible to recover viable leptospira following freeze drying despite using very careful pre- and post-drying steps. Kauffmann and co-workers from the Center for Disease Control and Prevention, Atlanta, Georgia reported on a 10-year study of the DNA relatedness between serovars of the family Leptospiraceae. A total of 288 leptospire strains representing 255 serovars were studied. The strains were found to belong to 14 different species, including 2 newly proposed species. Their observation that neither serogroup nor serovar reliably predicts the species of a leptospire strain confirms the importance of strain designation in reporting research results. Korver and co-workers from the Royal Tropical Institute or Foundation for the Advancement of Public Health, The Netherlands reported on a way of standardizing the microscopic agglutination test by using lyophilized leptospires and monoclonal antisera. They have invited other laboratories to participate in proficiency testing of the MAT using reagents that they will supply free of charge.

Dr. Donahue also reported that only 4 cases of equine leptospirosis were diagnosed in central Kentucky during the 1994 foaling season.

Thanks were extended to the U.S. Department of Agriculture for their response to last year's resolution to provide services and reagents upon request.
REPORT OF THE COMMITTEE ON LIVESTOCK IDENTIFICATION

Acting Chairman / Vice Chairman: Dr. Donald R. Bridgewater, Northglenn, CO
Chairman: Mr. John F. Wortman, Jr., Albuquerque, NM

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The committee met Wednesday, November 2, 1994, at 1:30 PM in the Emerald Room, Amway Grand Hotel, Grand Rapids, Michigan.

There were 9 members and 27 guests present. There were ten guests that requested to become members of the committee. Five topics were addressed. The topics covered were:

1. Dr. James P. Davis, USDA, APHIS, VS, discussed the need for a “National Premises Identification System”

   He reported that animal identification has not significantly changed in the last 30 or so years. We still rely on eartags, backtags, tattoos, and brands to provide the means to trace diseased livestock to their premises of origin. As we reach the final stages of brucellosis, pseudorabies, and tuberculosis eradication, we cannot afford to become complacent in our efforts to trace the final vestiges of disease. Improved accountability is needed if we are going to indeed find the last pseudorabies, tuberculosis or brucellosis infected herd. We may need to consider identification changes that enhance the development of a national premises identification system. This system will provide a means to rapidly trace animals to their premises of origin.

   Before we change identification systems we need to be sure that improvements in livestock identification are indeed needed. Any change will have wide spread ramifications to industry such as cost, record keeping and possibly new regulations. As present the Pathogen Reduction Act has been introduced in both the House and Senate. This bill will require the ID of all livestock at slaughter and records kept to identify the animal back to the premises or origin.

   Today when we talk identification systems we are talking about a sys-
tem that will meet APHIS needs for disease control, FSIS needs for all livestock ID'ed for food safety, the packers need for payments based on grade/yield, the producers need for a value added product, and international needs for import-export animal identification.

A national premises identification system in the future must meet the needs of animal ID for disease control, quality assurance programs, and food safety. This system will utilize new technologies such as, electronic implants, bar-coded eartags, GIS tracking systems and personalized information on plastic tags identifying the premises of origin.

This premises identification would be driven by state assigned premises identification codes conforming to a standard format.

2. Dr. Joe Annelli, USDA, APHIS, VS, discussed a "Strategic Plan to Implement Swine Producer Applied Premises Identification System".

He reported that the system, which can be used on the farm, in the markets, in the slaughter house, and conceivably to the final product, is being explored at the present time. New technologies, such as bar-coding, which will allow electronic data transfer of premises identification at each critical point in the pork chain will be an important part of the new system. He also addressed the Immediate, Short-Term, and Long-Term Goals necessary to implement the system. A copy of the report is included in the proceedings.

3. Mr. Phil Dukas, Chief Executive Officer, National Dairy Herd Improvement Program (DHIA) discussed "Perspectives on Dairy Cattle Identification".

He provided background information addressing DHIA's database which contains the identification and management records on approximately 50% of the nation's dairy cows. He reported on the identification methods and national database pointing out that although the current system appears to be meeting most demands, an evolving dairy industry may develop new requirements for an integrated identification system. A copy of the report is included in the proceedings.

4. Dr. Phil Pickerill, USDA, APHIS, VS, discussed "Trends In Feedlot Identification", prepared by Dr. Dick Ferris and Mr. Tim Dean, both with USDA, APHIS, VS.

He reported that the Identification of cattle in feedlots has always been important to allow accurate record keeping of performance of cattle on feed. For a variety of reasons, the trend in feedlot identification is toward an identification device that will enable the feedlot to determine more about the origin of their cattle.

Issues as drug residue, hide branding, and injection site blemishes continue to concern the industry. As these and other issues continue, and new
REPORT OF THE COMMITTEE

concerns are added, the need to for accurate and complete records is heightened. Whether tracking the origin of cattle on feed with excellent performance records, finding the source of an excessive number of "poor doers", or locating the origin of diseased cattle condemned at slaughter, identification is the key.

As an increasing number of feedlots have gone to computerized record keeping, the ability to handle more specific information has increased. It therefore becomes less burdensome to record not only the owner of a consignment of cattle, but allows the utilization of identification devices that provided the link to more detailed information. This may include recording previously applied identification such as firebrands, freeze brands, backtags and bangle tags, as well as specifics on owner, shipper, point of origin, shipping weight, death losses, treatment schedules, breed and sex.

Disease conditions, drugs residues, bacterial contamination, and a host of other issues will continue to provide reasons to ensure development of the most complete and detailed method of identifying feedlot cattle.

5. Mr. Dan Montanari, MIS Director, Origen, Electronic Tracking Systems, discussed “Utilization of Bar Codes to Correlate Live Animal to Carcass for Quality Assurance and Consumer Protection”

He reported Origen™ is a Patent pending method for tracking the production history of a food product. This process was originally developed for use by Coleman Natural Meats, Inc. to verify the production claims for its "natural" beef products. It has since been expanded to track and identify food products from "Farm to Table".

With the aid of a bar-code ear clip attached to an animal at birth, the identity, or origin of an animal can be established. By referencing this number, information can be collected and used for various purposes. These purposes include, but are not limited to: 1. Monitoring production efficiency 2. Maintaining veterinary and health records 3. Recording breed and genetic information 4. Tracking pathogens 5. Identifying ownership from birth to slaughter 6. Serialized inventory control of both animals and meat products 7. Identification of production methods 8. Traceback and/or recall of meat products.

Origen™ is also designed to be device independent. In other words, it is not dependent on a single type of identification device. Although it currently uses bar coded ear clips, it is designed to work with radio frequency ear tags, data buttons or implanted transponders. Origen™ is also designed to maximize tracking at any point in the production cycle. It can track and identify an animal from the ranch to slaughter, and can even maintain animal identification through processing of individual meat cuts. The same number originally assigned an animal on its ear tag is the same number that appears on each primal cut produced from that animal. It is this ability to identify an animal at both the pre-harvest and post-harvest stages that
LIVESTOCK IDENTIFICATION

makes Origen™ unique.

Open Discussion: Current Issues in Animal Identification

During this open session, Dr. Ralph Knowles, veterinary consultant to Destron-Fearing, discussed the “ParlorScan™ Wireless Electronic ID System”.

He gave the following report: A cornerstone for USDA’S Food Safety and Disease Control and Eradication Programs is animal identification. Present animal identification methods are not adequate to serve these needs. I believe that electronic identification of livestock is the most practical identification method for food safety and disease control and eradication purposes.

Remote Ag Data has developed Parlor Scan™ for use in dairy cattle. The parlor Scan system is used in milking parlors to positively and uniquely identify cows and link their identity to their milk production and other information. Cows wear MicroBand™ electronic leg bands which contain miniature, passive (no battery) radio frequency transponders. Each transponder contains a unique identification number. The MicroBand is made of a 1/2 inch wide strap with the transponder in a small capsule less than an inch square.

Cows are "scanned" using a hand-held wand attached to a small transceiver clipped to the milker's belt. Another transceiver is plugged into the desk-top computer (back in the office) and acts as the second half of the wireless communication link to the parlor.

The desk-top computer holds the database (cows and related information) and houses software that error-checks and links identification to the proper record in the herd. This computer is also tied to the milk meters in the parlor, and sends cow numbers to each meter as cows are scanned in the parlor. As cows finish milking, their metered milk weights and identification are stored in the desk-top database.

COWHAND™ Puts Your Entire Herd in the Palm of Your Hand

Now you can "load" your entire herd into a checkbook sized computer and take the information with you wherever you go. Look up any cow and view information on her reproduction, health, production, and history. And you can record events at cowside, too. Just use function keys located under the screen to quickly record any event and comment.

When you get back to the office, just connect the palmtop computer to your desk-top computer and update your herd management system automatically - no more writing it down now and keying it in later.

Comments:

The issue of a common standard for livestock identification led to a
very active discussion. The major points were: 1. Industry needs to take the lead and work together on the development of common standards. 2. The producers need to become more involved in this decision making process. 3. Additionally, a review of the present livestock identification systems needs to be conducted.

There being no further business to come before the Committee, the meeting was adjourned at 5:30 P.M.
USDA, APHIS, Veterinary Services is committed to participate with pork producers, livestock marketing groups, packing plants, and regulatory agencies in developing an effective, efficient, affordable, and dependable system of identifying swine. To demonstrate this commitment, a new position of National Swine Identification Coordinator has been established on the Swine Health Staff. A system of producer applied premises identification which can be used on the farm, in the markets, in the slaughter house, and conceivably to the final product is being explored at the present time. New technologies, such as bar-coding, which will allow electronic data transfer of premises identification at each critical point in the pork chain will be an important part of the new system. Pilot projects and feasibility studies are now underway.

**Overall Vision**

**STEP 1. Premises Identification**
All swine premises are given an identification number which is uniformly acceptable and linked to a geographic location.

**STEP 2. Producer Applied Identification**
The premises identification number is incorporated into the identification system in current use in the production unit or applied separately to animals leaving the unit when no form of identification is used.
The likelihood of mis-identification of animals is reduced when the animals are identified before leaving the farm.
There are several methods of identification which may be used now and others which have potential for future use: Bar-coded backtags; bar-code imprinted eartags; bar-coded labels applied to ear tags (purchased from vendors or printed on the premises); slap tattoos for swine not skinned at slaughter; and electronic implants.

**STEP 3. Identification Recorded critical control points**
As efficient methods of scanning animals are developed and implemented, animal identification numbers can be collected to allow accurate sampling at critical control points within the production unit as well as those on the outside such as livestock
SWINE IDENTIFICATION: IMMEDIATE, SHORT-TERM, AND LONG-TERM GOALS

hauling points, livestock markets, and slaughter plants.

STEP 4. Sample Data Collection
Rapid and accurate collection of data will allow scientifically based sampling for many purposes: Animal disease surveillance; zoonotic disease surveillance; residue and contamination monitoring; carcass quality improvement; genetic evaluation; and identification of beneficial swine rearing practices.

STEP 5. Data Transfer
Samples can be accurately and rapidly identified; computer data files can accompany the samples to the testing laboratory; and laboratory results can be electronically transmitted to appropriate locations.

Plan of Action (This Year)

1. Promote the wide use of on-farm identification systems. The basis for an accurate farm record system; allows development of productive decision-making strategies; and provides information to improve productivity and profitability.

2. Assemble an active Swine Identification Working Group ready, willing, and able to devote time, talents, and resources in aggressively establishing a swine identification system. Composed of individuals representing: Producers; swine breeding companies; livestock markets; livestock dealers; livestock haulers; slaughter establishments; state and federal agencies (swine disease control, market development / protection, food safety); and university swine research.

3. Continue to vigorously encourage correct application of swine backtags in markets while promoting the importance of quality traceback in all surveillance programs.

Short Term Goals (1-2 Years)

1. Begin to establish uniformly acceptable premises identification numbers for all swine production units.

2. Begin pilot projects to test the feasibility and reliability of various methods of producer-applied premises identification. Projects now under way in Indiana, North Carolina, and Missouri. The projects will evaluate relative merits of different devices in terms of: Retention rates; longevity; readability; price; and labor requirements.

3. Begin implementing the use of bar-coded backtags for slaughter surveillance.

4. Begin installing bar-coded readers as available in pseudorabies testing laboratories to facilitate information gathering and reporting.

5. Continue to be involved in monitoring the latest developments in electronic identification and coordinating activities related to future implementation.
6. Improve the reporting of pseudorabies surveillance results.
7. Participate in a national swine identification conference.
8. By October 1, 1995, the newly formed Swine Identification Working Group will:
   A. Complete analysis of:
      (1) The identification requirements and responsibilities of each stakeholder.
      (2) The proportion of the total benefit each stakeholder will enjoy in an integrated swine identification system.
   B. Accept a proposal to define an integrated identification system based on the needs analysis of the Working Group.
9. By October 1, 1996, an integrated swine identification system will be presented for consideration and acceptance.

Intermediate Goals (3-5 Years)
1. National Premises Identification system full implemented, i.e., all swine premises are identified and geographically located.
2. Begin implementation of the integrated swine identification system.

Long Term Goals (5+ Years)
1. Approach 100% identification traceability.
2. All critical control points equipped to collect animal identification information.
PERSPECTIVES ON DAIRY CATTLE IDENTIFICATION

Phillip Dukas
National Dairy Herd Improvement Association, Inc.
Columbus, Ohio

Overview
The U. S. DHIA system processes management records for 4.7 million dairy cattle monthly, located on over 50,000 farms throughout the United States. Accurate identification is essential to DHIA efforts, and to other off-farm uses of the records, such as by the artificial insemination industry, purebred associations, veterinarians, and consultants. Although the current system appears to be meeting most demands, an evolving dairy industry may develop new requirements for an integrated identification.

Background
DHI maintains a national distributed database containing the identification and management records on approximately 50 percent of the nation’s dairy cows. In addition, a significant number of young stock are identified and entered into the database at birth. These databases reside at the nine approved Dairy Records Processing Centers (DRPC’s) throughout the U.S.. Each month, approximately 2,000 DHIA technicians visit each farm on a monthly basis to collect milk weight, identification, and other management information. Although no regulatory efforts are involved, certain Official DHIA Rules are maintained.

DHIA allows four approved identification methods:
1. Uniform-Series Metal Eartag
   This nine-character (exS2VBG1234) metal eartag is used jointly by state veterinarian, DHIA Affiliates, and artificial insemination organizations for disease surveillance and herd management, as covered by a 1975 AHPIS-Veterinary Services memorandum. Responsibility for maintaining the unique series is covered by this cooperative agreement. All parties have been well-served under this arrangement for a number of years.

   Recently, one state regulatory agency restarted the unique-series after using two of the available three alpha characters. Since the first two digits are the state number, this practice dropped the available unique combinations to 6.7m (175m are available using all three characters). Although this may have been adequate capacity to traceback, it resulted in identification conflicts at DHIA and in genetic evaluations, posing a challenge to the cooperative use of this identification method.

   Representative from APHIS, the state veterinary service, and DHIA worked together to find a satisfactory solution, which involved use of the third alpha character. A short-term editing procedure was also implemented by DHIA to minimize the impact of the identification conflicts, but routine communication in the future will be needed to maintain the integrity of the cooperative identification system in the U.S..
2. Registration Number

In 1993, 440,000 animals were registered under programs operated by the seven dairy breed associations. Although many registered animals may carry a metal eartag, it is not required by the breed associations.

3. Verified Identification Number

The grade identification program of National DHIA also requires the unique-series metal eartag.

4. Electronic Identification (proposed)

National DHIA and the Holstein Association proposed criteria for the approval of electronic identification systems. These criteria appear in the 1990 Proceedings of the USAHA (page 46). The International Standards Organization (ISO) recently adopted a modified version of these approved criteria. However, full walk-through systems operating under the ISO standard are not yet commercially available. In addition, the commercial availability of an operating EID system does not necessarily guarantee development and maintenance of an integrated ID database.

The National Database

All DHIA Affiliates (previously referred to as state DHIA's) have signed a membership agreement that ensures the contribution of their member's performance data to the national database. In addition, the Quality Certification program of National DHIA requires the electronic transfer of cattle identification, using a standard format, with in two days after a request by another DHIA member purchasing the cattle. These steps represent progress in turning the decentralized database into a seamless network with appearance and benefit of a single database, yet allowing the marketplace benefits of an internally-competitive system.

This model could also be used as a basis for a broader national database across user groups, involving health agencies, DHIA, breeds, and AI. Options include:

1. An Identification Locator

This polling system would identify the location of detailed animal information, but would not actually contain the animal data. This development is underway at National DHIA, and could be integrated into a larger effort.

2. Identification Database based on Market Incentives

Previous attempts to fund an enhanced ID database, with or without electronic ID, have not been successful. However, changing market conditions, increased liability, and integrated marketing structures may have increased the demand for a unified identification database, driven by market incentives.

In addition, evolving database and communications technology have lowered impediments that may have inhibited development of an integrated database in the past.

3. Identification Database based on USDA-APHIS Requirements

A third option, requiring participation in a comprehensive database, may be possible. However, without adequate and clear market incentives, it may remain politically challenging to implement.
REPORT OF THE COMMITTEE ON NOMINATIONS AND RESOLUTIONS

Chairman: Dr. Thomas J. Hagerty, St. Paul, MN

Dr. J. Lee Alley, AL; Mr. Philip E. Bradshaw, IL; Dr. John P. Huntley, NY; Dr. Maxwell Lea, Jr., LA; Dr. J. C. Shook, PA; Dr. Patton L. Smith, CA; Dr. Max A. Van Buskirk, PA; Dr. Robert J. Velure, ND; Dr. Richard D. Willer, AZ.

PRESIDENT .................................................. H. W. Towers, Jr., Delaware
PRESIDENT-ELECT ................................................. M. R. Marshall, Utah
FIRST VICE-PRESIDENT .................................. L. L. Williams, Nebraska
SECOND VICE-PRESIDENT ..................... J. W. Bryan, South Carolina
THIRD VICE-PRESIDENT ......................... R. H. McCapes, California
TREASURER ................................................... J. C. Shook, Pennsylvania

Regional Delegates

Northeast ................................................. Robert Eckroade, Pennsylvania
........................................................................ V. P. LaBranche, Massachusetts
North Central .......................................................... D. D. Gingerich, Iowa
............................................................................ L. Lodoen, North Dakota
South .................................................................... W. C. Baisley, Georgia
................................................................................... M. C. Turner, Texas
West ...................................................................... O. H. Timm, California
......................................................................... R. H. McCapes, California

RESOLUTION NUMBER: 1
SOURCE: COMMITTEE ON ANIMAL WELFARE
SUBJECT MATTER: STATE REGULATION OF THE PET INDUSTRY

Prior to the 99th Annual Meeting, the U.S.A.H.A. shall develop a model state law for the fair and reasonable regulation of pet production, distribution, boarding, grooming, shelter, and pound facilities.

The model state law shall promote a common, uniform national standard for pet animal welfare and shall discourage conflicting state and local regulations.

RESOLUTION NUMBER: 2
SOURCE: COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUS
SUBJECT MATTER: CERTIFICATION OF CATTLE HERDS AS BOVINE LEUKOSIS VIRUS FREE

USDA, APHIS actively encourage State Animal Health Officials to implement the "Standards For Certification of Cattle Herds As Bovine Leukosis Virus Free" and provide national recognition for herds achieving BLV-free status.
RESOLUTION NUMBER: 3
SOURCE: COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY
COMMITTEE ON PROFESSIONAL OVERSIGHT
COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE
COMMITTEE ON FOOD SAFETY
SUBJECT MATTER: FOOD SAFETY

USAHA resolves that USDA, Food Safety, and USAHA work together in establishing a working group of state and federal animal health officials, state and federal food safety officials, livestock and poultry producers, research workers, and other related groups to address the issues of pre-harvest and post-harvest food safety and other related food safety issues.

RESOLUTION NUMBER: 4
SOURCE: COMMITTEE ON FOOD SAFETY
SUBJECT MATTER: NATIONAL VETERINARY EXTENSION PROGRAM DIRECTOR

USAHA strongly urges the U.S. Department of Agriculture to fill the critical position of National Veterinary Extension Program Director with a qualified veterinarian as soon as possible.

RESOLUTION NUMBER: 5
SOURCE: COMMITTEE ON SALMONELLA
SUBJECT MATTER: SALMONELLA DIAGNOSTICS

USAHA should develop a symposium on Salmonella Diagnostics to be held in conjunction with the 1995 USAHA/AAVLD Meeting.

RESOLUTION NUMBER: 6
SOURCE: COMMITTEE ON RABIES
SUBJECT MATTER: SUPPORT COOPERATIVE RABIES CONTROL PROGRAMS

The United States Animal Health Association should encourage the cooperation between state, provincial, and national governments along the United States-Canadian border in rabies control programs.

RESOLUTION NUMBER: 7
SOURCE: BRUCELLOSIS COMMITTEE AND WILDLIFE DISEASES COMMITTEE
SUBJECT MATTER: BRUCELLOSIS

The United States Animal Health Association (USAHA) petitions USDA,APHIS, VS, to expedite the evaluation of alternative procedures for raising the level of immunity to bovine brucellosis in cattle, bison, and elk while at
REPORT OF THE COMMITTEE

the same time being able to distinguish vaccine-related problems.

RESOLUTION NUMBER: 8
SOURCE: BRUCELLOSIS COMMITTEE
SUBJECT MATTER: BRUCELLOSIS

Interstate movement, quarantine, and quarantine releasing requirements should be established for all domestic ruminants, zoological species or other species from populations known to be infected and believed capable of transmitting *Brucella abortus* to livestock.

RESOLUTION NUMBER: 9
SOURCE: BRUCELLOSIS COMMITTEE
SUBJECT MATTER: BRUCELLOSIS

The USAHA is unequivocally opposed to the exportation of brucellosis exposed bison from the Greater Yellowstone Area (GYA) or any other place or facility, either private or governmental, except under strict guidelines. Under such guidelines animals may be moved or exported, in cooperation with APHIS, to a research facility; or to a quarantine facility that is located in or adjacent to the GYA and that is under state quarantine.

The research guidelines would require strict controls by the USDA approved research agency, including, but not limited to the following:

1. Would not allow the research animals to be grazed under range conditions.
2. Would require strict accountability of all animals.
3. Would be a terminal use of the animals involved in the research project.

The quarantine facility guidelines are:

1. Strict state quarantine procedures and accountability of all animals, as developed by the state animal health agency in cooperation with APHIS.
2. No animal may be released from such quarantine facility, except for movement directly to slaughter, until such animals have unequivocally been determined to be free of brucellosis.

RESOLUTION NUMBER: 10
SOURCE: BRUCELLOSIS COMMITTEE
SUBJECT MATTER: BRUCELLOSIS

APHIS should examine the authorities and procedures for a special classification for brucellosis affected national wildlife parks and refugees that would place responsibilities on government land and wildlife managers to prevent transmission of brucellosis from their herds and to participate in the national effort to control and eliminate brucellosis.

If such authorities exist, APHIS work with affected wildlife and land management agencies and the Greater Yellowstone Interagency Brucellosis
Committee, to develop a draft proposal for establishing a classification system for government lands harboring brucellosis infection for review by the Committee on Brucellosis in 1995.

RESOLUTION NUMBER: 11
SOURCE: BRUCELLOSIS COMMITTEE
SUBJECT MATTER: BRUCELLOSIS

USAHA should request USDA, APHIS, VS, to take the necessary steps to assure that all test eligible cattle are subjected to a brucellosis test in the U.S. within 30 days prior to being exported to Mexico for slaughter.

RESOLUTION NUMBER: 12
SOURCE: COMMITTEE ON PSEUDORABIES
SUBJECT MATTER: PSEUDORABIES

Revised Goals, PRV Eradication
1994—All states Stage II or higher
1995—23 states Stage IV or higher
1996—32 states Stage IV or higher
   42 states and major portions of 5 split-status states in Stage III or higher
1997—40 states in Stage IV or higher
   All other states in Stage III
1998—43 states Stage IV or higher
1999—49 states Stage IV or higher
2000—Iowa to Stage IV, all 50 states free of the disease

RESOLUTION NUMBER: 13
SOURCE: FOREIGN ANIMAL DISEASES COMMITTEE
SUBJECT MATTER: SCREWWORM PRODUCTION FACILITY RESOLUTION

USAHA requests that USDA urgently identify the resources necessary to assure the building of a new sterile screwworm production facility in Panama.

RESOLUTION NUMBER: 14
SOURCE: IMPORT/EXPORT COMMITTEE
SUBJECT MATTER: MYCOPLASMA, UREAPLASMA, AND HEMOPHILIS SOMNUS

USDA, APHIS, VS, should include in the Pattern Protocol for the Importation of Bovine Semen a statement that imported semen be processed with an antibiotics and extender combination that has been scientifically proven efficacious to control the dissemination of Mycoplasma, Ureaplasma, and Hemophilus somnus, and other bovine venereal diseases - OR - that semen to be exported to the United States be cultured in the country of origin in the laboratory designated by the national government of the
exporting country and shown to be negative for Mycoplasma, Ureaplasma, and Hemophilus somnus, and other bovine venereal diseases.

RESOLUTION NUMBER: 15
SOURCE: SHEEP AND GOAT COMMITTEE AND IMPORT/EXPORT COMMITTEE
SUBJECT MATTER: SCRAPIE AND EMBRYO TRANSFER

The USAHA urges the USDA to make available the funds necessary in support of the collaborative research project between Utah State University and the neuropathogenesis unit in Edinburgh, Scotland with the objective of resolving the discrepancies in the results of both groups.

RESOLUTION NUMBER: 16
SOURCE: COMMITTEE ON INFECTIOUS DISEASE OF CATTLE, BISON AND LLAMAS
SUBJECT MATTER: MODEL REGULATIONS FOR INTERSTATE TRANSPORTATION OF LLAMAS

USAHA endorses the model regulation and recommends that it be distributed to the state veterinarians of the United States for their consideration.

RESOLUTION NUMBER: 17
SOURCE: SHEEP AND GOAT COMMITTEE
SUBJECT MATTER: SCRAPIE

USAHA opposes FDA's proposed rule docket number 93N-0467 and encourages FDA's continued cooperation in monitoring surveillance programs that are now in place.

RESOLUTION NUMBER: 18
SOURCE: JOHNES DISEASE COMMITTEE
SUBJECT MATTER: INCREASED FUNDING FOR RESEARCH ON PARATUBERCULOSIS

USAHA; 1) request USDA increase funding for studies on the molecular epidemiology of paratuberculosis in the Animal Health Program of the National Competitive Grants Research Initiative, 2) request USDA include for funding consideration by the Food Safety Program of the National Competitive Grants Research Initiative, studies on M. paratuberculosis, and 3) request USDA increase funding to USDNARS for studies on the ability of M. paratuberculosis to survive food processing procedures such as pasteurization.

RESOLUTION NUMBER: 19
SOURCE: JOHNES DISEASE COMMITTEE
SUBJECT MATTER: ACCREDITATION OF LABORATORIES FOR PARATUBERCULOSIS TESTING
USAHA requests USDA/APHIS/VS/NVSL to assume responsibility for accrediting laboratories to run diagnostic tests for the National Paratuberculosis Certification Program, and to run a check test for those laboratories on an annual basis.

RESOLUTION NUMBER: 20
SOURCE: JOHNES DISEASE COMMITTEE
SUBJECT MATTER: NATIONAL PARATUBERCULOSIS CERTIFICATION PROGRAM

USAHA encourages all member organizations to actively promote participation in the National Paratuberculosis Certification Program and to work to educate veterinarians and livestock producers about paratuberculosis biosecurity concepts.

RESOLUTION NUMBER: 21
SOURCE: COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE
SUBJECT MATTER: RECYCLING FOOD WASTE

USAHA urges USDA to 1) continue to examine the risks and benefits of recycled commodity feeding with regard to foreign animal diseases and public health diseases including organizing a national symposium to explore the future of expanded recycled commodity feeding and its impact on swine health; 2) increase funding available for Swine Health Protection regulatory activities; 3) increase funding for research for alternative methods of recycling food waste.

RESOLUTION NUMBER: 22
SOURCE: TUBERCULOSIS COMMITTEE
SUBJECT MATTER: DESIGNATED TUBERCULOSIS EPIDEMIOLOGIST/CHANGE UM&R

USAHA request that USDA modify the Uniform Methods and Rules for Tuberculosis (UM & R) to provide the following definitions and prescribe the responsibility and authority of the designated tuberculosis epidemiologist.

Definitions:
Designated Tuberculosis Epidemiologist - an epidemiologist who has demonstrated the knowledge and ability to perform the functions required under the standards of the Bovine Tuberculosis Eradication Uniform Methods and Rules. The Designated Tuberculosis Epidemiologist must be selected jointly by the cooperating State animal health official, the Area Veterinarian in Charge (AVIC), and the Regional Tuberculosis Epidemiologist.
Individual Herd Plan - a written disease management plan that is designed by the herd owner, the owner’s veterinarian if requested, and a State
or Federal veterinarian, and approved by the cooperating State Animal Health official and AVIC to eradicate tuberculosis from an affected herd. The herd plan will prescribe appropriate herd test frequencies, tests to be employed, and any additional disease or herd management practices deemed necessary to eradicate tuberculosis from the herd in an efficient and effective manner.

Responsibility and Authority of the Designated Tuberculosis Epidemiologist - The Designated Tuberculosis Epidemiologist has responsibility to determine the scope of epidemiological investigations, assist in development of individual herd plans, and to coordinate disease surveillance and eradication programs within their geographic responsibility. The Designated Tuberculosis Epidemiologist has authority to make independent decisions concerning the use and interpretation of diagnostic tests and management of affected herds when those actions are supported by sound disease eradication principles.

RESOLUTION NUMBER: 23
SOURCE: TUBERCULOSIS COMMITTEE
SUBJECT MATTER: BORDER STATES CONSENSUS DOCUMENT RE: MEXICAN CATTLE

United States Animal Health Association urges USDA to adopt recommendations included in the Border State's Consensus Document, which phases in specific requirements that must be met by each individual Mexican state within prescribed time periods, for cattle to continue to be exported into the United States.

RESOLUTION NUMBER: 24
SOURCE: TUBERCULOSIS COMMITTEE
SUBJECT MATTER: IDEXX GAMMA INTERFERON TEST

USAHA recommends to USDA, APHIS, VS that the INF be used in parallel with the caudal fold test (CFT) in known infected herds at the 1.25 to 1.8 level. The Designated TB Epidemiologist will classify responding animals as negative, suspect, or reactors. Animals responding over 1.8 will be classified as suspect or reactor. Suspects will be branded, and either moved to high risk herds as defined in an approved herd plan or to slaughter. Animals classified as suspects or reactors, and moved to slaughter according to Uniform Methods & Rules guidelines will qualify for indemnity.

USAHA recommends to USDA, APHIS, VS that the INF be used as an experimental presumptive test and be approved on a temporary basis (2 years) in a status that will allow both federal and state governments to use it and provide indemnity funds, as they do for intradermal tuberculin tests. The parameters of this use in an experimental presumptive basis will be as follows:
1. That the INF test is used as a stand alone test in herds of unknown status and that it be used at the 1.25 level. Any responding animals will be classified as suspects. An official presumptive supplemental test (comparative cervical test or CFT) will be applied to those responding animals.

2. That those entities interested in seeking experimental status for the INF work in concert with USDA APHIS Veterinary Services and Agriculture Research Services; and the Scientific Advisory Subcommittee of the Committee on Tuberculosis to help develop other appropriate parameters.

RESOLUTION NUMBER: 25
SOURCE: HEMOPARASITIC DISEASES COMMITTEE
SUBJECT MATTER: COMMITTEE ON DIAGNOSTIC TEST

USAHA recommends that USDA expedite development and approval of a new diagnostic test for anaplasmosis.

RESOLUTION NUMBER: 26
SOURCE: AQUACULTURE COMMITTEE
SUBJECT MATTER: IMPLEMENTATION OF A NATIONWIDE COMPREHENSIVE AQUATIC ANIMAL HEALTH PROGRAM

Therefore, it is imperative that in order to protect the economic viability of the more than $1.5 billion U.S. aquaculture industry, APHIS act immediately to ensure that the U.S. aquaculture industry is not irreparably harmed by failure of the USDA to implement a nationwide comprehensive aquatic animal health program that satisfies the requirements of important U.S. trading partners.

Made and seconded. Passed unanimously.

RESOLUTION NUMBER: 27
SOURCE: AQUACULTURE COMMITTEE
SUBJECT MATTER: FUNDING FOR PIVOTAL STUDIES FOR APPROVAL OF AQUACULTURE DRUGS

USAHA supports Congressional appropriation and allocation of funds for pivotal studies required to obtain FDA approval of critical aquaculture drugs, and urges the United States Department of Agriculture and/or the United States Fish and Wildlife Service to establish procedures to ensure maximum effective use of the monies to be used in the approval of aquaculture therapeutic and disease control compounds.
REPORT OF THE COMMITTEE ON PARASITIC DISEASES AND PARASITICIDES

Chairman: Dr. W. E. Pace, Tallahassee, FL
Vice Chairman: Dr. B. H. Bokma, San Juan PR

L.H.Biehl, IL; R.O.Drummond, TX; T.Galvin, TX; F.Gvillo, CA; T.J.Holt, NY; J.A.Jarvinen, IA; R.D.Jones, SD; S.E.Kunz, TX; L.F.Moore, KS; J.E.Novy, APO; R.E.Omohundro, TX; P.A.Pickerill, TX; R.L.Pyles, NM; J.L.Schlater, IA; M.G.Scroogs, TX; J.E.Strickler, GA; B.Terhaar, IA

This committee met at 1:30 P.M. on Monday, October 31, 1994, in the Heritage Hill Room, Amway Grand Plaza Hotel, Grand Rapids, Michigan.

The meeting was called to order by Dr. Pace, Chairman. There were 17 attendees, 11 of which were members.

Dr. Sidney E. Kunz, USDA, ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory, reviewed tick research being conducted by ARS. Coumaphos breakdown was slowed and long-term efficacy was enhanced by the acidification of dipping vats. Breakdown and disposal of spent pesticide remain issues of concern.

Ivermectin on corn demonstrates excellent control of ticks on deer and elk and blood levels of 30 ppb were maintained with daily feeding. By the third year, complete control of Boophilus annulatus was achieved on deer. Elk have remained free for 2 ½ years.

With ivermectin in biodegradable microspheres, subcutaneous injection can provide effective control in cattle for up to 6 weeks. This may become a price competitive control method if approved by FDA and EPA. On heavily infested pastures, cattle became free of ticks and had markedly improved performance over controls. Ivermectin reduced tick engorgement and the hatchability of eggs and prolonged the attachment of female ticks. These two techniques show considerable promise for the management of tick infestations and also certain worm infections in llamas.

Another issue discussed was horn fly resistance to permethrin and the need to use organophosphate pesticides in a rotational fashion. Some 36 pesticides are being phased out of use, 9 of which are commonly used for livestock parasite control. Coumaphos is being continued but permethrin is scheduled for deregistration.

Dr. Bob H. Bokma, USDA APHIS VS, reviewed the status of the Puerto Rico Tick Program. He pointed out success in reducing infestations of Boophilus microplus in many areas, but 50 per cent herd infestation rates remain in others. Reinfections and treatment failures remain high and can be attributed to many illegal cattle movements. Implementation of technical recommendations is not seen as feasible due to increased funding needs. Different approaches being discussed with the Commonwealth and industry include augmented industry involvement and integrated pest management. A sheep farm and a riding stable were added to herds under
treatment for *Amblyomma variegatum* on St. Croix, U.S. Virgin Islands. No new ticks have been detected in the three herds found infested in 1993. These also remain under treatment.

Dr. Alex Thiermann, USDA, Marketing and Regulatory Programs, outlined action plans for a partnership approach to eradicate the tropical bont tick from the Caribbean. The Interamerican Institute for Cooperation on Agriculture will coordinate surveillance activities in the CARICOM counties during phase 1. Funding is from USDA, the Food and Agriculture Organization (FAO), and potentially from other sources. FAO will coordinate the treatment activities in the CARICOM counties, presumably using pour-on flumethrin. The USDA and France carry out similar activities in their territories. Dermatophilosis has inflicted many cattle losses on several islands.

The screwworm program has moved successfully into Nicaragua, considered the last major hurdle for eradication into Panama. The headquarters and plant are scheduled to move to Panama. Future activities would include the major Caribbean Islands of Jamaica, Hispaniola, Trinidad, and Cuba.

Dr. Katherine Kocan, Oklahoma State University, gave a very informative and thought-provoking paper on the future control of some ticks and four major tick-borne diseases of cattle, using a variety of different techniques in an integrated fashion. She outlined current knowledge on attacking ticks with plants, insects, nematodes, antibodies in animal blood, pasture management, and chemicals. She also described methods to get at the disease-causing tickborne hemoparasites via the use of antibodies in animal blood and with chemicals such as ivermectin and tetracycline.

Dr. Jack L. Schlater, USDA, APHIS, VS, NVSL, reported on NVSL findings of exotic ectoparasites. Screwworms were detected in a horse presented for importation at Miami. There were two findings of *Amblyomma variegatum* from sheep and a horse in St. Croix. *Hyalomma* ticks were collected from trophy hides imported from a former eastern block republic. There were 32 collections of exotic ticks from reptiles. These were from six states and include *Amblyomma, Aponema,* and *Ornithodoros* which are tick vectors for heartwater and African swine fever.

No resolutions were passed by the committee. After lively discussion, members agreed on the need for strengthening this committee's focus. There is a unique need for a committee with industry, commercial and governmental participation for the control and eradication of ecto- and endoparasites. These needs seem separate from the focus of other committees such as hemoparasitic diseases. Active participation by the Environmental Protection Agency for discussion of pesticide registration issues and by members from parasitology societies would enhance consideration of options for the control of parasites. The committee is alarmed at the reduction in pesticides available for parasite control. The Chairman stated that he will seek EPA participation in future meetings.

The committee adjourned at 5:00 P.M.
Failure to control ticks and tick-borne diseases is a major factor limiting livestock production. Worldwide economic losses from ticks and tick-borne diseases are estimated to be in the billions of dollars annually (Sonenshine 1991). Losses from the four major tick-borne hemoparasites affecting cattle, *Anaplasma marginale*, *Cowdria ruminantium*, *Theileria parva* and *Babesia* spp., constitute a significant portion of these tremendous economic losses (Bram 1975, Sonenshine 1991, Kocan 1994). Currently, anaplasmosis is the only major tick-borne disease of cattle enzootic in North America. Losses due to this hemoparasite of cattle are estimated at 300 million annually. *Babesia* spp. is present in Mexico and remains a constant threat to cattle production in Texas.

Patterns of tick-transmitted diseases are constantly changing (Kocan & Kocan, 1991). Population of rural areas results in displacement of wild animal populations on which ticks normally feed. Vertebrate hosts available for tick feeding then become replaced by domesticated livestock, pets and humans. The new trade agreements will result in freer movement of livestock and thus may allow for importation of ticks into the United States from other countries. Also of concern is the uncontrolled importation of wildlife, posing risk for introduction of hemoparasitic diseases. Texas, for example, now has large populations of exotic ruminants that have potential to serve as reservoirs for hemoparasites, some of which may be transmitted to cattle and, therefore, may impact cattle production in the future.

In recent years, new tick-transmitted parasites have been described and the distribution of others have changed markedly. *Ehrlichia* sp., formerly found only in dogs, has been described and is a human health problem in several areas of the United States. Lyme disease, *Borreliaburgdorferi*, is endemic and a serious threat to human health in many parts of the United States, no longer being limited to the northeastern Atlantic states. A newly-described *Babesia* has been recently described in humans (Thomford 1994). The distribution of anaplasmosis in cattle in the U.S. has changed markedly and the disease has been reported in most all states.

An integrated approach for control of ticks and tick-borne disease may be necessary due to shortcomings of individual methods (Jangejan 1990, Floyd et al. 1987, Tatchell 1988; Uilenberg 1990, Dipeolu 1991, Kocan 1994). Ticks develop resistance to acaricides, and parasites may change in viru-
lence depending on the vector or mode of transmission. Also, genetic recombination of parasites via sexual reproduction in ticks can lead to increased numbers of field strains. Climatic conditions influence fluctuations in tick and wild animal populations that serve as reservoir hosts. Chemotherapy may be indicated when enzootic stability is disrupted, but widespread use of drugs alone is often too expensive for routine control. Interruption of management strategies may lead to disease outbreaks, and some breeds of cattle have less tick resistance than others, reducing the opportunity for introduction of more productive breeds of cattle into areas where tick-borne diseases are endemic. Therefore, current control strategies for ticks and tick-borne diseases depend on individual circumstances that are influenced by financial resources, degree of acaricide resistance, development of immunity to tick infestation and/or availability of vaccines against tick-borne pathogens. Tick eradication may be feasible on islands such as those in the Caribbean because movement of ticks are more restricted. However, ticks can be transported among islands by attachment to birds (Camus & Barre 1992). Eradication of 1-host ticks is more feasible than 2- and 3-host species, and in fact was accomplished early in this century for the 1-host tick, Boophilus annulatus, in the southern United States of America. However, tick eradication does not appear to be likely in continental areas such as the United States, Europe and Africa (Uilenberg, 1979).

Although vaccines for tick-borne hemoparasites of cattle are being developed, the focus for control of these parasites remains treatment of tick vectors with acaricides (Young et al. 1988). Acaricides are expensive, require stringent application regimens and management strategies, and may contribute to environmental pollution. In addition, ticks, like other organisms, develop resistance to chemicals and cattle, especially calves, are susceptible to and suffer from their toxic effects. Finally, these chemicals may not actually result in reduction of tick populations because, in the absence of tick pheromones being deposited on cattle, ticks may fail to aggregate on acaricide-treated cattle (Norval et al. 1989). Even if successful vaccines are developed against hemoparasites, control of ticks is needed because, they, in themselves, may cause serious reductions in cattle production (Young et al. 1988).

Transmission of tick-borne hemoparasites is facilitated through a remarkably balanced relationship among the organisms, ticks and vertebrate hosts. In all known life cycles, initial infection of tick tissues occurs in midgut epithelial cells and transmission is effected as ticks feed after parasites have developed and multiplied in salivary glands. Multiplication in salivary glands seems precisely coordinated with the tick feeding cycle when infective stages develop and are transmitted during feeding. Hemoparasites can persist in ticks for long periods by remaining dormant while their hosts are inactive.

It is easy to overlook the importance of the vector, but development of
FOCUSING ON TICKS FOR CONTROL OF HEMOPARASITIC DISEASES

control strategies should focus on the combination of tick/pathogen/cattle interactions. Despite the great difference in size between tick and vertebrate hosts, ticks are an indispensable part of the developmental cycle of the organisms they transmit. Sexual reproduction of protozoan hemoparasites occurs in ticks and, thus, it is the invertebrate host phase where genetic recombination occurs that results in field strains that vary in their antigenic composition.

Clearly, a new generation of methodologies is needed for effective control of tick-borne diseases of cattle. Molecular technologies will most likely play a central role in development of anti-tick/anti-parasite vaccines. However, development of effective vaccines and other control strategies will depend, in part, on defining the molecular basis for tick-parasite interactions (Crampton et al. 1992). Control of ticks and hemoparasites will most likely require use of integrated methodologies aimed at both the hemoparasites and tick vectors.

Strategies for Control of Ticks and the Pathogenes They Transmit: Strategies for interrupting development and transmission of pathogens in ticks may focus on (1) mechanisms that affect tick feeding and biology, with consequent reduction in parasite populations being secondary, (2) mechanisms that affect the hemoparasite directly by reducing infection, development or transmission by ticks or (3) a combination of the two. These control strategies, including tick control, development of vaccines against ticks and hemoparasites, and the effect of chemotherapeutic agents on hemoparasites in ticks, will be briefly reviewed. A more comprehensive review is included in the Proceedings of the 9th International Hemoparasite Research Workers Conference (Kocan 1994).

Tick Control: Tick control has been a major means of limiting tick-borne diseases. Intensive tick control resulted in eradication of Texas cattle fever from the United States and East Coast fever from southern Africa and is still practiced intensively in many parts of Africa (Norval et al. 1992a). Current tick-control methods involve use of acaricides, biological control (including use of tick-killing plants, entomopathogenic nematodes and insects), habitat modification, and development of tick-resistant hosts. Tick resistant hosts have not been useful for large-scale tick control (Norval et al. 1992a; Samish & Glazer 1990, 1992). Indigenous breed of cattle in Africa have some resistance to ticks but the mechanism of innate resistance is not understood, and the use of more resistant cattle has not been commonly exploited in control programs (Norval et al. 1992a). Anti-tick vaccines are being developed and show promise for control in the future (Wikel 1988; Wikel et al. 1993).

Acaricides: Use of acaricides for tick control, as recently reviewed by Norval et al. (1992a), is most commonly accomplished by dipping animals in tanks or vats containing a solution of acaricide. Dipping is more effective than spraying in achieving satisfactory coverage of cattle with the pesti-
KOCAN

cide. Other means of applying acaricides that are less commonly used include spot or pour-on application, slow release acaricide boluses, and acaricide-impregnated ear-tags. Ivermectin, an avermectin derived from a soil fungus, has been shown to be effective for tick control in Africa (Schroder et al. 1985; Wilson 1993). This drug may also affect hemoparasites carried by the ticks by interrupting tick function even if the hemoparasites are not directly affected by the drug.

According to Norval et al. (1992a), development of resistance to acaricides by ticks is a major draw-back of their continual use. One-host Boophilus ticks develop resistance more rapidly than do other types of ticks presumably because of their shorter generation time and because of the continual exposure of 1-host ticks to pesticides. Continual use of acaricides interferes with development of endemic stability that can exist in native breeds of cattle when calves are exposed to naturally-transmitted tick-borne diseases. These native cattle populations are at special risk for disease outbreaks when acaricide treatment is interrupted for any reason (Norval et al. 1991a). In addition, acaricides are expensive to purchase and labor intensive to apply. Acaricide application may not reduce tick populations in a given area because treated cattle do not attract ticks; without tick attraction/aggregation pheromones to attract ticks to treated cattle, populations remain essentially unaffected.

**Pheromone-Mediated Tick Control:** Certain pheromones are important in tick mating and these compounds result in attraction of males to tick-susceptible hosts (Norval et al. 1991b; Norval et al. 1992a; Norval et al. 1992b; Sonenshine 1991; Sonenshine et al. 1992). An aggregation-attachment pheromone (AAP) emitted by *Amblyomma* males enhances aggregation and attachment of both unfed nymphs and adults (Norval et al. 1992b). The presence of pheromone on previously infested cattle allows unfed ticks to select cattle on which ticks have successfully fed (Norval et al. 1989). The AAP may be used in control strategies for *Amblyomma* ticks by incorporation of pheromone-like compounds into plastic tags infiltrated with acaricide. These tags slowly release pheromone, attracting ticks, acting as “tick decoys.” Attachment of the decoys to the tails of cattle provided effective control against *A. hebraeum* adults for up to two months (Norval et al. 1992b).

**Development of Vaccines Against Ticks and Tick-Borne Hemoparasites:** Development of vaccines for cattle against ticks or the hemoparasites which they transmit may be a feasible approach in control of both vector and hemoparasite because small amounts of vertebrate host immunoglobulins appear to cross the midgut epithelium of invertebrates and enter the hemolymph without breakdown. A complete review of passage of immunoglobulins from vertebrates to invertebrates can be found in Sauer et al. 1993.

Vertebrate hosts exposed to repeated tick infestations develop resis-
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tance that results in effects detrimental to ticks such as reduced engorge-
ment, delay in onset of oviposition and reduction in egg mass (Trager 1939
a & b; Garin & Grabarev 1972; Allen & Humphreys 1979; McGowan et al.
1980; Ackerman et al. 1980). An approach, currently under development,
for control of ticks involves interruption of their feeding by immunization of
vertebrate hosts with antigens found on arthropod tissues that are not ex-
posed to vertebrates during tick feeding (termed concealed antigens) (Wikel
1988 & 1993; Wikel et al. 1992; Willadsen & Kemp 1988; Dhadiall et al.
against “concealed” tick antigens appears to be a productive approach be-
cause these antigens would not be introduced into the vertebrate host dur-
ing feeding, thus avoiding cutaneous hypersensitivity reactions which have
been a problem when using salivary gland antigens as vaccines (Willadsen

Ticks have numerous cell membrane receptors and hormones that could
serve as targets for vaccines (for a complete review of this subject see
Sauer et al. 1993). Successful use of this approach is dependent on iden-
tification and characterization of internal receptors and cellular components
that are essential and unique to the tick and that would be accessible to and
affected by specific antibodies introduced when ticks feed on vertebrates.
Three major classes of membrane receptors include (1) gated ion chan-
nels, (2) agonist-stimulated kinases, and (3) receptors that interact with
GTP-binding (G) proteins. Targeting any of these receptors might interrupt
fluid secretion, inhibit turnover of plasma membranes or block receptor-
mediated endocytosis. Results might include interference with vitellogenin
incorporation of oocytes or uptake of the lysed blood meal by digestive
cells of the tick midgut (Sauer et al. 1993).

Vaccines Against Hemoparasites in Ticks: Development of vac-
cines against tick stages of cattle hemoparasites will depend, in part, on
whether the various developmental stages in ticks express unique anti-
gens. If so, antigens could be targeted for use in a recombinant vaccine
that might interrupt parasite development, reduce the challenge dose for
cattle, or even eliminate parasite transmission. Precedent for this approach
comes from research on malarial parasites on which stage specific anti-
gens have been described (Nussenzweig & Nessenzeig 1985; Carter et
al. 1988).

Stage-specific proteins have been demonstrated on Theileria, and in-
clude a 32 kDa antigen specific to T. annulata piroplasms (Kachani et al.
1992) and sporozoite-specific coat antigens of T. parva (Musoke et al.. 1982;
Dobbeelaere et al. 1985 a & b); Nene et al. 1992) and T. annulata (Miranpuri
1986; Williamson et al. 1989; Kachani et al. 1992). The sporozoite stage of
T. parva was neutralized with a monoclonal antibody directed against the
sporozoite-specific antigen, suggesting that it would be a good candidate
for development of a vaccine to prevent infection of bovine lymphocytes (Musoke et al. 1982; Dobabelaere et al. 1984). Furthermore, sporozoites of different stocks were neutralized with one monoclonal antibody against sporozoite surface protein (Dobbleaere et al. 1984; Musoke et al. 1984). A recombinant anti-sporozoite vaccine, currently being developed for *T. parva*, induces protection in cattle challenged with tick-transmitted sporozoites (Musoke et al. 1992). The effect of anti-sporozoite antibodies on the development in and transmission of *T. parva* by ticks has not been reported. Because sporozoite antigen is first expressed after 2-3 days of tick feeding as schizogony commences (Dobuelaere et al. 1985a) this vaccine may also interrupt development of sporozoites. This potential second vaccine target in ticks, as well as the primary one in cattle, might be exploited to reduce the sporozoite challenge dose while reducing the likelihood of overwhelming the bovine immune system.

Potential targets of *Babesia* spp. vaccines include tick stages, suggesting a need for the characterization of babesial gametes and sporozoites. However, the complexity of the *Babesia* life cycle in ticks and difficulty in producing highly infected ticks has slowed research progress on tick stages of the apicomplexan (Hodgson 1992; Hodgson et al. 1992). It is likely that tick stages of *Babesia* will have stage-specific antigens as has been shown for other hemoparasites. Although *Anaplasma* and *Cowdria* have specific developmental cycles in ticks, unique antigens, differing from those expressed by organisms within erythrocytes, have not been described for the tick stages of these organisms.

Chemotherapeutic Agents: Drugs currently play an important role in integrated control strategies for hemoparasites. Discovery of anti-parasitic drugs for use in cattle was facilitated by growth of hemoparasites in cell culture, allowing for in vitro drug screening. Oxytetracycline is effective against *Anaplasma*, *Cowdria*, and *Theileria*. When oxytetracycline is administered with a live sporozoite vaccine (infection-treatment method), cattle develop immunity and are protected from clinical reaction (Dolan 1981; McHardy 1984, 1989). However, parasite stocks vary in their sensitivity to drugs and eventually may develop drug resistance. A drug sensitivity test is routinely used to characterize *T. parva* stocks (Norval et al. 1992a). Parvaquone is also used for treatment of clinical theileriosis. Tetracyclines, administered via feed additives or salt blocks, are commonly used for control of anaplasmosis in the United States. Resistance of *Anaplasma* to tetracyclines has not been reported. Several compounds are effective against *Babesia* spp. including diamidine derivatives and imidocarb dipropionate (Young et al. 1988).

The use of drugs in the past has focused on elimination of hemoparasites in the vertebrate host, but drugs may also affect development and transmission of parasites in ticks when ingested with the bloodmeal. The effect of drugs on hemoparasites in tick vectors appears to be variable.
mission sometimes can be blocked but tick infection appears to continue. Ticks are exposed to drugs when they ingest them via the bloodmeal when feeding on drug-treated cattle. The amount of drug in ticks may vary with the drug level in cattle and the feeding activity of the tick.

**Summary:** Development in and transmission of hemoparasites by tick vectors are phenomena closely synchronized with the tick feeding cycle. In all known life cycles, initial infection of tick tissues occurs in midgut epithelial cells and transmission is effected as ticks feed after parasites have developed and multiplied in salivary glands. Four hemoparasites of cattle, *Anaplasma marginale*, *Cowdria ruminantium*, *Theileria parva*, and *Babesia* spp., are all dependent on ticks for biological transmission. Mechanical transfer of infected blood via fomites and mouthparts of biting arthropods is also a major means of transmission for *A. marginale* but not of the others. Potential control methods for hemoparasites that target parasites as they are developing in their respective tick host include tick control, vaccines (against ticks and parasites), and drugs (against ticks and parasites). Successful application of control strategies will be dependent upon thorough understanding of parasite developmental cycles, biology of the tick vectors and the immune response of cattle to ticks and to hemoparasites. The most effective control measures will be those that are targeted against both ticks and the hemoparasites they vector.

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FOCUSING ON TICKS FOR CONTROL OF HEMOPARASITIC DISEASES


The Committee met at 1:30 p.m. on Tuesday, November 1, 1994 in the Kendall Room at the Amway Grand Plaza Hotel in Grand Rapids, MI; 14 committee members and 8 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. Invited speakers included Dr. Stephen F. Sundlof, Director, Center of Veterinary Medicine, Dr. G. A. Mitchell, Director, Office of Surveillance and Compliance, Center for Veterinary Medicine and Dr. J. S. Gloyd, Associate Director for Scientific Activities, American Veterinary Medical Association.

Dr. Sundlof conducted a presentation entitled "A New Vision for CVM". His objectives and discussion centered around a shift in emphasis of being mission-oriented vs. enforcement/rule driven. The CVM is currently undergoing strategic planning training and development of a new mission statement. A proposed new mission statement would be: To ensure the safety of the food supply and provide the pharmaceutical needs of animals through approval and post-marketing monitoring of safe and effective animal drugs. Dr. Sundlof also cited CVM's vision statement as a base to establish a process to allow CVM approval of safe and effective new animal drugs for all species regardless of market/profit potential of the drug. He stated that the prevention of "bad drugs" from entry into the system is one objective, but that also those drugs which are safe and effective need to be allowed to enter the marketplace.

Further comments included a discussion of the economic health of the animal drug industry. It is a mature market; there are a few major new products on the R & D horizon. The avermectins and fluoroquinolones will be divided in the existing animal drug market. The forces at work suggest that 4-6 more animal health firms will be purchased by larger organizations within the next year. For CVM to keep pace with these changing events,
they plan to implement a program of (i) strategic planning (ii) performance monitoring and (iii) total quality management. In this new mode, Dr. Sundlof suggested that responsibilities of the New Animal Drug Evaluation group will do the following:

- transform from a rule driven to a mission driven organization
- find innovative ways to utilize science in decision making
- develop processes which are flexible and efficient
- encourage information sharing
- encourage decision making at all levels
- encourage experimentation
- encourage entrepreneurship
- seek opportunity
- accept failure as a necessary component of the learning/growing process
- become more customer oriented

In the Office of Surveillance and Compliance, Dr. Sundlof suggested the need to (i) minimize illegal activities, such as marketing of unapproved drugs, manufacturing the unapproved drugs, or excessive extra-label drug usage which serve as a disincentive to drug approval process, (ii) maximize use of information technology, (iii) take proactive measures to prevent unacceptable drug use practices before they become problems, (iv) educate constituents/stakeholders to minimize unwarranted written/verbal assaults, (v) develop coalitions with constituents and (vi) involve constituents in "ownership" of decision-making process.

Responsibilities of the Office of Science will include focus of research efforts at decreasing scientific uncertainties associated with decision making in the NADE. There will also be focus on identification of mechanisms of drug actions.

As a general principle, Dr. Sundlof feels that many of his objectives for the CVM may be enhanced by the veterinary profession overall holding itself responsible for the upgrading and maintenance of higher professional standards.

During 1994, Dr. Sundlof reported a significant improvement in new drug approvals. As compared to 1993, they are:

<table>
<thead>
<tr>
<th></th>
<th>FY 93</th>
<th>FY94</th>
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<tbody>
<tr>
<td>Total Number</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>New Chemical Entities</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>New Species</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Generic Applications</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

The CVM has an objective of shortening the approval process from 59 months to 24 months; Dr. Sundlof expects the sponsoring companies to improve submissions to enable the goal of "submit once, review once" to become commonplace. The CVM wishes to work with companies more at the onset of drug trials (i.e. planning and protocol development aspects) to
PHARMACEUTICALS

expedite the review process later. To encourage this type of activity, CVM will exercise a "refuse to file" policy. He expects the refuse to file decision to be made within 60 days if the submission is not of sufficient quality to merit a full review. To enhance timely reviews, CVM is (i) expediting methods in which data can be submitted in electronic formats, and (ii) implementing phase and component review to allow companies to submit data in packages rather than as a whole submission.

CVM is considering feedstuffs as a vehicle for the administration of prescription drugs. For some drugs, it is an issue of drug availability (i.e., fluoroquinolones would be easily formulated into a feed ingredient). Such an approach would be exclusively for therapeutic drugs; these would be formulated at appropriate levels in a given volume of feed to be fed over a limited period of time. There is considerable controversy from several segments of the agri-business community on this issue, and currently a compromise consensus has not been obtained. At this time, CVM has no plans for retroactive inclusion of currently approved feed-additive drugs into this program. Outside input from various segments of agri-business is welcomed and encouraged to attain a suitable solution to this issue.

CVM's interpretation of the recently passed legislation which legalizes extra-label use of drugs by veterinarians was discussed. This includes: (i) statutory recognition to set a safe level for drug residues, (ii) development of a residue method (if CVM determines a potential food safety problem, an analytical method must be developed), (iii) record keeping by practitioners, (iv) definitions of a VCPR (veterinary/client/patient/relationship), and (v) prohibition of extra-label use if the Secretary determines the use poses a risk to public health.

Hazard Analysis Critical Control Point (HACCP) was also discussed briefly. A Critical Control Point is a point, step, or procedure at which control can be applied and a food safety hazard can be prevented, eliminated, or reduced to acceptable levels. HACCP is the subject of a lot of discussion in producer organizations and among Federal Agencies. Activity at the Federal level is detailed below:

- HACCP for seafood---proposed final rule (FDA) FR 1/28
- HACCP for landfood--ANPR (FDA) FR 8/4
- HACCP for FSIS slaughter plants (FSIS) promised by the end of the year
- ACCP for live animals Pathogen Reduction Act (APHIS)
- HACCP for live animals (GAO) GAO report 9/28/94

Dr. G. A. Mitchell presented a discussion of CVM's proposed guidance of drug compounding and proposed guideline on product advertising and product promotion. These are available in toto below as appendices A & B.

Dr. Gloyd reported on the USP Practitioner Reporting Network. This is being offered and evaluated as an adjunct to the current CVM reporting
system for adverse or failure to perform types of drug complaints/incidents. A copy of each report will be processed by the USP and sent to the manufacturer/libeler and to the appropriate regulatory agency (i.e., FDA, USDA, EPA). This reporting system will augment, but not replace, the FDA Form 1932-A system this is currently in use. Case numbers assigned by the USP will be cross-referenced with FDA case numbers; FDA will cooperate and utilize the USP system as appropriate with each case. A copy of this proposed report form is attached to the Committee report as Appendix C.
## THE PROMOTION AND ADVERTISING OF ANIMAL DRUGS

G.A. Mitchell, D.V.M.
Director, Office of Surveillance and Compliance
Center for Veterinary Medicine/FDA
at USAHA Pharmaceutical Committee on November 1, 1994

### Category of Promotional Material

<table>
<thead>
<tr>
<th>Category of Promotional Material</th>
<th>Guidance for Review</th>
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<tbody>
<tr>
<td>Advertising</td>
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<tr>
<td>•</td>
<td>Must be submitted at time of initial publication. [21 CFR § 510.300(b)(3)]</td>
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<tr>
<td>•</td>
<td>Must include brief summary relating to side effects, contraindications and effectiveness. (Reminder advertisements are exempted.) [21 CFR § 202.1(e)]</td>
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<tr>
<td>Labeling</td>
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<tr>
<td>•</td>
<td>Must be submitted at time of initial dissemination. [21 CFR § 510.300(b)(3)]</td>
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<tr>
<td>•</td>
<td>Must include full product disclosure information, (i.e., copy of the approved package insert). [21 CFR § 201.105(d)(1)]</td>
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<tr>
<td>OTC Advertising</td>
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<td>•</td>
<td>FTC has regulatory authority.</td>
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<tr>
<td>•</td>
<td>May cause the product to become misbranded under Sections 502(n) or 502(f)(1) of the Act.</td>
</tr>
<tr>
<td>OTC Labeling</td>
<td></td>
</tr>
<tr>
<td>•</td>
<td>Must be submitted at time of initial dissemination. See 21 CFR § 202.1(l)(2) for examples of labeling.</td>
</tr>
</tbody>
</table>
Promotional activity during the pre-approval period.

It is a violation of 21 CFR § 511.1(b)(8)(iv) for the sponsor, or anyone acting on behalf of the sponsor, to disseminate in any forum, any audiovisual, textual, or graphic presentation prepared by, or on behalf of the sponsor which suggests that the drug is, or will be shown to be, safe and/or effective for any use in animals prior to NADA/ANADA approval.

While promotional activity prior to approval is expressly prohibited, the intent of the regulation is not to stifle the full exchange of scientific information. The following activities are recognized by CVM as non-promotional and are viewed as legitimate exchange of scientific information:

1. Promotional Activity

   The need for clarification of this issue, as it pertains to new animal drug products, arose during the debate between proponents and opponents of products derived by biotechnology. The following types of political activity are considered by CVM to be non-promotional:
   - Participation in formal public debate, e.g. legislative hearings, using the summarization and presentation of published scientific literature.
   - Release of information in response to political activity which is directed toward influencing the passage or enforcement of laws restricting or precluding the development or future marketing of a product. Such information should: be directed towards the issues in the debate, meet the usual standards of scientific rigor when the results of studies are presented, refrain from making any conclusions about safety or effectiveness, and be aimed at groups clearly targeted by the political activity. Further, any release of information needs to be appropriate to the scope of the debate, and generally occur during the time frame that the issue is active.

2. Scientific Forums

   The FDA's policy regarding industry involvement in, and financial support of, scientific forums is under internal review at this time. Any final policy statement that is published will apply to CVM, and will affect organizers of educational meetings for the veterinary profession.

3. Response to Unsolicited Requests for Information Beyond Published Literature

   In response to unsolicited requests, a sponsor may release information beyond published scientific literature. The response should be objective, balanced, and appropriate to the incoming query. It should meet usual standards of scientific rigor when results of studies are released. The information provided should be accompanied by a cover letter stating clearly that the product is under investigation to determine whether it may be safe and effective for its proposed use and that the product has not been approved by the FDA.
4. Contacts With the Media

Responses to questions independently proposed by news media will generally not be considered promotional activity if the responses are limited to summarizations of findings published in scientific literature.

Use of Superlative Terms to Describe an Drug Product

21 CFR § 202.1(e)(6) states that an advertisement for a prescription drug is false, lacking in fair balance, or otherwise misleading if it contains a representation or suggestion that a drug is better, more effective, useful in a broader range of conditions or patients (man or animal), safer, has fewer, or less serious side effects or contraindications than has been demonstrated by substantial evidence or substantial clinical experience. Such as advertisement is misbranding within the meaning of section 502(n) of the Act.

CVM will examine the use of superlative terms such as "best", "exceptional", "maximum", and "unsurpassed" in advertising and OTC labeling. The terms "broad spectrum" and "less resistance" require substantiation. CVM suggests limiting the use of the term "broad spectrum antimicrobial" in labeling and promotional activities to drug products demonstrating in vitro and in vivo activity against multiple species of both gram positive and gram negative bacteria. Substantial scientific evidence and/or substantial clinical experience will be requested to justify such statements.

Promotion of Products for Unapproved Uses

Only the indications for which an NADA/ANADA is approved may be promoted, even if clinical efficacy in other areas has been suggested by use in the field or reports in scientific literature. A supplement to the NADA/ANADA must be submitted, and approved, before new indications may be promoted. This applies to both and OTC products.

Improper or Unsubstantiated Product Comparisons

When used in promotional material which compares products, claims that a given product has fewer problems with residues in edible tissues than competing products are inappropriate. The new animal drug approval process is designed to ensure that all approved drugs are safe and effective when used according to label directions. Therefore, drug residue comparisons are meaningless.

Claims of Derived Economic Benefits From Therapeutic Drugs

CVM recognizes that the basis for use of therapeutic agents in food producing animals is related to the potential to mitigate economic losses due to clinical and/or subclinical disease processes. However, promotional material for therapeutic drugs may not be worded to place primary emphasis on derived economic benefits. Use of improved carcass quality claims such as "leaner", "more carcasses grading USDA Choice", "forms muscle not fat", etc. in promotional labeling or advertisements is unacceptable unless a sponsor holds an approved NADA/ANADA or NADA/ANADA supplement providing for the claim in the approved labeling.

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Mention of derived economic benefits in promotional material will be considered unacceptable unless the following criteria are met:

- The results of the use of the drug must be closely linked with the approved claims for the drug. There should be a clear statement that the effect of the drug is treatment of a primary disease problem, e.g., parasite burden or infectious pneumonia.

Use of In Vitro Data

The use of in vitro data is not permitted in the labeling for OTC drug products because such information is inherently misleading to the lay users. Promotional use of in vitro data is only permitted for antimicrobial products, and is only permitted so long as it is fairly balanced.

Human Safety Issues

If a product bears a human warning on the label, the warning must be included in the product disclosure information section of any promotional piece. Format and prominence of the warning should be the same as that present on the approved labeling.
The FDA's Compliance Policy Guide (CPG) on compounding and illegal manufacturing is being developed for use by FDA investigators and as guidance for use by veterinarians and pharmacists. It is based on the Federal Food, Drug, and Cosmetic Act, the regulations, case law, established agency policy in respect to pharmacy compounding of human drugs, and statements made at a public Symposium on Veterinary Compounding that was held in September 1993. Symposium participants represented human and veterinary medicine, academic and private practice, small and large animal veterinary practitioners, and others. In sum, the speakers represented a large array of stakeholders with interest in veterinary compounding.

The Center for Veterinary Medicine (CVM) acknowledges the medical need for compounding, within certain aspects of veterinary practice, to enable the profession to use drugs in a responsible manner. The current state of veterinary medicine requires products to treat hundreds of conditions and diseases in more than 100 different species, some of which are known to have unique physiological characteristics. The CVM, other federal and state agencies, and producer groups encourage drug sponsors to obtain approvals for all applications of new animal drugs. In some instances, public funds are used to assist in research that is filed in a Public Master file so that the number of drug approvals can be increased especially for uses in minor species. However, the business and market realities in the animal health industry and limitations in the availability of public money can only be expected to provide a fraction of the products explicitly labeled for each of these indications and public funds are inadequate to sponsor all the needed studies. Consequently, veterinarians are required to relieve pain and suffering, treat diseases or conditions, and save animal lives in clinical situations in which no FDA approved product is properly formulated and labeled to address the specific medical need.

While under certain circumstances CVM acknowledges the existence of the medical need for the compounding of animal drugs, the Center knows of adverse reactions apparently caused by compounded products, that have resulted in animal deaths and increased Agency concern that compounding
practices could lead to potentially harmful residue in food derived from food-producing animals. As an example, recent deaths of cattle due to endotoxin were attributed to the compounding of a parenteral product from spectinomycin products which had been approved for oral use. Another development of great concern to the Agency is illustrated by pharmacies that compound large quantities of dozens of different unapproved new animal drugs. Many of these are essentially copies of FDA-approved products and are actively advertised, marketed, and sold at a lower price than the approved product. The firms claim to be legally practicing pharmacy within their state rights as registered pharmacists. However, in these extreme cases, it appears to FDA that these firms are engaged in illegal manufacturing, using their pharmacy license as a means to circumvent the animal drug approval system, and mass marketing animal drug products produced under little or no quality control or other manufacturing standards of purity, potency or stability.

There is a potential for causing harm to the public health and the health of animals when drug products are compounded, distributed and used on a large scale in the absence of adequate adherence to the principles of contemporary pharmaceutical chemistry and current good manufacturing practices. The pharmacokinetics of compounded products are unknown and extemporaneous withdrawal times may result in potentially harmful residues in food.

Health concerns about the use of products compounded for human administration prompted FDA's Center for Drug Evaluation and Research, to prepare a Compliance Policy Guide (CPG 7132.16). It addresses the subjects of legitimate compounding and illegal manufacturing by pharmacists. The purpose of CPG 7132.16 is to provide enforcement guidance for FDA inspectors and guidance to pharmacists to reduce the unnecessary compounding of drugs for use in man.

The Federal Food, Drug, and Cosmetic Act and related Federal regulations contain, in section 510, registration requirements for establishments engaged in the manufacture, preparation, propagation, compounding or processing of drugs or devices. Sections 510(g)(1) and (2) exempt from these establishment registration requirements, pharmacies and practitioners that are engaged in compounding or otherwise fabricating drugs exclusively for use in their practice. This section does not exempt these firms or individuals from the other sections of the Act.

Under the Act and the related regulations, notably 21 CFR § 207.3(a)(8), compounding and manufacturing are not recognized to be different although historically compounding has been associated with pharmacists and practitioners. Compounding or other manipulation of drug substances creates a new unapproved dosage-form in virtually all cases. Unless specifically approved, each new dosage form is a new animal drug and use of which is a violation of the Act. The courts have confirmed FDA's interpretation of these statutes and also acknowledged FDA's discretionary practice of al-
lowing limited compounding within a practice if the need is great and the risk is small.

Let's begin the examination of the compounding of veterinary drugs with a discussion of definitions.

1. The Act and regulations do not define compounding.
2. The term compounding means any manipulation to produce a dosage form drug except for that manipulation which is provided in directions for use on the labeling of the approved drug products, e.g., reconstitution of a sterile powder with water for injection.

(a) Acceptable compounding is compounding that is done by a practitioner (or pharmacist on a practitioner's prescription) within the confines of a legitimate practice, where the need is great and the risk to public and animal health is small, i.e., the criteria described in this paper are met.

(b) Unacceptable compounding is compounding, by a pharmacist or practitioner, within the confines of a legitimate practice, where the risk to public or animal health is great, irrespective of whether or not the need is great, i.e., the criteria described in this paper are not met.

3. Anything done outside the confines of a legitimate practice is manufacturing.

4. A Bulk Drug is an active ingredient (in unfinished form) intended for manufacture into a usable drug product dosage form.

We have developed an algorithm, similar to that expressed in the CPG on extra-label use of drugs, that provides guidance on the acceptability to FDA of compounding when it is the appropriate solution to a clinical problem. It describes the decision steps which should be followed so that compounding would not be a regulatory concern to CVM. Some common examples of these situations include: combinations of anesthetic drugs for titrated administration to effect; preparation of dilute dosage forms for small, young, or exotic species patients; and some antidote preparations.

The practitioner would, using the following algorithm, affirmatively answer each standard before deciding to compound. A negative answer to any one of the standards would negate the need to compound.

1. Would the health of the animals be threatened or would suffering result from failure to treat?
2. Is there a need to create an appropriate dosage form for the species, age, anatomy, size, medical condition, or safety of the patient or practitioner, increase effectiveness, decrease side effects, or minimize the need for restraint of dangerous animals?
3. Is there no marketed approved animal drug dosage form which, when used as labeled, or in an extra-label manner in conformity with criteria listed in the Extra Label Use CPG 7125.06, or human-label drug, CPG 7125.35, that might acceptably treat the condition?

When the decision is made to compound, then all the following criteria
are expected to be met by the practitioner.

(a) The compounding is to be performed by the veterinarian or by a pharmacist on the receipt of a valid prescription. Veterinarians must exercise professional judgement to determine when compounding requires the services of a pharmacist. The assistance of a pharmacist is necessary when the complexity of compounding exceeds the veterinarian's formal educational knowledge, skill, facilities, or available equipment and in States in which the term compounding does not appear in the Veterinary Practice Act or its rules.

(b) A valid veterinarian/client/patient relationship must exist.

(c) Either the veterinarian or the pharmacist may dispense the compounded product.

(d) No violative residues occur from use of the compounded article.

(e) The safety and efficacy of the compounded new animal drug is consistent with current standards of pharmaceutical and pharmacological practices, that is, known incompatibilities and inappropriate combinations are avoided and minimum current good compounding practices are met for the preparation of drug products by State-licensed pharmacies for dispensing and/or administration to humans or animals (5/26/93 NABP).

(f) Procedures are instituted to assure that appropriate patient records for the treated animals are maintained.

(g) All drugs dispensed to the animal owner by the veterinarian or pharmacist, bear labeling information and an expiration date which is adequate to properly use the product.

A complete label should bear the following information:

1. Name and address of the attending veterinarian
2. Date dispensed and expiration date. The expiration date should not exceed the length of the prescribed treatment.
4. Identity of treated animals.
5. Directions for use.
6. Cautionary statements if needed.
7. Withdrawal/withholding times if needed.
8. Condition or disease to be treated.

1. Here are some specific situations that we would consider to be evidence of illegal manufacturing, or if within the confines of a legitimate practice, unacceptable compounding.

(a) The volume and process resembles manufacturing. An operation "resembles" manufacturing when, for example, the volume and distribution of a finished product is clearly beyond the normal requirements of a veterinary or pharmacy practice of the same client base, and/or large quantities of product are stored for periods of time that cause stability concerns.
(b) Promotion and/or distribution of compounded medicaments that are essentially similar to FDA-approved products, regardless of whether intended for food or non-food animals;
(c) Compounding from bulk drugs, with the rare exception of those medicaments that are explicitly permitted to be compounded by CVM through compassionate investigational status or other means (certain antidotes and other substances).
(d) Preparation for sale of unapproved new animal drugs which employ fanciful or trade names, colorings or other additives, or in any way imply that the compounds have some unique effectiveness or composition;
(e) Advertising, promotion, display, resale, or other means of marketing prepared unapproved new animal drugs;

2. Here are some examples of situations we would consider to be of great risk to public health or animal health and thus unacceptable compounding.
(a) Instances where violative residues occur in meat, milk, eggs, honey or aquaculture products;
(b) A residue depletion time for food-producing animals is not anticipated or if anticipated the pharmacokinetic justification is considered to be a scientifically unacceptable rationale.
(c) Preparation of drug products that are essentially similar to products that have been removed from the market or otherwise prohibited due to safety or efficacy concerns, such as: chloramphenicol, DES, or dimetridazole for use in food animals.
(d) The compounding of drugs for simultaneous use in large numbers of animals, especially food-producing animals, e.g., herds, flocks, or schools, is likely to draw regulatory attention.

3. The next are examples of instances which would likely satisfy the definition of acceptable compounding, when the decision algorithm is met and the dispensed product is properly labeled.

1. Dosage form dilutions and reductions.
   A small animal practitioner determines that an approved animal or human drug for which there are well recognized indications for use is not marketed in an appropriate dosage strength for a small kitten, ferret, or other very small animal. The veterinarian engages a licensed pharmacist to prepare a compounded dosage form of an appropriate strength, e.g., place a portion of a tablet in a gelatin capsule or simple aqueous to aqueous dilution to increase the accuracy of the dose. It is an arm's length transaction, either through a valid prescription order and dispensed by the pharmacist to the client, or delivered to the veterinarian who dispenses it to the client. The pharmacist prepares the medication on a practitioner-driven basis, does not promote the availability or prepare large quantities of this specific formulation. These scenarios are of minor regulatory concern to CVM unless the scale becomes too large or if the compounded products cause a safety problem that is brought
REPORT OF THE COMMITTEE

to our attention

2. Antidotes and poison treatment drugs.
   An emergency practice would like to use 4-methylpyrazole for the
treatment of ethylene glycol toxicity in dogs, a common presenta-
tion in their region, especially in the spring. The CVM allows the
veterinarian discretion for use of this compound for this indication if
several conditions are met. The practitioner engages a compounding
pharmacist, and obtains a published protocol for preparation of
the dosage form. The pharmacist can supply a sterile, finished
dosage form to the practitioner on an as-needed basis.

3. Topical dosage forms, ophthalmic, and intra-articular preparations.
   A well known combination of both human and animal-labeled drugs
is used for treatment of ulcerative keratitis in horses and felines.
This combination of acetylcysteine, atropine, chloramphenicol or
gentamicin, and artificial tears, as documented in several textbooks,
is made up on demand for the veterinarian by a pharmacist that
has the capability to compound a sterile preparation, as required
for an ophthalmic product.

4. Anesthetics, pre-anesthetics, and drugs that are administered to
effect.
   Several well known combinations of ketamine, xylazine and
guaifenesin, combined in one syringe as described in numerous
textbooks and articles on equine anesthesia and are commonly used
at "horse-side" by equine practitioners. Preparations for use in guns
that fire a dart loaded with a tranquilizer into a dangerous animal
housed in a zoo or roaming in the wild.

5. Large volume electrolyte products for treatment of shock and de-
hydration.
   A pharmacist, at a veterinary teaching hospital prepares, through
a complex sterile process and using good compounding practices,
10 and 20 liter containers of physiological saline solution and other
large volume parenteral for intravenous infusion or oral administra-
tion into dehydrated and otherwise ill large animal hospital patients.

6. Compounding, at animal side, by the practitioner.

7. The admixture of a vaccine and drug at animal side.

8. Compounding that is based on information in the USP, BP, recog-
nized pharmacy textbooks, or a formal foreign agency approval in
a country listed in Section 802(b)(4)(A).

9. Dosage forms and water medications for public/private aquaria.
   Articles compounded by a staff or consulting veterinarian or by a
pharmacist on the order of the veterinarian for treating aquatic ani-
mals in public and large private aquaria where approved drug prod-
ts are unsuitable for use as labeled or extra-label, provided that
all local and state environmental requirements are met and worker
safety is not endangered.
## USP PRACTITIONERS' REPORTING NETWORK™

*An FDA MEDWATCH partner*

The Veterinary Practitioners' Reporting Program is presented in cooperation with the American Veterinary Medical Association (AVMA)

### 1. Product Name (brand and generic)

<table>
<thead>
<tr>
<th>1. Product Name (brand and generic)</th>
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### 2. Dosage Form/Strength

<table>
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<tr>
<th>2. Dosage Form/Strength</th>
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### 3. Lot Number/Exp. Date

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<th>3. Lot Number/Exp. Date</th>
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### 4. Name and Address of Manufacturer/Labeler

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<th>4. Name and Address of Manufacturer/Labeler</th>
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</table>

### 5. Diagnosis and/or Reason for Product Usage

<table>
<thead>
<tr>
<th>5. Diagnosis and/or Reason for Product Usage</th>
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</thead>
</table>

### 6. Administered by

- [ ] Veterinarian
- [ ] Owner

### 7. Product Administration

<table>
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<tr>
<th>7. Product Administration</th>
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</thead>
</table>

#### Concurrent clinical problems/products administered including pesticides, chemicals, feed additives, etc.

### 8. Species

<table>
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<th>8. Species</th>
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</table>

### 9. Breed

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<th>9. Breed</th>
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</table>

### 10. Age

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<th>10. Age</th>
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### 11. Sex

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<th>11. Sex</th>
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### 12. Weight

<table>
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<tr>
<th>12. Weight</th>
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</table>

### 13. Reaction/Problem Information

#### a. Number of animals affected

<table>
<thead>
<tr>
<th>a. Number of animals affected</th>
</tr>
</thead>
</table>

#### b. Time between initiation of therapy with suspected product and onset of reaction

<table>
<thead>
<tr>
<th>b. Time between initiation of therapy with suspected product and onset of reaction</th>
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</thead>
</table>

#### c. Time between last administration of suspected product and onset of reaction

<table>
<thead>
<tr>
<th>c. Time between last administration of suspected product and onset of reaction</th>
</tr>
</thead>
</table>

#### d. Outcome

- [ ] Recovered from reaction
- [ ] Died from reaction
- [ ] Other (comment below)

#### e. Was the reaction treated?

- [ ] No
- [ ] Yes (comment below)

#### f. When the reaction appeared, treatment with suspected product:

- [ ] Had already been completed
- [ ] Was discontinued due to reaction
- [ ] Was discontinued and replaced with another product
- [ ] Continued
- [ ] Stopped
- [ ] Recurred
- [ ] Other (comment below)

#### g. The reaction:

- [ ] High
- [ ] Medium
- [ ] Low

#### h. Level of suspicion that drug caused the reaction:

- [ ] High
- [ ] Medium
- [ ] Low

#### i. Reaction/Problem Information

<table>
<thead>
<tr>
<th>i. Reaction/Problem Information</th>
</tr>
</thead>
</table>

### 14. Describe the reaction or product quality problem. Add details about case history including laboratory results, and outcome (attach separate sheet if necessary)

### 15. Veterinarian's Name and Address

<table>
<thead>
<tr>
<th>15. Veterinarian's Name and Address</th>
</tr>
</thead>
</table>

### 16. Owner's Name or Case ID

<table>
<thead>
<tr>
<th>16. Owner's Name or Case ID</th>
</tr>
</thead>
</table>

### Telephone Number

<table>
<thead>
<tr>
<th>Telephone Number</th>
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</thead>
</table>

### 17. A copy of your report is routinely sent to the manufacturer/labeler and to the appropriate agency (FDA, USDA, or EPA). Please indicate to whom USP may voluntarily disclose your identity (check box(es) that apply)

- [ ] The manufacturer and/or labeler as listed above
- [ ] The Food and Drug Administration or appropriate agency
- [ ] AVMA
- [ ] Other persons requesting
- [ ] None of these

### Signature of Reporter

<table>
<thead>
<tr>
<th>Signature of Reporter</th>
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</table>

### Date

<table>
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<tr>
<th>Date</th>
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</thead>
</table>

Return to USP Practitioners' Reporting Network (USP PRN™)

12601 Twinbrook Parkway
Rockville, Maryland 20852
or FAX to: 1-301-816-6532
or Call Toll Free 1-800-4-USP PRN
REPORT OF THE COMMITTEE

VETERINARY PRACTITIONERS’ REPORTING PROGRAM

Animal Safety and Quality Products Through the USP Practitioners’ Reporting Network™ (USP PRN™)

To further enhance the USP Practitioners’ Reporting Network, the United States Pharmacopeia has added the Veterinary Practitioners’ Reporting Program. Through the combined efforts of the American Veterinary Medical Association and the USP, this new program is aimed at detecting product quality problems, medication mishaps, and adverse reactions in drugs, biologics, chemicals, pesticides, and other products used in the practice of veterinary medicine. This program covers products used in companion and other household pets, as well as food producing and farm animals, zoo animals, and exotic pets. The USP shares reported information with the FDA Center for Veterinary Medicine (CVM) to provide a safer environment for the various animal populations in the U.S. Reports not regulated by the Center are forwarded to the appropriate agencies such as USDA, EPA, etc.

Reportable Products and Problems

<table>
<thead>
<tr>
<th>Products</th>
<th>Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td>Ineffective/lack of efficacy</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Product failure/malfunction</td>
</tr>
<tr>
<td>Pet dips</td>
<td>Suspected counterfeit drugs</td>
</tr>
<tr>
<td>Feed additives</td>
<td>Product mix-ups</td>
</tr>
<tr>
<td>and any other product used in your practice</td>
<td></td>
</tr>
</tbody>
</table>

Problems:

- Ineffectiveness/lack of efficacy
- Product failure/malfunction
- Suspected counterfeit drugs
- Product mix-ups
- Inaccurate/confusing labeling
- Therapy errors:
  - Misadministration, similar products and names, mislabeling, wrong product administered, etc.

All product concerns should be reported!

Contribute to a Nationwide Database

The report you submit becomes part of a database that serves to provide insight into product problems that can affect the quality of veterinary care. This database captures all fields of reported information to allow for tracking, analysis, a comparison of product problems occurring nationwide. The product information received through the Program is available under the USP Document Disclosure Policy.*

*"Items may be required for disclosure of any submission pursuant to the USP Document Disclosure Policy.

Just a Phone Call Away

Just call 1-800-4-USP PRN to reach a USP health care professional, who will take your report and respond to your concerns. Your phone/faxed or written report will reach a USP professional for a potential health hazard or product defect to the FDA and the product manufacturer for review and any necessary follow-up. Reports may be submitted anonymously and USP will act as your intermediary in corresponding with the FDA, manufacturer and the AVMA. Your report can result in product improvement, correction, or even product recall. Your report can directly impact the development and revision of USP standards and information in the animal product arena. Practitioners are asked to provide specific information on species, age, breed, sex, histopathology, clinical pathology or necropsy results, etc., which add value to all reported incidents.

USP: A Partner in MedWatch.

The USP PRN is a partner in MedWatch, the FDA’s medical products reporting program. As a partner, USP PRN contributes to the FDA’s efforts to protect the public health by helping to identify serious adverse events for the Agency. This means that all reports are shared with the FDA on a daily basis or immediately if necessary.

USP PRN™ The USP PRN™ is designed to collect experiences and observations from health care providers through five separate reporting programs:

- The USP Drug Product Problem Reporting Program
- The USP—ESMP Medication Errors Reporting Program
- The USP—SNM Drug Product Problem Reporting Program for Radiopharmaceuticals
- The Medical Device and Laboratory Product Problem Reporting Program
- The USP—AVMA Veterinary Practitioners’ Reporting Program

Your input could make the difference in protecting patients from questionable drug and radiopharmaceutical products, defective medical devices, and possible future medication errors.

USP PRN... CALL US WHEN YOU NEED US.

*The Medical Device & Laboratory Product Problem Reporting Program is funded by the Food and Drug Administration under contract 223-F1-6011.

Thank you for your attention. I look forward to the question and answer session.
REPORT OF THE COMMITTEE ON PROFESSIONAL OVERSIGHT

Chairman: Dr. John R. Ragan, Nashville, TN
Vice Chairman: Dr. Daryl K. Thorpe, Pierre, SD

Dr. J. Lee Alley, AL; Mr. Joe B. Finley, TX; Dr. Donald W. Luchsinger, VA; Mr. J. O. Pearce, Jr., FL; Dr. Richard D. Willer, AZ; Dr. Saul T. Wilson, Jr., AL.

Report of the Professional Oversight Committee Vice Chairman - Dr. Daryl Thorpe.

The Committee considered the following items presented.

Dr. J. Lee Alley introduced a resolution calling for a working group of state and federal animal health and food safety officials, along with livestock industry and representatives to guide the development of food safety issues and actions. Dr. Donald W. Luchsinger moved adoption of the resolution. The motion was passed.

Dr. C. Carter Black appeared before the Committee to discuss the 7-day observation period for accredited veterinarians to write a health certificate. Dr. Luchsinger states that this problem is being resolved.

Dr. J. Lee Alley discussed the accreditation requirements of college veterinarians doing program works in state and federal programs. The committee felt that these veterinarians should meet the same standards as private veterinarians.
During the past year there has been twenty-nine changes of status of States in the pseudorabies eradication program. All but two of these changes have been advancements. The status of all States and an approximate number of pseudorabies infected herds in each State is shown in figure 1. Twenty-four States have no known pseudorabies infected herds and thirteen of those States have been classified as Stage V (free).

On July 1, 1994, the United States had 6,205 pseudorabies infected swine herds. This was down from 6,888 a year earlier. During the year 2,789 herds were released from quarantine, but 2,106 newly discovered herds were placed under quarantine. The final results were a reduction of 683 infected herds. Figure 2 shows a graph of States with infected herds with or without cleanup plans. Nationally, 95 percent of all infected herds are working with a cleanup plan. This is an improvement over the 90 percent that existed one year ago. To maintain our national goals we need to have 100 percent of our herds on a cleanup plan with the only exception being those herds that have been discovered less than ninety days. Figure 3 is a chart showing the source of new herd infections during the quarter ending in June 1994. Figure 4 shows the prevalence trends. The number of infected herds is beginning to decline and should continue to decline at an accelerated rate once all testing has been completed in Iowa and all herds are working on a cleanup plan. Figure 5 shows the apparent incidence rate per quarter. The rise in incidence in late 1993 was due primarily to increased testing. However, the further increases in 1994 were due to increased spread of the disease.

Figure 6 shows a graph of percentages of breeding swine being sampled by different States that are doing first point or slaughter surveillance. Our pseudorabies program standards states that this type of surveillance should sample at least 10 percent of the breeding swine in a State during a one year period. States that have advanced to Stage V (free) may reduce their surveillance to no less than 5 percent. States which are above 20 percent can reduce the amount of surveillance as their programs advance.

Infected herds have been identified by the following methods during the past year July 1, 1993 to July 1, 1994.

<table>
<thead>
<tr>
<th>On Farm Testing</th>
<th>Herds tested</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughter traceback</td>
<td>1,449</td>
<td>3.4%</td>
</tr>
</tbody>
</table>
U. S. PSEUDORABIES ERADICATION PROGRAM REPORT

First Point traceback 64 17.0%
Tracing from infected herds 179 23.5%
Tracing into infected herds 123 27.6%
Circle testing infected herds 824 16.9%
Other epidemiology 4,679 14.9%

<table>
<thead>
<tr>
<th>Area Testing Herds Tested</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding herds 17,451</td>
<td>10.0%</td>
</tr>
<tr>
<td>Grower/Finisher 1,488</td>
<td>8.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Herd Status Testing Herds Tested</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeder pig monitoring 23,342</td>
<td>2.1%</td>
</tr>
<tr>
<td>Qualified Negative Herd 17,646</td>
<td>.5%</td>
</tr>
<tr>
<td>Q-N Vaccinated Herd 1,768</td>
<td>6.7%</td>
</tr>
<tr>
<td>Sale/Exhibition 38,918</td>
<td>.6%</td>
</tr>
<tr>
<td>Retest of Imported Swine 18,180</td>
<td>.4%</td>
</tr>
<tr>
<td>Diagnostic 3,840</td>
<td>7.6%</td>
</tr>
</tbody>
</table>

Slaughter surveillance has some advantages in collection of a large number of samples with limited help, but the program is dependent upon reliable identification and a rapid, economical, sensitive and highly specific test. The test results must be reported in a timely manner to the responsible person for assigning traceback investigations. Traceback investigations and necessary herd testing must be completed promptly. This is not being accomplished in all situations. Every Laboratory Director, State Veterinarian, or Area Veterinarian in Charge who is involved with this program has a responsibility to search out the problems and correct them. In an effort to improve our laboratory results and turn around time the Frankfort, Kentucky and Centralia, Illinois, laboratories cooperated on studies using the PCFIA gl test and the ELISA gl test.

The PCFIA gl was used on PCFIA or Autolex positive samples at the Frankfort, Kentucky laboratory. Serum from 216 pseudorabies positive screening test samples were sent on to the National Veterinary Service Laboratory for serum-neutralization (S-N) testing. Of the 216 positive screening test samples 175 (81%) were positive on S-N test at 1:4 or greater. When the 216 positive samples were tested by PCFIA gl only 105 (48.6%) were positive. At Centralia, Illinois laboratory all slaughter samples are screened with the S-N test. Two hundred positive samples were then tested with the ELISA gl. One hundred thirteen (56.5%) were positive to ELISA gl. Some advantages of the gl differential tests on these samples were:
1. Non-infected gl vaccinated swine were negative.
2. 40% and 43.5% of the S-N positive were negative vaccinates.
3. Fewer traces will be sent to the field.
4. Quicker turnaround time for testing.

The Cost/Benefit Study has been completed by Dr. Gay Y. Miller and 100 copies are available at this meeting. Additional copies are available.
from USDA,APHIS Veterinary Services, Swine Health Staff, Suite 204-PB, 6525 Belcrest Road, Hyattsville, Maryland 20782. I urge you to pick up a copy. For the benefit of my presentation today I have drawn the following conclusions from Dr. Miller’s report;

1. Profitability is greater in an uninfected herd.
2. The Pseudorabies Eradication Program must progress faster in the next 5 years or the goal of free by the end of year 2000 will not be reached.

In conclusion, I want to urge all producers, State/Federal regulators, and practicing veterinarians to rapidly finish all initial testing, to enroll every known infected herd in a cleanup plan that is specific for that individual herd, and to continually upgrade our national pseudorabies surveillance so as to locate any newly infected herds. Cleanup plans may be very simple for some herds requiring only a test and removal of the positive. In other herds a repopulation of breeding stock may be the best. There will be other herds that require total herd vaccination and management changes to accomplish herd cleanup. For a herd to eliminate pseudorabies virus from a swine herd in three years or less, certain progress steps must be attained and then maintained. First, the herd must produce and finish negative pigs. If segregation alone does not maintain negative pigs, vaccination with management changes, if indicated, must be considered. Vaccination will be necessary in certain swine dense areas. Vaccination is cost effective as demonstrated in Figure 7. The vaccinated pig which is challenged will reach market weight sooner than the unvaccinated pig that becomes infected. The unvaccinated infected pigs will create a lot more virus to perpetuate the disease. Secondly, negative gilts must be added to the herd which can be bred, gestated, farrowed and still remain negative. When these two important measurement steps are accomplished the herd will soon cleanup. Failure of either of these steps will indicate that the herd plan needs to be re-evaluated and other changes or plans implemented. The positive grow/finisher is, in my opinion, the greatest single source of pseudorabies virus that continues to re-cycle within the herd and even spread to other herds in the community. In almost all instances every pig, no matter what the source, can grow up free of pseudorabies if properly managed. The grower/finishers that are sero-converting to positive status need to be identified and plans initiated to correct the problems whether it is with a single herd or a group of herds. All program managers must focus on these important progress indicators to make sure that the herd plans progress as intended.

Once all infected herds are on progressive cleanup plans, there will be less pseudorabies virus created, there will be fewer newly infected herds discovered, there will be an accelerated number of herds released from quarantine, and the goal of total eradication of pseudorabies can become a reality at an earlier date than predicted.
Pseudorabies
Program Stages and No. Infected Herds-Oct. 1, 1994

Stage I
(1 State)

Stage II
(8 States)

Stage III
(22 States)

Stage IV
(5 States)

Stage V
(13 States)
Pseudorabies

States with Infected Herds

* Iowa reported 3771 infected herds (3570 with cleanup plans) June 30, 1994
Pseudorabies
Source of New Herd Infections-June 1994

Area Spread 35.7%
Feral Swine 0.8%
Unknown 42.3%
Feed/Bedding 0.6%
Purchased Breeders 9.7%
Purchased Feeders 10.9%
Pseudorabies

Prevalence Trends

Thousands


- with Iowa
- without Iowa
Pseudorabies

Apparent Incidence Rate per Quarter

Rate = # newly discovered infected herds per quarter
Combined 1st Point and Slaughter Surveillance
July 1, 1993 to July 1, 1994

Percentage of breeding swine sampled

States

Mn ND Mo KS SD TX De IL Ky Md Nj Oh Pa Va Wi Wv Al Fl Ga La Ms Pr Sc Ca Nv Or
Changes in Body Weight Post-Challenge

Weight (pounds)

Number of Days Post-PrV Challenge

- Vaccinates
- Non-Vaccinates
REPORT OF THE COMMITTEE ON PSEUDORABIES

Chairman: Mr. Don D. Gingerich, Parnell, IA
Vice Chairman: Dr. George W. Beran, Ames, IA

Ms. JoAnn Alumbaugh, IA; Dr. Paul Anderson, MN; Dr. Joseph Annelli, MD; Dr. C. Carter Black, GA; Mr. Neal Black, MN; Dr. Decatur Blanchard, NC; Mr. Philip E. Bradshaw, IL; Dr. Donald Bridgewater, CO; Mr. Robert Dykhuis, MI; Dr. George C. Edwards, NC; Dr. Colleen Y. Erbel, TN; Dr. Walter D. Felker, IA; Dr. Thomas W. Freas, IN; Dr. Merwin L. Frey, IA; Dr. Anthony M. Gallina, IL; Dr. Larry M. Granger, MI; Dr. Thomas J. Hagerty, MN; Dr. Edwin Hahn, IL; Dr. Mark Hammer, NC; Dr. Howard Hill, IA; Mr. Donald Hoogestraat, SD; Dr. Irwin H. Huff, ND; Dr. Richard D. Hull, IL; Dr. John W. Hunt, MO; Dr. John P. Huntley, NY; Dr. Owen James, MT; Dr. Charles L. Kanitz, IN; Dr. John P. Kluge, IA; Mr. Willard Korsmeyer, IL; Mr. John H. Lang, WI; Dr. Beth Lautner, IA; Mr. James W. Leafstedt, SD; Dr. James L. Lindstrom, TX; Dr. Herbert E. Little, CA; Dr. Bret D. Marsh, IN; Dr. Charles Massengill, MO; Dr. Thomas J. McGinn, III, NC; Dr. I. Lee McPhail, OH; Dr. William L. Mengeling, IA; Dr. Rita D. Michaels, MO; Dr. Harry F. Moberly, Jr., IL; Mr. Armand D. Moles, MO; Dr. F. J. Mulhem, MD; Dr. R. R. Ormiston, IN; Dr. Donald H. Person, MN; Dr. Nancy Pfeiffer, NE; Dr. John R. Ragan, TN; Ms. Nancy Robinson, MO; Mr. Jeff Schnell, IA; Dr. George P. Shibley, KS; Mr. Michael L. Snyder, ME; Dr. Thomas E. Socha, NE; Dr. Charles E. Starkey, AR; Mr. Jim Stocker, NC; Dr. Arnold C. Taft, Bowie, MD; Dr. David G. Thawley, MN; Dr. Dennis L. Thompson, CA; Dr. E. Thurber, NE; Dr. Paul O. Ugstad, NE; Dr. James W. Van Buren, MI; Mr. Joseph A. Vansickle, MN; Mr. Willard H. Waldo, NE; Dr. Douglas L. Weiss, MN; Dr. Larry L. Williams, NE.

Forty two committee members were in attendance. There were more than 35 guests.

Gary Simpson presented the National Pork Producers Council report. He reported the federal appropriation decreased from $8.63 million (FY '93) to $8.48 million (FY '94). The major challenge for administering the program will be to keep up with the rapid industry changes, especially interstate commerce. Swine I.D. will be essential to the program. Surveillance will be costly but must be done.

Dr. Arnold Taft gave the USDA/APHIS report of program progress and future objectives. He reported 29 status changes during the last year. Currently, 24 states have no known infected herds.

The Control Board report was presented by its chairman Phil Bradshaw. The control board reviews applications for state status. The board reviewed 42 applications for state status change. Missouri recently applied for stage III status and all states are now at stage II or higher.
Dr. Joe Annelli gave a swine I.D. report. The I.D. system of the future will have a premises identification, will be producer applied, and will have a system of data transfer between different entities of the industry. His immediate goals are to promote the wide use of on farm identification, the coordination of a swine I.D. working group and encouraging the application of current I.D. systems. He reminded the committee that a LCI identification conference will be held December 1994 in St. Louis.

The report for the Differential Diagnostic Test Sub-Committee was given by Dr. Gene Erickson. A proposal by USDA was considered by the subcommittee, regarding whether the GI differential diagnostic assay should be classified as an official diagnostic test. Based upon the experience of the committee members and the data presented on sensitivity of the GI assay, the subcommittee endorsed the proposal and recommends that the GI assay be recognized as an official diagnostic test. Dr. Ludeman abstained from this action of the committee.

State reports were given from 11 states. All reported significant progress in the previous 3 years, especially during the previous 12 months. The concern for most of the states was cleaning up the last few remaining infected herds in a state or area.

The next topic for discussion was vaccination to control PRV in a wild swine population, reported by Dr. David Stallknecht. This project investigates the proportion of the wild swine population which can be reached through an intensive trap, vaccinate and release strategy. Although this project has been productive with studying bait uptake, there is evidence the virus is still circulating. More work will be completed during the winter of 1994.

Dr. Fernando Osorio gave a report on differential vaccines. The marker vaccines have not shown to recombine with wild strains of the virus. However, different deletion types showed varied immunogenic efficacy. GX only deleted vaccines provided more protection to prevent latent infection compared to GI deleted vaccines. This indicates GX only deleted vaccines may be useful in herds difficult to cleanup and where diagnostic capabilities are not necessary.

Dr. Ned Hahn gave the NC 197 regional committee report. The committee has contributed 72 publications, over 80 presentations and the development of a uniform data dictionary. The committee found that GI may play a role in establishing latency. Geographic information systems (GIS) is currently being used in NC and MN. The committee has also studied maternal antibody interaction with vaccination timing. Producers and veterinarians should consider using GI+ vaccines within infected, hard to cleanup herds.

The committee endorsed the concept of a proposal by the Council of State and Territorial Epidemiologists: Recommend that the U.S. Fish and Wildlife Service and the appropriate state agencies adopt regulations pro-
hibiting the importation and interstate movement of certain wildlife (for use as companion animals or for hunting purposes). These include, but are not limited to, wild canids and carnivorous animals, wild rodents and feral swine. The full committee also approved adding a prohibition on intrastate movement to the concept.

The following program standard sub-committee report was approved as amended. Recommended changes in the Program Standards (January 1, 1994) were approved as follows:

- Page 19. Stage III D. Duration of Status Revise and add to the provision so that it would read: Twelve to 14 months following assignment of stage III status by Veterinary Services, a State/Area must (1) certify that it meets the requirements of a higher program stage, or (2) indicate that it continues to meet Stage III requirements, utilizing the same certification procedure as followed initially, and demonstrate progress in herd cleanup consistent with the goal of eradication by the year 2000, by at a minimum, meeting the following two provisions: (A) herd cleanup plans written on all herds within 30 days of quarantine, and (B) all quarantined herds tested at least semi-annually with a 95/10 test, herd plans reviewed semi-annually and revised as necessary. States failing to recertify as required will automatically lose their Stage III status. (Note: underlined words are suggested additions/changes)

- Page 20, Stage IV, section E. Swine Import requirements, #3. Feeder Pigs, revise to read as follows: 3. Feeder Pigs a. Direct shipment from a farm of origin or a market in a Stage IV or V state/area, or b. Direct shipment from farm of origin in a Stage III state or area, or c. Direct shipment from a qualified pseudorabies-negative herd, or d. Entry is allowed into Stage IV states from feeder pig monitored herds in Stage II states under the following conditions: (1) That the swine enter on permit directly to designated feedlot; (2) that the swine originate from an approved feeder pig market or direct from a qualified negative (QN) herd, a qualified negative vaccinated (QVN) herd, a feeder pig monitored (FPM) herd, or a feeder pig monitored vaccinated (FPMV) herd; (3) That the swine be quarantined until they are sent to slaughter.

- Page 25. D. Establishment and maintenance of QN growout premises on which no adult breeding swine are maintained. Add to the provision regarding Herd B, the intermediate growout facility, the words "If herds A, B and C are in the same state and program stage this testing is not required." (Note: This would eliminate the need for testing in the growout facility if all the herds are in the same state and program stage.) Add to the provision regarding Herd C (sales point) a sentence from the definition of a 95/10 test on page 6, to make the provision read as follows: QN status may be maintained by a monthly negative official pseudorabies serologic test of 50 swine selected at random from those that have been in the herd at least 30 days, except that in all-in/all-out units, one test of 50 head is required of
REPORT OF THE COMMITTEE

each group. Each segregated group of swine on an individual premises must be considered a separate herd.

- Page 27, Subpart III—The Pseudorabies Monitored Feeder Pig Herd
  Section C. Strike the words “test of 30 pigs.” and substitute the following language “random sample test (95/10) as determined by an official pseudorabies epidemiologist;” and add a sentence at the end from the definition of an official random-sample test (95/10) on page 6. The section would then read as follows: C. A remote growout nursery to which pigs have been moved within 1 week of weaning from a pseudorabies monitored feeder-pig herd may qualify as a pseudorabies monitored feeder pig herd on the basis of a negative official random sample test (95/10) as determined by an official pseudorabies epidemiologist. Each segregated group of swine on an individual premises must be considered a separate herd for testing purposes.

- Page 27, Subpart III—The Pseudorabies Monitored Feeder Pig Herd
  Section D. Revise to read as follows: D. An official random sample (95/10) as determined by an official pseudorabies epidemiologist must be conducted on each group of pigs moving through the remote growout nursery. In the case of a continuous-flow facility, monthly tests (95/10) must be conducted.

- Page 28, Subpart IV—The Pseudorabies-Monitored Vaccinated Feeder Pig Herd
  Section C. The provision regarding a remote growout nursery would be amended to strike the word “of” after the words “30 days” and insert words “prior to” and striking the words “to the” and inserting the words “out of”. Also the words “test of 30 pigs.” would be stricken and the following inserted—“random sample test (95/10) as determined by an official pseudorabies epidemiologist” and add a sentence at the end from the definition of an official random-sample test (95/10) on page 6. The paragraph would then read as follows: C. A remote growout nursery to which pigs have been moved within 1 week of weaning from a pseudorabies monitored vaccinated feeder pig herd on the basis of a negative approved differential pseudorabies random sample test (95/10) as determined by an official pseudorabies epidemiologist. The required tests must be conducted within 30 days prior to movement out of the remote growout nursery. Testing must be conducted on each group of pigs moving through the remote growout nursery or, in the case of a continuous-flow facility, monthly tests (95/10) may be conducted. Each segregated group of swine on an individual premises must be considered a separate herd for testing purposes.
We are in year six. Only four to go. The State-Federal-Industry Cooperative Pseudorabies (PRV) Eradication Program continues to be a model of producer and government teamwork. The PRV Control Board has advanced states through the stages at a more rapid pace than ever before. Urgency is still an important word for our vocabulary and actions in the month ahead.

The program the next year will be focused on state's progress. We also want to carefully consider cooperation between states that is essential as neighboring states advance through the stages at different paces. The structure of the industry is demanding more interstate movement of pigs. We must work together and resolve the differences that occur as one state moves ahead of another. The eradication program will not work if interstate commerce is restricted to the extent that producers lose marketing opportunities. Neither can any state relax their regulations to the extent that allows for unnecessary exposure to happen. The need for state veterinarians to communicate and to be considerate of the needs of their neighbors without risking their state's program is essential. Producer involvement is of utmost importance in working out these differences.

The industry is in the midst of massive transition. Industry growth has surpassed it's ability to market pork product at a profit for the time being. The question is how long will we be in this stress situation. Producers are also deciding who will stay in business and who will drop out. It seems apparent that pork production will be in the hands of fewer producers and that the uniformity and quality of pork products will continue to be improved. Production technology has reached a new plateau. Although there are few secrets in the hog business, each producer must implement the best technology available to produce the best quality product at the least cost possible. The positive side of all this is that pork products are enjoying an increasing appreciation by consumers. We are a growth industry and you can expect to see professionalism to remain at a very high level.

The health of swine herds is recognized as an essential element of profitable pork production. PRV is a disease problem in the U.S. swine herds which is recognized to cause considerable loss. The Cost-Benefit Analysis of the National Pseudorabies Eradication Program written by a team from Ohio State University headed by Dr. Gay Miller is available for distribution. The study again shows the economic benefit to the nation for a successful PRV eradication program.

Funding continues to be a concern for state, federal and industry leaders. The federal funding for fiscal year 1995 as appropriated by the U. S. Congress is $8.484 million Fiscal year 1994 funding was $8.653 million.
We are now on the down hill side of the bell shape curve. The program is driven more by economics than regulations, as producers recognize the economic significance of maintaining PRV free herds. Producers may have to pay a larger share of the cost where state and federal funding is lacking. Producer support is at an all time high and must continue if we are to achieve eradication by the year 2000.

States should now have in place their detailed plan on how they plan to complete eradication in the next 4 years. Most infected herds have been identified and are on clean-up plans. Surveillance after clean-up is our next great challenge. We can be sure that there will be at least some break backs after clean-up. There will be more in high density swine areas. Our ability to detect these reinfected herds and clean them up immediately will be critical to the success of the program. Reinfection, if not detected and stamped out immediately, can cause producers to lose confidence in the program. Surveillance will cost, but we can not afford to overlook it. Each state must design a plan most suited for their own situation. The identification of all swine, especially cull sows and boars, will play a key role. The industry needs to reach some conclusions soon on which identification system they will use. Keep in mind that the quality of surveillance for the remainder of the program will make or break the program and each state must take the responsibility for their own.

Stimulation to reach program goals will come from two sources. One, of course, is the producer organizations. They should be asking state regulatory officials why they are still in stage I, II or III of the program. We also will rely on APHIS staff and regional, state and federal veterinarians to stimulate advancement. The drive must maintain it’s intensity. NPPC remains committed to complete this program and on time. Please feel free in contacting the NPPC office if you need help with anything.
The pseudorabies eradication program in Minnesota is based on mandatory herd monitoring, quarantine of infected herds, herd cleanup, restricted movement of swine, and continuing surveillance. The state is split into two stages. Sixty-six northern counties are in Stage III. The remaining twenty-one southern counties are in Stage II.

Minnesota rules that are more stringent than Program Standards include the requirement to isolate and retest all breeding swine imports, mandatory monitoring of all swine herds, and restricted movement of pigs from infected herds. Surveillance in Stage III counties is by slaughter surveillance and mandatory herd monitoring. Surveillance in Stage II counties is by mandatory herd monitoring.

On September 30, 1994, there were 577 infected herds in the state. This was a decrease of 85 infected herds from the same date in 1993. Cleanup of infected herds is progressing well and all infected herds have cleanup plans on file. Fifty quarantined premises are located in Stage III counties with the remaining 527 infected premises located in Stage II counties.

If the rate of quarantine release and the rate of pseudorabies spread stay constant, Minnesota will be pseudorabies free by November, 1998.
The Regional Committee NC197 is a CSRS Committee whose mission is to conduct research in support of the National Eradication of Pseudorabies. The Committee has been in existence for five years and has recently submitted a renewal and obtained approval for the next five years.

The contributors to NC197 represent investigators from six different states and two federal agencies as shown in figure 1. The nature of this committee requires collaboration and cooperation among the participating agencies in order to achieve the stated objectives. I need to say something about the funding for NC197 because the success of this Committee and its support of the Eradication Program is dependent, to a very large extent, on the level of funding that we have obtained. It has been clearly shown that those stations and agencies that have adequate resources are the ones that have been most productive over the past five years. The current funding for NC197 through the CSRS and the Agricultural Experiment Stations is shown in figure 2. Annual support, as you can see, is very minimal and has been largely provided through only two of the stations. Some stations get small amounts of travel money enabling them to attend our annual meetings, while some stations receive nothing. If our funding was restricted to this amount of money, our research would be greatly hampered. Fortunately, through outside competitive grants, it has been possible for individual investigators and collaborative projects to obtain funding through the USDA or through the National and State Pork Producers Associations. Some state monies have been obtained although, of course, over the last few years, this source has shown a reduced level of funding for the committee's activity.

The contributions that have been made by NC197 to the Eradication Program have been very significant. Over 72 publications and 80 presentations have been made during the five years that the Committee has been in existence. This work has contributed research knowledge and advice to the Eradication Program. Some of the highlights include the establishment of a uniform, data dictionary that has been used to pursue epidemiologic research at state levels. Reporting has been facilitated since certain databases have now been established that allow direct handling of the course of the Eradication Program. Several studies have concerned the effectiveness of different eradication techniques. Research focusing on the pilot projects has shown that eradication in large herds is possible. A number of
studies have outlined and determined the factors that influence spread from one farm to another. These observations and discoveries have been put into practice and will serve as an aid to reduce the future spread of the disease. The efficacy of differential diagnostics in vaccines has also been examined by researchers within this Committee's structure. The deficiencies in the gG (gX by old nomenclature) diagnostics and the relative ability of certain vaccines to achieve high levels of immunity have been shown to be important for the diagnostic process and for effective prevention of disease. Research on the immunosuppressive properties of the pseudorabies virus have shown which targets in the immune system are susceptible and the role of PRV infection on the increased incidence and problems caused by other diseases. New technology such as polymerase chain reaction-based detection systems have increased the sensitivity of diagnostics well beyond the level that was present five years ago. These techniques have been applied to look at low prevalent reactors that often have low levels of antibody. This technology will prove important in the area of pathogenesis and reactivation of virus. Research on the importance of glycoprotein gE (gI) in PRV vaccines has added important information in the controversy of whether to go to a gE vaccine as a uniform approach in this country. The influence of glycoprotein E on PRV immunotropism has been shown, where virus strains that do not have glycoprotein gE replicate more rapidly than gE+ virus in certain macrophage compartments. The role of this glycoprotein in vaccine inhibition of latency has also been shown where virus that contains this glycoprotein seems to colonize nervous tissue more and thereby prevent subsequent colonization by wild-type virus. The immunogenicity of the gE- deleted vaccines has also been considered and shown to be somewhat less than those virus strains that include glycoprotein E.

Geographic information systems have been applied in several studies that have been proven to be extremely important in following the infection, looking at the spread and conditions that affect spread to new premises. Because of these findings, certain recommendations can be made by this Committee that should have an important impact on the Eradication Program. The first recommendation is that the use of gE+ vaccines should be continued in situations were clean-up is difficult. Secondly, field trials should be done comparing major vaccines in pigs with maternal antibody. Thirdly, geographic information systems could be applied at the state and federal levels to tailor strategies in the final stages of the eradication process.

The current objectives of the Committee are shown in figure 3. Because of the large number of field and vaccine strains that are currently in use and the chance that some of these particular virus strains may be moving doing the later stages of the eradication program, one of our efforts has been centered on characterizing them for molecular epidemiology. At the same time, research continues to improve the detection systems. Because of the latency problems associated with pseudorabies, a second goal
REPORT OF NC197

is to determine the mechanisms of viral persistence. Studies on latency and the role of vaccines in latency are centered on discovering how the virus maintains itself in the host and reactivates during the later stages of the persistent infection. Because the spread of PRV from feral pigs to domestic pigs will become increasingly important as the eradication of the disease in domestic pigs occurs, our goal in this area is to examine the mechanisms of this spread and possible transmission of PRV from the feral swine reservoir. Intervention strategies are being applied with variable success. As the final stages of the Eradication Program are reached, it will be very important to modify and tailor intervention strategies toward the unique requirement of the end game. As in any process, costs will become increasingly important. An analysis of the cost benefits of the eradication and surveillance will be increasingly important as we reach the final stages. These objectives will provide help to surmount the challenges of the program during the next five years.

In conclusion, the information that I have provided indicates that NC197 is capable and ready to conduct appropriate research in support of the Eradication Program. Adequate funding must be found, however, because without it, the level of activity and the contribution of NC197 will be minimal. As the Eradication Program winds down in the next five years, there are going to be new problems of disease in feral reservoirs, of perhaps altered wild-type virus that has adapted to vaccines and other surprises that need to be addressed through research. Many of the speakers at this meeting have called for particular research in one situation or another. NC197 is ready to help contribute to answer some of those questions.
Funding for NC-197

Figure 2

Annual support

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NC-197 Objectives

- Characterize of field, laboratory, and vaccine strains of PRV and detection systems.
- Determine mechanisms of viral persistence.
- Evaluate the potential spread of PRV from feral to domestic swine.
- Identify intervention strategies to eliminate PRV in the final stages of eradication and prohibit renewed spread.
- Determine the costs of continued eradication and surveillance.

Figure 3
(SLIDE 1) Introduction
I would like to thank you today for giving me this opportunity to discuss the progress North Carolina's producers have made toward the eradication of Pseudorabies from our state.

(Slide 2)
As you may or may not know, this year North Carolina surpassed all other states except Iowa in hog production. We are now officially the second largest producer of pork in the country. Since last year, USDA indicates the number of sows in North Carolina has grown to 826,000. A 31% increase over last year. This tremendous growth presents our industry with numerous dynamic challenges.

Don Gingerich has asked us to address several questions regarding our state's program. The first question is:

QUESTION #1: WHAT IS NORTH CAROLINA'S PRV ERADICATION PLAN?
Even with this rapid growth in our state, we have found that our Basic Eradication Program has served us well and remains mostly unchanged. North Carolina is a split stage state (II/III) and its program is founded on the following:

(Slide 3) Step 1. Maintain Surveillance:
* 100% Stage II Breeding herds tested annually.
* Stage III Breeding herds tested at first point of concentration.

(Slide 4) Step 2. Stop Spread:
* Yearly monitoring and vaccination of all PRV/PRV Exposed finishing floors. *Concentration or relocation of most positive finishing spaces from a
geographically diffuse area into a limited geographic area thus limiting exposure to a small region.

* Control of PRV exposed animal movements.
* Control of marketing channels of PRV Exposed animals.
* Use of vaccine in herds at risk of exposure.

(SLIDE 5) Step 3. Clean up of infected animals/positive herds.
* 100% of all positive herds have herd plans.
* All positive herds are tested every 6 months to assure plan working.
* Measurable goals are set to assure progress of plan.

QUESTION #2: WILL NORTH CAROLINA ACCOMPLISH ERADICATION BY 2004 AS PREDICTED BY OHIO STATE UNIVERSITY? IF SO, HOW DO YOU INTEND TO REACH OR BEAT THIS GOAL?

(SLIDES 6) Yes, we will eradicate PRV by 2004.
1. Through the uses of the previous THREE STEP APPROACH mentioned above.
2. Through the use of three year cleanup plans (three years from the date of virus circulation ending). We make every effort to assure that gilts and following parities stay negative through frequent monitoring. We assist in identifying individual positive animals or parities so producer can target culling of these animals over non infected animals of similar production status.
3. By encouraging use of Isolation facilities. (Lessons learned from GIS break.)
4. By examining all aspects of production, new and old, to determine the effect on disease transmission in our state. (ie. dead animal disposal, salvage trucks, BBQ trucks, service personnel, Trash dumpster pickups, etc.)

QUESTION #3 HOW MANY INFECTED HERDS DOES NORTH CAROLINA CURRENTLY HAVE AND HOW MANY ARE ON HERD PLANS?

(SLIDE 7) North Carolina currently has 138 quarantined sow farms representing 109,500 sows and 366 nursery/finisher premises representing 1,062,735 finishers and 51,840 nursery pigs. All have herd plans. Any newly infected herds have a plan in place within 30 - 90 days. Herd plans are reviewed at least yearly and sows herd plans are tested and/or reviewed twice yearly to assure progress is being made. The 240,000 quarantined finishing spaces associated with the 30,000 sows released this year are scheduled for release over the upcoming year.
QUESTION #4 HOW MANY HERDS HAVE BECOME REINFECTED AFTER CLEANUP?

(SLIDE 8) North Carolina has had 12 sow farms rebreak after cleanup. 5 of those rebreaks were caused by imported breeding animals this past year. Finishing floors in North Carolina are always in flux and are therefore frequently quarantined, released and requarantined depending on producers pig flow needs. This relates to changes in pyramid flow as sow herds are released or as other sow herds are quarantined.

QUESTION #5. WHAT IS NORTH CAROLINA'S POLICY ON THE USE OF VACCINE?

(SLIDE 9) SENTINEL HERDS - Non-infected vaccinating herds:
Any herd that is deemed at high risk is allowed to vaccinate. To do so however, a request must be submitted to the State Veterinarian's Office requesting the use of a specific vaccine. Switching of vaccine is not allowed without prior review and approval of the state veterinarian. If approval is given the herd must agree to comply with movement restrictions (no feeder pig markets/ sales). The herd must also be tested/monitored twice a year. The veterinarian selling the vaccine must take responsibility for the number of doses distributed and must be on farm 2 times per year to train/teach proper handling and administration techniques for the vaccine.

(SLIDE 10) NORTH CAROLINA VACCINATION OVERVIEW:
Vaccination is required of all positive herds unless herd plan calls for rapid depopulation.
403,000 sows are currently vaccinating for Pseudorabies.
109,500 of those sows are quarantined/infected with Pseudorabies.
309,000 are vaccinated non-infected herds (Those that vaccinate for PRV but are not infected.)

(SLIDE 11) Quarantined finishing floors are required to be tested annually. If found to be infected, they are required to vaccinate.
1,172,741 PRV FINISHERS
559,860 VACCINATING
24 VACCINATED NON-INFECTED HERDS

QUESTION #6 WHAT NORTH CAROLINA PROGRAM STANDARDS ARE OVER AND ABOVE THE FEDERAL PROGRAM STANDARDS? JUSTIFY WHY.

(SLIDE 12) 1. We release finishers after two negative turns and 1 negative test. Instead of two negative tests 90 days apart. Justification - Three site
production, all/in all/out of finishers.


3. Whole herd test of some 1000 head farms. Justification - state/industry cooperation to accomplish task immediately vs. dragging out end of situation over several years.

4. More stringent statistical tests for release 95-2 level of confidence verses 95-5 level in both stage II and III areas. Justification - The consequences of failure are much more significant when dealing with large integrated operators. In addition, even on the largest of herds this extra level of insurance requires only 100 or so more animals to be tested on each farm.

5. Qualified status required on interstate movement of breeding animals. We will not except 30 day test of individual animals. Justification - Once again, the consequences of failure are much more significant when dealing with large integrated operators.

SUCCESSES OF THE PAST YEAR:

This year we have made some tremendous strides in our program with the release of over 38 sow farms with a capacity of 500 or more. It was only 5 short years ago that most of the nation was saying that it was impossible to clean-up the large sized herds that are common to North Carolina. The doom sayers were wrong:

(SLIDE 13) This is FARM 2101 - 1200 SOWS
(SLIDE 14) This is FARM 2102 - 1000 SOWS
(SLIDE 15) This is FARM 2104 - 1500 SOWS
(SLIDE 16) This is FARM 2105 - 1000 SOWS

The last four farm that I showed you were all part of 100% DEPOP/REPOP program to improve the genetics and disease status of these farms.

(SLIDE 17) This is FARM 2148 - 1350 SOWS
STATISTICALLY RELEASED
100% test of infected parities

(SLIDE 18) This is BACHELOR SOW FARM - 1000 SOWS RELEASED
WHOLE HERD
Culled positives
2 negative 10% tests
Culled positives
Two consecutive negative 95-2 statistical tests.

(SLIDE 19) This is the MARSHAL HORNE FARM - 500 SOWS
STATISTICALLY RELEASED
100% test of infected parities
Culled positives
Two consecutive negative 95-2 statistical tests.

(SLIDE 20) This is **PIGGY FARM** - 700 SOWS RELEASED.
  **WHOLE HERD**
  Culled positives
  2 negative 10% tests

(SLIDE 21) This is **SAND FARM** - 700 SOWS RELEASED.
  **WHOLE HERD**
  Culled positives
  2 negative 10% tests

(SLIDE 22) This is then **SESSOMS FARM** 500 SOWS
  **STATISTICALLY RELEASED**
  100% test of infected parities
  Culled positives
  Two consecutive negative 95-2 statistical tests.

(SLIDE 23) This is **SUMMERLIN I & II** - 1200 SOWS
  **STATISTICALLY RELEASED** - BOTH FARMS SIMULTANEOUSLY
  100% test of infected parities
  Culled positives
  Two consecutive negative 95-2 statistical tests.

(SLIDE 24) This is **AYDEN 3&4** 4000 SOWS/10,000 NUR./17,000 FIN.
  DEPOP/REPOP OF SOWS
  MOVED NURSERY AND FINISHERS TO OTHER SITES.
  CLEANUP OF THESE OTHER SITES IS COMPLETED AS WELL.
  FARM HAS SINCE EXPANDED TO OVER 7000 SOWS

(SLIDE 25) This is **THE MCGUIRE FARM** - 1700 SOWS RELEASED.
  **WHOLE HERD**
  Culled positives
  2 negative 10% tests

(SLIDE 26) This is **FRENCHS CREEK**-2400 SOWS/7200 NUR. /10,000 FIN.
  100% INFECTION.
  CLEANED UP IN LESS THAN 1 YEAR FROM DAY OF BREAK
  DEPOP/REPOP
Challenges Facing North Carolina's Pseudorabies Program.

(SLIDES 27) We need to Increase not Decrease our emphasis on biosecurity: With the rapid expansion of North Carolina's swine industry biosecurity requires continued emphasis and vigilance. Pseudorabies is a wonderful identifier of biosecurity break downs because it is easily recognized. 95% of the breaks in the last year could have been prevented through proper biosecurity. Each break had at least one break down in biosecurity but most had many possible sources of infection.

As an industry we must develop biologically secure methods of dealing with arising problems, marketing programs and management methods. For example:

* Dead animal disposal-
* Improper vaccination techniques/programs/schedules
* Getting producers to spend the dollars to stop virus circulation immediately not just the clinical signs.
* Monitoring and evaluating evolving industry practices:
  - Dead Animal Disposal Units
  - Salvage trucks and barbecue trucks making multiple runs without regard to farm disease status.
* Lack of enough isolation units.

Conclusion:

As you can see North Carolina while still having a way to go, continues to move ahead in its PRV program. Thank you again for giving me the time to show the commitment, the effort and the progress North Carolina producers have made toward the eradication of Pseudorabies.
A feral swine PRV vaccination trial was recently completed on Ossabaw Island, Georgia. In this simulation of a large-scale population immunization, feral swine were hand vaccinated with a commercial gene deleted vaccine using a mark-recapture system. During the summers of 1992 and 1994, 60 to 80% of the herd was vaccinated. Prevalence of antibodies to the field strain of PRV in the vaccinated population, however, showed no significant decrease when compared to the control (unvaccinated) population. Additional work on Ossabaw Island is in progress to increase our knowledge on the epizootiology of this virus in feral swine populations. Attempts are also underway to improve virus detection systems using virus isolation and PCR techniques. Other work currently in progress, include evaluations of non-target species associated with oral bait delivery systems, the mapping of feral swine populations in Florida, Georgia, Texas, and California, and the identification of hunting preserves in the United States which utilize captive feral swine.
I am going to briefly respond to five of the six issues suggested by Chairman Gingerich, and then spend more time addressing, in detail, the issue regarding reinfected herds. I will address that issue last.

Basic Pseudorabies Eradication Program

Nebraska’s basic pseudorabies eradication program is simple, and similar to other state programs, in that it is based upon the detection of infected herds, and cleaning up infected herds. The program is consistent with the pseudorabies program standards. One phase of the Nebraska program that was extremely beneficial to our progress was the “assessment” period. During this two year period, all herds were required to conduct a monitored test for two consecutive years at the owner’s expense. This period gave us a good handle on the disease prevalence and location in the state at a minimum of state-federal funds.

In retrospect, we believe that we could have done better if we would have required monitoring of all herds at owner’s expense until the area reached Stage III status. Requiring such testing would have simplified our surveillance requirements in the Stage II area, and would have given more incentive for producers to work toward achieving Stage III status.

Coupled with good case finding, must be an aggressive clean up program. We require all quarantine herds to have a herd plan written within 30 days of the quarantine. Quarantine herds must be tested semi-annually, and our field staff must review and update the herd plan every six months.

Goal to Become Pseudorabies Free

We are still optimistic that we will be able to eradicate pseudorabies by the year 2000. Our progress has been very good. However, during the last two quarters, a slowing trend has been reflected in herds being released from quarantine.

Overhead

One reason for this leveling out of the trend line could be a lack of producer incentive. To correct this, we are proposing a change in Nebraska’s pseudorabies regulations which will require that a herd be off of quarantine (or be making acceptable progress in getting off of quarantine) within 24 months of the quarantine date. Herds that have not met this requirement will be required to begin whole-herd testing every six months and remove positive animal within 15 days. It is proposed that this testing will be at
owner's expense. If the owner refuses to test, the Bureau would have authority to test the herd and charge the cost of testing back to the owner. An additional incentive is to provide a herd cleanup allotment so the producer would have an option to use the money for depopulating the quarantined herd or testing during the 24 months. This would work by the Bureau of Animal Industry establishing, at the onset of the quarantine, an allotment that would be based on the number of breeding animals in the herd, times a factor, which would give an allotment amount. That amount could be used for cleanup testing or depopulating during the 24 months.

Nebraska Herds on Quarantine

There are currently 232 herds on quarantine, all of which are on herd plans.

Vaccine Usage

We encourage usage of vaccine in quarantine herds. Beginning in July, 1992, we limited the use of pseudorabies vaccine to g1 deleted vaccines with a companion test kit.

Regulations in Addition to Program Standards

Nebraska's pseudorabies regulations are consistent with the State-Federal-Industry Program Standards. However, a proposed change in Nebraska pseudorabies regulation which includes provisions for a herd cleanup allotment, establishes a 24-month time limit with mandatory test and removal, is an addition to the program standards. We believe, this will offer producers another option and encourage them to work harder for eradication.

Reinfected Herds

Since 1989, 1,346 swine herds have been quarantined for pseudorabies and 1,756 have been released from quarantine. Of the 1,346 herds quarantined, 126, or 8.8%, had previously been quarantined.

The summary of quarantined herds from January 1, 1989, to September 30, 1994, is as follows:

Herd Quarantine Status

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<tr>
<td>Previously quarantined</td>
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<td>Released from quarantine (1/1/89 - 9/30/94)</td>
<td>1,756 410</td>
</tr>
<tr>
<td>On Quarantine (9/30/94)</td>
<td>232</td>
</tr>
</tbody>
</table>
The following table is a summary of previously quarantined herds by year:

<table>
<thead>
<tr>
<th>Year</th>
<th>New Herds Quarantined</th>
<th>Herds Previously Quarantined</th>
<th>Percent Previously Quarantined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>310</td>
<td>15</td>
<td>4.84%</td>
</tr>
<tr>
<td>1990</td>
<td>267</td>
<td>17</td>
<td>6.37%</td>
</tr>
<tr>
<td>1991</td>
<td>374</td>
<td>35</td>
<td>9.36%</td>
</tr>
<tr>
<td>1992</td>
<td>239</td>
<td>33</td>
<td>13.80%</td>
</tr>
<tr>
<td>1993</td>
<td>80</td>
<td>16</td>
<td>20.00%</td>
</tr>
<tr>
<td>1994(%)</td>
<td>76</td>
<td>10</td>
<td>13.16%</td>
</tr>
<tr>
<td>TOTALS</td>
<td>1,346</td>
<td>126</td>
<td>9.36%</td>
</tr>
</tbody>
</table>

Herds quarantined in 1993 reflect a reinfection rate of 20% which seems unrealistically high. We looked at this year more closely to attempt to see if there was an explanation such as type of test or type of herd plan, etc., that could cause such a high reinfection rate.

The following tables summarize the data for the 16 reinfected herds found in 1993:

Interim between release of quarantine and re-quarantine:

<table>
<thead>
<tr>
<th>Interim</th>
<th>Number of Herds</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 6 months</td>
<td>3</td>
<td>18.75%</td>
</tr>
<tr>
<td>7-12 months</td>
<td>2</td>
<td>12.50%</td>
</tr>
<tr>
<td>13-24 months</td>
<td>4</td>
<td>25.00%</td>
</tr>
<tr>
<td>Greater than 24 months</td>
<td>7</td>
<td>43.75%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Primary type of test used to release the quarantine:

<table>
<thead>
<tr>
<th>Type of Test</th>
<th>Number of Herds</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>37.50%</td>
<td></td>
</tr>
<tr>
<td>GPX</td>
<td>37.50%</td>
<td></td>
</tr>
<tr>
<td>IDEXX/CLINEASE</td>
<td>6.25%</td>
<td></td>
</tr>
<tr>
<td>Depopulation</td>
<td>12.50%</td>
<td></td>
</tr>
<tr>
<td>Not available</td>
<td>6.25%</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>
Type of herd plan at the time of quarantine release:

<table>
<thead>
<tr>
<th>Type of Herd Plan</th>
<th>Number of Herds</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test and remove</td>
<td>7</td>
<td>43.75%</td>
</tr>
<tr>
<td>Random sample testing</td>
<td>7</td>
<td>43.75%</td>
</tr>
<tr>
<td>Depopulation</td>
<td>2</td>
<td>12.50%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Reasons given by field veterinarians for herds to be re-quarantined:

<table>
<thead>
<tr>
<th>Reason</th>
<th>Number of Herds</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neighborhood spread</td>
<td>9</td>
<td>58.25%</td>
</tr>
<tr>
<td>Purchased feeder pigs</td>
<td>2</td>
<td>12.50%</td>
</tr>
<tr>
<td>Did not roll the herd</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>Mystery</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>No response</td>
<td>3</td>
<td>18.75%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Summary

A review of the limited data for reinfected herds, found in Nebraska in 1993, does not readily identify factors which could have attributed to a 20% reinfection rate.

The point I would like to make, however, is that the question "how many herds have become reinfected after cleanup?" might imply to some that reinfected herds were not cleaned up the first time. Although incomplete cleanup could be a reason for reinfection, there are other factors, some of which are not easily identified, which may be more significant in causing reinfection.

I hope the data I have presented will help illustrate the complex nature of pseudorabies eradication.

Another point that I would like to make is that although the Pseudorabies Quarterly Report provides statistically valuable information as to what is happening in regard to a state's pseudorabies program to determine why it happened, it is necessary to do in-depth study, involving the herd veterinarian, producers, and regulatory veterinarians.

I believe, we can accomplish our goal of pseudorabies eradication by the year 2000. We must remember our commitment to the swine industry when this program was initiated and that was to "eradicate pseudorabies and not the swine producers." We can do that by keeping the program
simple, using a commonsense approach in working with the producer, and continuing to do the best job we can with the tools we have.

We have made great strides using that approach, and if we remain flexible enough to make adjustments as we go along to accommodate the unexpected, and new technology, we will be successful.
Basic PRV Eradication Plan - Indiana

A. Identify Infection
   1. Down-the-Road Testing
      a. annually in Stage II
      b. biennially in Stage III
      c. 9,525 herds tested annually
      d. 21,664 cumulative herds in database
   2. Change of ownership testing
   3. Qualified herd testing, currently 494 herds
   4. Exhibition Testing
   5. Import retests - as of 3/94 retests waived on swine from Stage III Qual., State IV or Stage V
   6. Circle Testing - Two (2) mile area around infected herds

B. Clean-up
   1. Quarantines written and Herd Clean-up Plan (HCP) completed within 90 days of quarantine.
   2. State-Federal Cooperation Plan - collaborative effort in all counties with PRV infected herds.
   3. Epidemiology Report completed on each herd quarantined.
   4. Fee-basis funds offered to producers with infected herds. Cost shared by state/federal budgets.
   5. Vaccine Program - State purchases 1/2 of vaccine for PRV positive finishing floors. We have committed to $300,000 for vaccine purchases. 30% of infected herds are participating in the vaccine program (132 herds).
   6. Items #4 and #5 made possible because of Indiana Port Producers Association's (IPPA) legislative effort that resulted in $500,000 to support the program.

C. Results
   1. Quarantined herds are at the lowest number in nine (9) years (January 1985, 433 quarantines).
   2. CY 94 shows a net loss of 43 quarantines (through October 1, 1994).
   4. Six (6) additional counties added to Stage III in 2/94, thereby reducing producer costs of testing by half.
   5. PRV funding from State legislature has been included in biennial budget request.
   6. Ongoing investigation of slaughter surveillance as possible method of surveillance for Stage III.
UPDATE ON THE PSEUDORABIES ERADICATION PROGRAM
IN THE STATE OF INDIANA

PSEUDORABIES QUARANTINED HERDS BY COUNTY
OCTOBER 1, 1994

17 COUNTIES
IN STAGE II,
386 QUARANTINES

75 COUNTIES,
IN STAGE III,
52 QUARANTINES

436 Total Quarantined Herds

COUNTIES WITH NO KNOWN INFECTION 55
COUNTIES WITH KNOWN INFECTION 37
### Swine Herds That Have Been Tested for Pseudorabies

**October 1, 1994**

<table>
<thead>
<tr>
<th>County</th>
<th>Negative Tested Herds</th>
<th>Quarantined Tested Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9,135</td>
<td>390</td>
</tr>
<tr>
<td>Total</td>
<td>9,525</td>
<td>1,250</td>
</tr>
</tbody>
</table>

**MAP:**
- **Top Number:** Includes herds testing negative for feeder pig law, down-the-road, and qualification.
- **Bottom Number:** Includes quarantined herds testing for down-the-road.

**Counties in Stage II:**
- 17 counties

**Counties in Stage III:**
- 75 counties

---

**Legend:**
- Numbers represent the count of tested swine herds per county.
UPDATE ON THE PSEUDORABIES ERADICATION PROGRAM
IN THE STATE OF INDIANA

PREMISES QUARANTINED FOR PSEUDORABIES
INFECTION BY YEAR

<table>
<thead>
<tr>
<th></th>
<th>NET GAIN (LOSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JANUARY 1981</td>
<td>242</td>
</tr>
<tr>
<td>JANUARY 1982</td>
<td>304</td>
</tr>
<tr>
<td>JANUARY 1983</td>
<td>325</td>
</tr>
<tr>
<td>JANUARY 1984</td>
<td>384</td>
</tr>
<tr>
<td>JANUARY 1985</td>
<td>433</td>
</tr>
<tr>
<td>JANUARY 1986</td>
<td>473</td>
</tr>
<tr>
<td>JANUARY 1987</td>
<td>571</td>
</tr>
<tr>
<td>JANUARY 1988</td>
<td>709</td>
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<tr>
<td>JANUARY 1989</td>
<td>822</td>
</tr>
<tr>
<td>JANUARY 1990</td>
<td>828</td>
</tr>
<tr>
<td>JANUARY 1991</td>
<td>814</td>
</tr>
<tr>
<td>JANUARY 1992</td>
<td>783</td>
</tr>
<tr>
<td>JANUARY 1993</td>
<td>657</td>
</tr>
<tr>
<td>JANUARY 1994</td>
<td>481</td>
</tr>
<tr>
<td>OCTOBER 1, 1994</td>
<td>438</td>
</tr>
<tr>
<td>COUNTY</td>
<td>VAC</td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>ADAMS</td>
<td>19</td>
</tr>
<tr>
<td>BOONE</td>
<td>2</td>
</tr>
<tr>
<td>CARROLL</td>
<td>77</td>
</tr>
<tr>
<td>CASS</td>
<td>21</td>
</tr>
<tr>
<td>CLINTON</td>
<td>47</td>
</tr>
<tr>
<td>DECatur</td>
<td>32</td>
</tr>
<tr>
<td>HOWARD</td>
<td>22</td>
</tr>
<tr>
<td>Huntington</td>
<td>5</td>
</tr>
<tr>
<td>Kosciusko</td>
<td>12</td>
</tr>
<tr>
<td>Miami</td>
<td>20</td>
</tr>
<tr>
<td>Rush</td>
<td>17</td>
</tr>
<tr>
<td>Shelby</td>
<td>5</td>
</tr>
<tr>
<td>Tippecanoe</td>
<td>11</td>
</tr>
<tr>
<td>Wabash</td>
<td>37</td>
</tr>
<tr>
<td>Wells</td>
<td>8</td>
</tr>
<tr>
<td>White</td>
<td>11</td>
</tr>
</tbody>
</table>

GRAND TOTAL  346  88%  348  89%  350  89%  391
### PRV Quarantined Herds County Report - Stage 3

<table>
<thead>
<tr>
<th>COUNTY</th>
<th>VAC</th>
<th>%</th>
<th>PRO</th>
<th>%</th>
<th>HCP</th>
<th>%</th>
<th>HERDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen</td>
<td>10</td>
<td>100%</td>
<td>9</td>
<td>90%</td>
<td>10</td>
<td>100%</td>
<td>10</td>
</tr>
<tr>
<td>Bartholomew</td>
<td>6</td>
<td>85%</td>
<td>7</td>
<td>100%</td>
<td>7</td>
<td>100%</td>
<td>7</td>
</tr>
<tr>
<td>Benton</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>DeKalb</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Elkhart</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>50%</td>
<td>1</td>
<td>50%</td>
<td>2</td>
</tr>
<tr>
<td>Fulton</td>
<td>2</td>
<td>50%</td>
<td>4</td>
<td>100%</td>
<td>4</td>
<td>100%</td>
<td>4</td>
</tr>
<tr>
<td>Jasper</td>
<td>2</td>
<td>100%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
</tr>
<tr>
<td>Jennings</td>
<td>0</td>
<td>0%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
</tr>
<tr>
<td>Lagrange</td>
<td>0</td>
<td>0%</td>
<td>4</td>
<td>100%</td>
<td>2</td>
<td>50%</td>
<td>4</td>
</tr>
<tr>
<td>Lake</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>La Porte</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Marshall</td>
<td>1</td>
<td>50%</td>
<td>1</td>
<td>50%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
</tr>
<tr>
<td>Morgan</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Newton</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Noble</td>
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<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>1</td>
</tr>
<tr>
<td>Pulaski</td>
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<td>0%</td>
<td>0</td>
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<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Ripley</td>
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<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Tipton</td>
<td>3</td>
<td>60%</td>
<td>5</td>
<td>100%</td>
<td>5</td>
<td>100%</td>
<td>5</td>
</tr>
<tr>
<td>Union</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Wayne</td>
<td>0</td>
<td>0%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
</tr>
<tr>
<td>Whitley</td>
<td>2</td>
<td>100%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
<td>100%</td>
<td>2</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>30</strong></td>
<td><strong>57%</strong></td>
<td><strong>47</strong></td>
<td><strong>90%</strong></td>
<td><strong>48</strong></td>
<td><strong>92%</strong></td>
<td><strong>52</strong></td>
</tr>
</tbody>
</table>
## State Progress in National Pseudorabies Program

States enrolled in the program and the stage of each are as follows:

<table>
<thead>
<tr>
<th>State I</th>
<th>State II</th>
<th>Split States</th>
<th>State III</th>
<th>Stage IV</th>
<th>Stage V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Illinois</td>
<td>Stage II/III</td>
<td>Alabama</td>
<td>Delaware</td>
<td>Alaska</td>
</tr>
<tr>
<td>Iowa</td>
<td>Indiana</td>
<td></td>
<td>Arkansas</td>
<td>Nevada</td>
<td>Connecticut</td>
</tr>
<tr>
<td>Kansas</td>
<td>Michigan</td>
<td></td>
<td>Arizona</td>
<td>South Carolina</td>
<td>Idaho</td>
</tr>
<tr>
<td>Missouri</td>
<td>Pennsylvania</td>
<td>Minnesota</td>
<td>Colorado</td>
<td>Washington</td>
<td>Maine</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>Rhode Island</td>
<td>North Carolina</td>
<td>Hawaii</td>
<td>Missippi</td>
<td>Montana</td>
</tr>
<tr>
<td>US Virgin Islands</td>
<td>US Virgin Islands</td>
<td>Stage III/IV</td>
<td>Louisiana</td>
<td>New Mexico</td>
<td>North Dakota</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maryland</td>
<td>New York</td>
<td>Oregon</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wisconsin</td>
<td>Utah</td>
<td>Wyoming</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Massachutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>New Hampshire</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>New Jersey</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ohio</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oklahoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>South Dakota</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tennessee</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Texas</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vermont</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Virginia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>West Virginia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 1 | 8 | 5/1 | 21 | 4 | 12 |

October 1994
REPORT OF THE DIAGNOSTICS AND BIOLOGICS
SUBCOMMITTEE PSEUDORABIES COMMITTEE


The subcommittee met on November 1, with 9 of 11 members present, to continue discussion of factors associated with problem cleanup herds. In 1993, the committee developed data for 42 herds that had not eliminated pseudorabies virus (PRV) within 3 years of initiation of a cleanup program. In evaluating that information, the committee decided that a matched cohort of herds was needed to determine if risk factors of the problem herds were common to herds that had successfully eliminated PRV with the same type of vaccine. Accordingly, data was developed for 14 herds of the same size and type of production. We anticipate additional herd data sheets to be completed within the next 3 months. Results of these evaluations will be evaluated for statistical significance and will be submitted to the Journal of the American Veterinary Association for publication.

Forty of the 42 problem cleanup herds used GI-deleted vaccine, while 2 used gpX-deleted vaccine. Average cleanup time for the 14 successful cleanup herds was over 3 years in duration, which will pose a problem for successful completion of the national PRV eradication program by the target date of the year 2000. For seven of the success herds using GI-deleted vaccines, the average time required for cleanup was in excess of 5 years, with an average time of 5+ years and a range of 4 to 6 years. From initial analysis of the committee's data it was clear that herd and vaccine management were the most critical factors for the time required to successfully remove PRV from a herd.

In addition to the risk factor evaluation, two committee members presented current research information on the sensitivity of differential diagnostic assays for 55 experimentally infected swine with latent PRV infection, and the ability of gpX-deleted and GI-deleted vaccine viruses to colonize trigeminal ganglia. Data was also presented on the ability of 2 types of GI-deleted vaccine virus to block infection of the trigeminal ganglia by a highly virulent strain of PRV (Becker).

GI and GIII assays had 100 percent sensitivity in comparison with screen ELISA, latex agglutination, autolex and PCFIA, as well as 1:2 serum neutralization. IDEXX gpX detected 50 of 55 latently infected animals, while Agdia gpX detected 52 of the infected animals.

Vaccine virus colonization was most dramatic for gpX-deleted vaccine (not GI, GX-deleted). In 4 of 10 vaccinated animals gpX-deleted vaccine had the same ability to infect trigeminal ganglia as extremely virulent PRV. On a cell-equivalent basis, Bartha GI-deleted vaccine virus at an intranasal
vaccination dose of $10^7$ TCID$_{50}$ was able to colonize ganglia with 50% of the efficiency of the virulent PRV inoculum (Becker at $10^5$ TCID$_{50}$ intranasal). That same dose of Bartha was able to completely block colonization of ganglia by the Becker virus.

A proposal by USDA was also considered by the subcommittee, regarding whether the GI differential diagnostic assay should be classified as an official diagnostic test. Based upon the experience of the committee members and the data presented in our subcommittee meeting on sensitivity of the GI assay, the subcommittee endorses said proposal, and recommends that the GI assay be recognized as an official diagnostic test. Dr. Ludeman abstained from this action of the committee.
Right to Farm Act

On July 11, 1981, the Michigan Legislature passed the Michigan Right to Farm Act. The purpose of the Act is to provide circumstances under which a farm shall not be found to be a public or private nuisance. The legislation was initiated in an attempt to give farmers protection from being sued by their neighbors who may object to the odors, dust, and other activities associated with normal farming operations. In order to gain the legal bar afforded by the law, a farm must meet two conditions:

1) The farm must conform to generally accepted agricultural and management practices (Practices).
2) The farm must have not been considered a nuisance prior to a change in land use around the farm.

Without written Practices the law proved ineffective since Circuit Court Judges were not able to ascertain generally accepted practices. This problem was overcome with the 1987 amendments to the Right to Farm Act. This amendment laid the groundwork for the Practices to be defined.

Right to Farm Practices

The responsibility for defining generally accepted agricultural and management practices as authorized by the Right to Farm Act rests on the Michigan Commission of Agriculture. After identifying a need for a specific set of management practices, the Commission requests that Michigan State University College of Agriculture and Natural Resources assemble a task force to draft the Practices. These task forces are comprised of experts from agricultural commodity groups, government agencies, and MSU. The Commission requests that the task force meet three main objectives when developing the Practices:

1) The Practices must be scientifically based.
2) The Practices must be protective of the environment.
3) The Practices must not put Michigan Farmers at a competitive disadvantage with farmers from other states.

To date, Practices have been adopted for Manure Management, Pesticide Utilization, and Nutrient Utilization. These Practices should not be construed as Best Management Practices, but, rather, as the base level of management necessary to ensure the protection of the environment and
public health. The Practices are not rules or regulations, therefore, neither the Michigan Department of Agriculture, the Michigan Department of Natural Resources, nor any other government agency has legislative authority to enforce them.

Conformance with the Practices is strictly voluntary, however, there are many advantages for farmers who follow them. In addition to gaining protection from civil nuisance suits, conformance with the Practices also provides farmers with exemption status from several state environmental laws and permitting policies. These include the Michigan Air Pollution Control Act, The Michigan Environmental Response Act, and State Discharge Permit Policy.

Environmental Complaint Response

On June 20, 1989, the Michigan Department of Natural Resources and the Michigan Department of Agriculture entered into a Memorandum of Understanding regarding the investigation of pollution complaints filed against farms. This memorandum names the Department of Agriculture as the primary investigator for all non-emergency agriculturally related pollution complaints. A non-emergency is considered to be all complaints that do not involve a significant direct discharge of pollutants to the waters or air of the state.

Under the memorandum, if MDA substantiates a pollution problem while responding to a complaint, the farmer is given 60 days to work with MDA on a voluntary basis to develop a remediation plan and implementation schedule to address the identified problem. Should the farmer choose not to correct the pollution problem per the conditions of the memorandum, the complaint goes back to the Department of Natural Resources for investigation under standard pollution investigation policy.

Environmental complaint response by MDA has averaged 140 new complaints per year over the past four years. Of these, approximately 60% were found to have a verifiable pollution or nuisance problem while the other 40% were classified as not verified. The majority of the complaints concern pollution to surface waters, followed closely by air pollution and finally ground water.

Role of the Farmer

Agriculture as an industry, and at the farm level, must be sensitive to the perception of the general public. Publicity surrounding recent media events, whether justified or not, has heightened the concern over modern production agriculture management techniques. The appearance and environment around a farm will effect the perception and attitudes of surrounding neighbors. If that appearance is negative, the resulting conflicts can also effect other farms in the area. Many of the agriculturally restrictive Township and Local Ordinances seen today were initiated by the problems
associated with a single area farm. It is imperative that all farmers do their part to protect the positive image of agriculture.

The Right to Farm Act does not give sanctuary to farmers who pollute. No industry, farm, or citizen has the legal right to cause pollution. Farmers shouldered the responsibility for the stewardship of their land and the air and water associated with it. Fortunately, because of their close ties to the land, farmers in general, are astute conservationists and very much aware of their responsibilities in caring for and maintaining the land.

In addition to following the Practices, simply being a good neighbor is an effective way to avoid litigation. This includes an effort to keep neighbors informed of modern farm practices and how they protect the environment. Common courtesy such as avoiding manure spreading on weekends and holidays, and paying attention to weather conditions such as wind direction will also help in avoiding nuisance suits.

Unfortunately, despite the best of efforts, some farmers may still find their management challenged. In these cases, the survival of the farm may depend upon the farmers ability to defend his management practices. Implementation of a Waste Utilization Plan, developed by the local USDA Soil Conservation Service is an important first step. In addition, good record keeping is paramount to the ability to defend an operation in court. A successful record keeping system should enable a farmer to demonstrate that he has managed his farm in conformance with the Right to Farm Practices. Record keeping systems are available through local Cooperative Extension Service Offices. Finally, if a farmer is named in nuisance litigation, he should procure the services of a competent attorney.

Voluntary conformance with the Right to Farm Practices provides Michigan farmers with protection from nuisance litigation and exemption from several state environmental laws. Also, in many cases, following the Practices will save producers money by maximizing the efficiency of nutrient utilization. You can obtain copies of the Michigan Right to Farm Practices by mailing a request to: Right to Farm, Michigan Department of Agriculture, P.O. Box 30017, Lansing, Michigan 48909.
Overview of Foodborne Disease

Foodborne disease is a common and preventible cause of morbidity in the United States. Although estimates of the yearly incidence of foodborne illness vary widely, all are large ranging from 6.5 to 33 million cases of illness (1), and 6,000 to 9,000 associated deaths (2,3). *E. coli* O157:H7 infections have been estimated to cause approximately 8,000 to 20,000 cases of illness and 150 to 390 associated deaths annually (2).

In addition, foodborne illnesses incur substantial costs to ill persons, food producers, and the national economy. These costs have been estimated at $7.7 to $8.4 billion annually (4).

O157 Illness and Its Complications

The public health consequences of O157 infections are important. The illness it causes in people is frequently severe and sometimes kills, although more culture positive asymptomatic persons are being reported. The illness is often misdiagnosed, the organism has been found on every continent, and the frequency of human illness is increasing.

The classic illness due to O157 consists of bloody diarrhea with severe abdominal cramps and little or no fever. O157 can also cause mild nonbloody diarrhea. Hemolytic uremic syndrome or HUS is the most important complication.

The Organism and Its Detection

*E. coli* are gram negative bacilli and are named based on the cell wall or “O” antigen and the flagella or "H" antigen.

*E. coli* O157:H7 is one of hundreds of strains of *E. coli* that are part of the normal flora of the intestinal tract of humans and other warm-blooded species. However, special medias (Sorbitol-MacConkey) are necessary to distinguish O157.

O157 Outbreaks and Transmission

Eighteen outbreaks of O157 were reported in the U.S. from 1982 to 1992 (5). It's important to remember that most patients with O157 infection are sporadic cases and not part of a recognized outbreak.

Three major modes of transmission of O157 have been identified—
food, water, and person-to-person. The last two suggest a low infectious
dose. In the 1993 outbreak in the Western U.S., studies indicated infec-
tions were caused by less than 100 organisms (6).

Most O157 outbreaks have been linked to food and ground beef has
been implicated most often. Unchlorinated municipal drinking water, swim-
mimg in a contaminated lake, apple cider, vegetables, and salad bars have
also been implicated.

Seventeen clusters or outbreaks of infection were reported from 13
states for 1993 (5).

Twenty-seven clusters or outbreaks of infection have been reported
from 20 states in 1994 (5). An increase in reporting is apparent as more
states develop the laboratory and epidemiologic capability to recognize
O157.

Cattle As a Reservoir

Since O157 colonized cattle show no apparent clinical signs or lesions,
O157 may not be considered a bovine pathogen and, in fact, cattle may be
an incidental host and not the reservoir for O157.

Dr. Dale Hancock at Washington State University established baseline
comparative data on the prevalence of O157 in Washington State cattle
populations. In his studies, findings included that post-weaning dairy heif-
ers were the most likely group to yield O157; data suggested that beef
cattle may be a larger reservoir than previously thought; shedding is tran-
sient; and herd O157 status can change (7,8).

As part of the 1991-92 USDA, APHIS, VS, NAHMS National Dairy Heifer
Evaluation Project, fecal sampling for O157 was conducted in dairy herds
in 28 states in order to estimate national and regional prevalence and to
provide insights regarding risk factors for infection. Findings included that
O157 was found in 19 or 1.8% of the sampled herds and no evidence of
geographic clustering or seasonal trend was evident (9).

A follow-up study to the NAHMS Dairy Heifer Survey examined the
shedding patterns in infected herds and management factors associated
with infection. Findings supported the change of herd O157 status (10).

Overall, from the APHIS and Washington State University studies, the
percent of O157 infected herds has been estimated to be < 10%. This
implied that control measures, whether they are environmental and/or nu-
tritional, may need to be applied to a small number of herds.

The NAHMS Project and the Washington State University studies found
the following tentative associations between management variables and
O157 herd colonization:

1. Use of fresh manure slurry on pastures grazed by heifers
2. Ionophore feed supplementation; and
3. Computerized grain feeding in the milking parlor

A study which began this summer in Oregon, Washington, and Idaho
by APHIS and Dr. Dale Hancock (Tri-State E. coli O157:H7 Project) will evaluate the association of two of these risk factors, manure slurry and ionophores, with dairy herd O157 status.

At present, no definitive cause and effect relationships are established concerning the ecology of O157 in cattle populations.

The USDA, Food Safety and Inspection Service (FSIS) Microbiological surveys include the 1992-1993 FSIS Nationwide Beef Microbiological Baseline Data Collection Program for Fed Cattle or Steers and Heifers. Of the 2,081 carcasses sampled, 4 or 0.23% were positive for O157 (17). Additional FSIS surveys underway include the cow/bull carcasses baseline sampling; the nationwide ground beef survey; and the disabled or downer cow study.

Research needs for a Preharvest or farm-to-slaughter O157 Food Safety Program include prevalence studies, diagnostic studies, and experimental modeling.

Some of these research areas are being addressed by the USDA, Agricultural Research Service, National Animal Disease Center, in Ames, IA.

In addition to the Tri-State E. coli O157:H7 project, the USDA, APHIS, VS, NAHMS, Cattle On Feed Evaluation (COFE) is underway in 13 primary cattle feeding states. COFE objectives, relative to O157 and Salmonella, include prevalence of shedding, determination of risk/protective factors, and shedding patterns.

In summary—
1. E. coli O157:H7 can cause severe illness and death in humans. Only a small inoculum is needed to cause disease.
2. Laboratory diagnosis of O157 is critical in evaluating and treating patients and in detecting outbreaks so that further transmission can be stopped.
3. Ground beef is the major food vehicle. However, illnesses have been linked to other foods, and other modes of transmission are being recognized.
4. There are tremendous gaps in our knowledge regarding pre- and postharvest critical control points for O157, therefore additional epidemiologic and research studies are needed.

References


REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND ENVIRONMENTAL QUALITY

Chairman: Dr. John C. New, Knoxville, TN
Vice Chairman: Mr. Larry D. Woodson, Topeka, KS

Dr. Ronald D. Anderson, NV; Dr. Bill F. Bamum, OK; Dr. George W. Beran, IA; Dr. Douglas L. Berndt, DC; Dr. Lee M. Brooks, GA; Dr. Thomas C. Bunting, IL; Dr. Stanley L. Diesch, MN; Dr. Don A. Franco, VA; Dr. Stanley L. Hendricks, MN; Dr. John P. Honstead, MD; Dr. William T. Hubbert, MD; Dr. William James, DC; Dr. William E. Jennings, TX; Dr. Tari P. Kindred, MD; Dr. J. C. Leighty, MD; Dr. Herbert E. Little, CA; Dr. Anne MacKenzie, CAN; Dr. Edward L. Menning, DC; Brig. Gen. T. G. Mumane, TX; Dr. Gary Oltmans, MN; Dr. Morris E. Potter, GA; Dr. John C. Prucha, MD; Dr. Robert D. Ragland, VA; Dr. William W. Rosser, TX; Dr. Parmesh K. Saini, MD; Dr. John C. Sawyer, CA; Dr. Dale F. Schwindaman, MD; Dr. Robert H. Singer, KY; Dr. C. D. Stumpff, KS; Dr. Lewis P. Thomas, WV; Dr. Manuel A. Thomas, Jr., TX; Dr. Lyle P. Vogel, IL; Dr. Janice Webb, FL.

The Committee met at 1:35 p.m. in the Thomapple Room, Amway Grand Plaza Hotel, Grand Rapids, Michigan, November 2, 1994. Dr. John New, University of Tennessee, convened the meeting. There were 29 people attending which included 15 Committee members. Dr. New explained how interested individuals could become members of the Committee and requested items for the USAHA Newsletter.

Dr. Karen M. Wemette, Staff Consultant to the AVMA Committee on Environmental Affairs, gave a report on the newly formed Committee. It has met three times to address the veterinarian's role related to environmental interaction with animals, legislative and regulatory issues associated with the environment, conservation, hazardous and toxic waste, and recycling. The Committee is charged with identifying the veterinarians' contribution to conservation of the environment, determining the effect of environmental regulations on the profession, and creating educational material relating to veterinary and animal environmental issues. Specific issues the Committee plans to focus on are the need for research on composting of large and companion animals as a means of carcass disposal, development of an environmental forum on NOAH, and the "greening" of veterinary medical offices. The need for a video tape on safe handling and disposal of pesticides and expanded AVMA preceptorship stipends for veterinary students are other areas of focus.

Dr. Thomas M. Gomez, Veterinary Medical Officer/Epidemiologist with USDA, APHIS, VS, is currently a staff epidemiologist with the Centers for Disease Control and Prevention. He made a presentation titled "Escherichia coli O157:H7 Infections-An Update." A summary of Dr. Gomez's presentation is included in the proceedings.
"The FSIS Microbiological Initiatives" was the title of a presentation by Dr. Jill Hollingsworth, Director, FSIS Health Affairs Staff. The presentation covered four areas. 1) Baseline Studies: A study of the pathogens found on cow and bull carcasses is being conducted similar to the recently completed steer/heifer carcass study. A poultry carcass study is currently underway and a swine carcass study is planned for 1995. A ground beef survey has been completed. It consisted of 1200 samples, half collected from retail sites and the other half from federal processing plants. None of the samples were positive for \textit{E. coli} 0157:H7. 2) \textit{E. coli} Testing: If \textit{E. coli} is found in raw, ground, or comminuted beef it is considered adulterated. Consequently, it must be cooked or condemned. If found in a commercial site or in the distribution system, a Class 1 recall is required. Tests for \textit{E. coli} 0157:H7 will be done on 5000 samples, half from retail sites and half from federal plants. Samples will also be taken from state plants and imported product. Half of the samples from federal plants will come from plants that have been randomly selected. The other half will come from plants that have been targeted because of previous positives, plants in areas where outbreaks have occurred, and other areas where more information is needed. An ELISA screening test will be used to classify samples as negative or presumptive positive. Presumptive positives will be tested further for confirmation. 3) Proposed Regulations: Draft regulations are being written to set guidelines and/or targets. Microbiological testing will be incorporated into daily practices within the inspection program but there are no plans this year to develop mandatory standards for specific pathogens. Instead, regulations will be aimed at reducing the occurrence of pathogens. For example, if 25 percent of carcasses are contaminated with a specific pathogen, the target might be to reduce (as a first step) the prevalence to 12.5 percent of plants within a specific time period. 4) Pathogen Reduction Legislation: Current laws would be enhanced. The Secretary, USDA, would be required to use the best available scientific data to reduce pathogens. New regulations would be issued within 2 years. Public health goals will be the basis of limits and microbiological testing will provide the data to set limits. End product testing will be part of the microbiological testing for verification but is no guarantee of safety of products. This type of testing is being done to stimulate the industry to do their own testing, encourage industry to come up with innovations and technology (as they have done before) to reduce pathogens, and to stop contaminated product from going to the consumer. Prevention will continue to be essential since cures are not available for some pathogens. Prevention strategies will allow FSIS to detect problems early and hopefully prevent deaths.

Dr. Mahlon W. Vorhies, Head and Director, Kansas State University Department of Veterinary Diagnostic Investigation, reported on "Inappropriate Addition of Distilled Alcohol Waste Residue to Dried Distillers Grain." The acute Kansas feedlot cattle deaths illustrate the value of diagnostic
In April, 1994, in southcentral Kansas, illness and deaths occurred in three feedlots. The clinical history included acute depression, anorexia, respiratory dyspnea, and locomotor problems (stiff and stilled gait). Lesions reported from necropsy included acute myocardial hemorrhage, intralobular pulmonary edema, swollen liver, with evidence of hemorrhage in areas of the intestine and hemoglobinuria. Review of the rations of the three farms illustrated a commonality of Rumensin supplementation, a forage or grain supplement and dried distillers grain (DDG). The Rumensin was from two different feed mills and different manufacturing lots. Chemical analysis revealed all of the Rumensin supplements were within the manufacturing label and no evidence of any error in supplementation was present from the computerized records. There were no similarities among the forage materials. The DDG was similar in that all premises received loads during the same manufacturing period. Analyses of the DDG, grain and forage samples were negative for chlorinated hydrocarbons, organophosphates, mycotoxins and selenium. Investigation of the DDG production revealed that an alcohol product had been distilled and the residue added back to the DDG during the drying process. Chemical analysis identified the analogues of erythromycin as well as other products in the alcohol material. In May, site visits revealed chronically affected animals remaining in the lots. They had large edematous briskets and other evidence of cardiovascular failure. Recent arrivals were not affected and served as on-site controls. A literature review revealed that ionophore toxicity can be potentiated with macrolide antibiotics. The question is raised about alcohol waste residue transportation across state lines and what governmental agencies have the responsibility for tracking such material. During July, 1994, a feeding trial was conducted at Kansas State University. The trial consisted of six groups. The basic premise was to evaluate the current DDG production against the suspected feed delivered to the three different farms which contained materials from the alcohol residue. The three groups which were fed DDG with alcohol addition or residue blended with the DDG, or the original suspected DDG, when fed with Rumensin created the exact clinical picture and lesions present at the three farms where the highest losses occurred. Deaths did not occur with any of the DDG or alcohol supplemented feed if Rumensin was not present in the ration. Therefore, we have concluded that some material present in the alcohol which contained erythromycin analogues when fed with Rumensin increased the toxicity of the ionophore at levels which were non-toxic if the alcohol product was not present. Summary of our clinical investigation and clinical trial suggests that Rumensin plus the alcohol residue containing erythromycin analogues was responsible for the death losses in the DDG contamination. Questions raised by this investigation concerned the disposal of biohazardous materials and the tracking of them by federal and state agencies. Another question that was raised is the
problem with contaminated products which result from feeding contaminated DDG to animals which do not die and have products which enter the food chain. This investigation illustrates the value of normal surveillance which occurs through the state-wide system of state supported diagnostic laboratory investigative activities.

Dr. Kurt D. Thelen, Right to Farm Program Manager, Michigan Department of Agriculture, made a presentation titled “Production Agriculture Environmental and Nuisance Management in Michigan: The Right to Farm Act.” His presentation is included in the Proceedings.

Dr. R. A. Robinson, Professor of Clinical Epidemiology, University of Minnesota, reported on “Disposal of Dead Piglets-A New Approach Using Homogenization.” The swine industry in Minnesota is facing a greater problem in the disposal of dead pigs with the trend to increasing herd size and total confinement operations. While the majority of deaths occur in the preweaning phase, the actual weight of carcasses to be disposed of is still very significant. Experiments were performed to determine acceptable loading rates into a liquid waste handling system with homogenized dead piglets and the fate of pig tissue in a typical handling system, and the survival of selected swine pathogens using artificially contaminated dead piglets. Pathogens used were pseudorabies virus, porcine reproductive and respiratory syndrome virus, and *Salmonella anatum*. A commercial homogenization system was used to dispose of dead piglets in existing swine waste handling systems. Addition of piglet dry matter to tanks containing liquid swine wastes at 0, 1, 2, or 4 percent of the dry matter in the tank had no effect on the breakdown of dry matter in the tanks. *S. anatum* introduced in swine waste via homogenized piglets survived for less than 7 days. Experiments using the viruses will be repeated because the amounts of virus used initially may have been too small to detect in liquid waste. These preliminary results indicate that introduction of homogenized dead piglets into existing swine waste handling systems is a practical disposal alternative for piglet carcasses.

Dr. Stanley L Diesch, Director of International Programs, University of Minnesota, reported on the Eighth Congress of the International Society for Animal Hygiene held in September, 1994, in St. Paul, Minnesota. A limited number of copies of the proceedings are available ($60 U.S.) from the Outreach Programs, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, 55108-6068, (612) 624-2268. The Ninth Congress will be held in Helsinki in August, 1997 and the Twentieth in the Netherlands in 2000.

The Committee business meeting began with an update on the 1993 resolution on tuberculosis in non-human primates. The Committee also discussed the possibility of merging with one or more other committees. There was enough support within the Committee to continue to explore options during the coming year. Dr. New requested suggestions for topics for the Reno meeting and then adjourned the meeting at 5:30 p.m.
The Committee on Public Relations met at 5:00 p.m. on Sunday, October 30, 1994, in the Thomapple Room of the Amway Grand Plaza Hotel. There were six committee members and one guest present.

The chairman gave a report outlining the activities of the committee during the year. Those activities included an attempt at making the quarterly newsletter more interesting and current by including articles of interest on subjects and events that had occurred since the last meeting. Copies of the proceedings were sent to all veterinary schools for inclusion in their libraries. As this year's meeting date approached, the chairman sent a letter to each dean of the colleges of veterinary medicine inviting them and members of their faculty to attend the upcoming meeting.

Mr. Neal Black reported that he had made several mailings to over three hundred media contacts throughout the country advising them of the date and location of this year's meeting. As the meeting date approached, he contacted the media people again with the same information and also included a convenient request form for them to select committee reports or speaker's texts they might like sent to them. Mr. Black reported that he had had requests for approximately three hundred committee reports and two hundred speaker texts. It was noted that press registrations are complimentary and that there had been a slight increase in their registration this year.

The chairman asked for any suggestions from the committee members on new initiatives that might be tried this year. One suggestion that was put forward was to have a Regional Column for items of local interest to be included in the newsletter. Another suggestion was to send a supply of U.S.A.H.A. brochures to each Area Veterinarian in Charge to be included with other hand-out items given to every newly accredited veterinarian.

The chairman appointed a subcommittee to look into the possibility of some form of a more modern, up to date, easily transportable and accessible form of promotion for our organization. This new promotion vehicle would be available for use in veterinary schools, state veterinary medical associations, industry meetings, etc. The chairman also stated that later in the year, he hoped to name a committee to start planning the 1996 centennial meeting.

There being no further business, the meeting adjourned at 5:50 p.m.
REPORT OF THE COMMITTEE ON RABIES

Chairman: Dr. Nancy A. Frank, Lansing, MI
Vice Chairman: Dr. Robert B. Miller, Gaithersburg, MD

Dr. Deborah J. Briggs, KS; Dr. H. Michael Chaddock, MI; Dr. Donald S. Davis, TX; Dr. E. P. J. Gibbs, FL; Dr. Keith N. Haffer, SD; Dr. Stanley K. Harris, IA; Dr. Richard E. Hill, IA; Dr. Owen James, MT; Dr. John C. New, TN; Dr. Charles E. Rupprecht, GA; Dr. Leon H. Russell, Jr., TX; Dr. Lyle P. Vogel, IL; Dr. James C. Wright, AL.

The Committee on Rabies met at 1:30 p.m. on Monday, October 31, 1994, in the Winchester Room of the Amway Grand Plaza Hotel, Grand Rapids, Michigan.

Our first speaker was Dr. Charles Rupprecht from the Centers for Disease Control and Prevention (CDC). Dr. Rupprecht updated the committee on rabies activities at CDC. In 1993, there were 9,498 cases of rabies reported to CDC. There continues to be 3 rabies epizootics occurring in the United States. These are raccoon rabies on the east coast, skunk rabies in the north and south central regions, and coyote rabies in Texas.

CDC continues to be involved with a variety of rabies activities. These include research, acting as a reference laboratory, providing support to state and local health departments, providing formal consultations, and participating in WHO activities. Current research areas include looking at the pathogenesis of rabies, studying rabies in coyotes, and looking at new vaccines and vaccine strategies for humans and animals.

Dr. Rupprecht reviewed human cases of rabies in the United States. Since 1981, there have been 21 humans diagnosed with rabies. Nine of these cases involved exposure abroad. Most of these 9 cases were due to a canine strain of rabies. Of the 12 cases involving exposure in the United States, 9 were due to a bat strain of rabies. This included 7 cases of Silver Hair bat rabies and one each of Big Brown and Mexican Free Tail bat rabies. So far in 1994, there have been 3 cases of rabies in humans in the United States. These occurred in California, Florida, and West Virginia. Details of these cases were discussed.

Dr. Rupprecht indicated that while raccoons are the most significant rabies reservoir in the United States, there have been no human deaths from raccoon rabies. This may be due to a number of factors including the relatively new occurrence of this reservoir, better post-exposure treatment, and better vaccination of dogs and other domestic animals. Bats continue to be the primary source of human rabies. CDC has several recommendations to address the bat rabies concern. These are public education, mandating companion animal vaccination, especially "bat catching" cats, pre-exposure vaccination of humans involved in high-risk activities, prompt
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and proper post-exposure follow up, speciation of bats submitted for rabies testing, and bat-human interaction management.

Our next speaker was Dr. Robert Miller from USDA, APHIS, BBEP. Dr. Miller reviewed the history and current status of the vaccinia vector rabies vaccine. The vaccine has been used in several trials in the United States. Further, trials and experimental programs utilizing the vaccine are being proposed and planned. Studies are underway in Texas to develop a program to utilize the vaccine to help halt the spread of coyote rabies. At this point, licensure of the vaccine is awaiting completion of the environmental assessment review process.

Our third speaker was Dr. Mary Grace Stobierski from the Michigan Department of Public Health. Dr. Stobierski reported on bat rabies in Michigan. Since 1980, bats have accounted for about 80% of the total positive rabies cases in Michigan. In 1993, of 2,045 animals tested for rabies, 246 (10%) were bats. Of all these animals tested, however, the vast majority of positive animals were bats (89%). Specifically, there were 16 positive bats, 1 positive cat, and 1 positive skunk. All the positive bats, as well as the vast majority of bats submitted for testing, were Big Brown bats, Eptesicus fuscus. When looking at exposure data, based on history provided on submission slips, 89% of exposures to any rabid animal were to humans and 11% were to animals. When looking at exposures to rabid bats, 40% were to humans, 53% were to animals [dogs (33%), cats (20%)], and in 7% there was no history of exposure to humans or animals.

Our next speaker was Dr. Leon Russell from Texas A & M University. Dr. Russell reviewed the history of rabies in Texas and transmission cycles in wild and domestic animals. Bat rabies occurs throughout Texas. Otherwise, there are currently local foci of skunk, fox, and coyote rabies. Coyote rabies is of primary concern at this time. This epizootic started in southern Texas in about 1988 and is moving north at about 100 km per year. The problem has spread to an area about 40 miles south of San Antonio. The strain of rabies involved in this outbreak is the Mexican DF strain. This strain originated in dogs, spilled over into coyotes, and is being spread back to dogs. It is of particular concern because it circulates in both coyotes and dogs. Dr. Russell discussed steps that are being taken to halt this outbreak. There are plans to utilize the oral vaccinia vector rabies vaccine to immunize coyotes and develop a protective barrier south of San Antonio. In addition, there are a variety of programs being undertaken to ensure rabies vaccination of dogs and cats.

The gray fox rabies epizootic in Texas is also of concern. This epizootic is located primarily in the 32 county sheep raising area of west central Texas. Between 1987 and 1988 there was an explosion of the gray fox population. There is potential that this foci of rabies could spread to the east. Various alternatives for dealing with this situation are being considered.
Dr. Russell also discussed post-rabies exposure treatment of domestic animals in Texas. Texas law allows for strict isolation for 90 days and vaccination of unvaccinated domestic animals exposed to rabies. Rabies vaccinations are given immediately and during the third and eighth weeks of isolation. Since 1988, 490 never vaccinated, and 259 not currently vaccinated (i.e. vaccinated less than 30 days or more than 12 months previously), dogs and cats have been handled using this protocol. There have been only 2 failures. These were 2 never vaccinated dogs which developed rabies before the third week vaccination. Dr. Russell reviewed an unpublished study of rabies challenged non-vaccinated dogs treated with rabies vaccine at 0, 3, 7, 14, and 28 days post challenge. Of 10 control dogs, 9 developed rabies. Of 10 treated dogs, only 1 developed rabies. Further study is needed of post-exposure management protocols.

Our fifth speaker was Dr. Deborah Briggs from Kansas State University. Dr. Briggs discussed a proposed study of rabies in ferrets. Despite the availability of a rabies vaccine for ferrets, there are still public health concerns about ferret bites. It is not known if ferrets shed rabies virus in their saliva, either with or without rabies vaccination. After much discussion, planning, and paperwork, it appears that the study may begin by the end of December 1994. There are numerous groups contributing to this study, including Kansas State University, CDC, USDA, 2 vaccine manufacturers, the ferret industry, and private ferret owners.

Following the presentations, a business meeting was held. Concern was expressed from the llama industry about the lack of a rabies vaccine approved for use in llamas. There were suggestions made for topics to cover at next year’s meeting. One resolution was proposed and passed.

The meeting ended at approximately 5:15 p.m.
THE SALMONELLA ENTERITIDIS PILOT PROJECT AND THE PENNSYLVANIA EGG QUALITY ASSURANCE PROGRAM

Wayne D. Schlosser, David J. Henzler, John Mason, David C. Kradel, Scott Hurd

The Salmonella Enteriditis Pilot Project
The Pennsylvania Salmonella Enteriditis Pilot Project (SEPP) began in April 1992 as a cooperative effort by the poultry industry in Pennsylvania, the Pennsylvania Department of Agriculture, Pennsylvania State University, the University of Pennsylvania, and the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. It had two main objectives:
1. Lower the incidence of egg related outbreaks of Salmonella enteritidi (SE) in humans.
2. Study the epidemiology of SE in egg-layer flocks.

An underlying assumption was that lowering the number of SE contaminated eggs going to the market would reduce the risk of egg related human outbreaks of Salmonellosis due to SE. Volunteer participating producers agreed to follow specific procedures believed to reduce the risk of maintaining SE in a flock or environment. These procedures included intensive rodent control, cleaning and disinfection between flocks, and increased biosecurity efforts.

Participating producers also agreed to test the environment of the layer house for SE. If SE was found in the environment, eggs from the flock were tested. Eggs from egg positive flocks were then diverted to pasteurization or hard cooking. The egg sampling scheme was designed to detect flocks that had positive eggs at a level of 0.5% with a confidence of 99%.

Findings from the SEPP
Below are some of the conclusions drawn from the study:
1. 50% of the flocks in the study were environmentally positive.
2. Environmental positivity appears to increase slightly as the flock ages.
3. There appears to be no seasonal difference in the prevalence of environmental or egg positivity.
4. Repeated environmental testing of a house may not give consistent results.
5. Sampling the manure gives more consistent results than sampling the egg machinery.
6. 50% of the environmentally positive houses had positive eggs during the course of testing.
7. The prevalence of contaminated eggs in the environmentally positive flocks was 2.75 per 10,000. Some flocks yielded no positive eggs. One flock had an overall prevalence of 35 per 10,000.

8. The prevalence of SE positive eggs appears to increase as the flock ages.

9. The average prevalence in blood spot eggs was twice the prevalence in nest run eggs.

10. Dirty eggs that have been immersion washed on the farm may have a higher prevalence of SE contamination.

11. Positive eggs were not consistently detected in environmentally positive houses.

12. 10% of the isolations from eggs were Salmonella other than SE.

13. Approximately 50% of the houses that were cleaned and disinfected were negative on environmental sampling following the cleaning and disinfection.

14. High rodent populations are significantly associated with environmental positivity and rodents continue to be an important risk factor for maintaining SE in layer flocks.

15. Molting may increase the prevalence of egg positivity in the immediate post molt period.

16. Eggs, rodents, and the environment often yielded, the same phage types within a house.

17. There is an association between the proportion of positive environmental samples and the prevalence of contaminated eggs.

18. There was not an association detected between strain of bird and environmental positivity.

19. Single houses had higher rates of egg positivity than did houses that were part of a complex.

Evaluating the SEPP

Measuring the success of the SEPP is necessary so producers and public health officials can decide if participation in similar risk reduction programs is a good use of resources.

The number of human outbreaks is not an adequate measure of the success of the program. Approximately 22% of the Pennsylvania flocks participated in the program at any one time. This represents only about 3% of the country's annual egg production of 60 billion eggs. Furthermore, the occurrence of human outbreaks is greatly influenced by food storage and handling practices. The dramatic decrease in SE outbreaks so far in 1994 is probably attributable to a number of causes.

The best way to evaluate whether the risk of SE outbreaks from table eggs has decreased is to determine if fewer SE positive eggs are being shipped to the table egg market or if producers are at a lower risk of producing contaminated eggs.
From the beginning of the project through February 1994 an estimated 360 million eggs from participating flocks were diverted to pasteurization or hard cooking. At an average prevalence of 2.75/10,000 this amounted to an estimated 100,000 positive eggs kept off the table egg market.

Another way to evaluate the SEPP is to look for improvement in participating flocks. Figures 1 and 2 show the monthly prevalence of positive samples from egg machinery and manure pits respectively.

Both graphs show a general decrease in the number of positive samples over the life of the project. This should correspond with a similar decrease in the number of positive eggs produced by the participating flocks. Since only eggs from environmentally positive flocks were tested, environmental results are used as a measure of the positivity of a layer house.

Tables 1 and 2 summarize the results of all environmental testing done in 55 houses that housed two consecutive flocks while participating in the SEPP. Environmental samples were taken from egg machinery, manure pits, and other areas of the house.

Table 1. Summary of environmental samples taken when the first project flock had any environmental positive and there was a second project flock placed in the same house. Data is summarized from flock ages 20-89 weeks.

* - no data available

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<tr>
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<td>504</td>
<td>35</td>
</tr>
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</table>

Table 1 shows that houses which were environmentally positive when the first flock was housed had a lower prevalence of SE in the environment in the second flock.
Unfortunately, table 2 shows that some houses that were negative in the first flock had some positivity in the second flock.

The Pennsylvania Egg Quality Assurance Program

The Pennsylvania Egg Quality Assurance Program (PEQAP) began in February 1994 as an outgrowth of the SEPP. Most of the flocks in the SEPP were transferred to the PEQAP when it began. Like the SEPP, the PEQAP continues as a cooperative, industry program intended to minimize the contamination of shell eggs with SE. As of August 1994 there were over 150 flocks participating in the program representing over 12 million birds.

Below are the specific program requirements for participation in the PEQAP:

- Pullets
  - Purchase chicks from U.S. Sanitation Monitored Salmonella enteritidis

Unfortunately, table 2 shows that some houses that were negative in the first flock had some positivity in the second flock.

### Table 2. Summary of environmental samples taken when the first project flock had no environmental positive and there was a second project flock placed in the same house. Data is summarized from flock ages 20-89 weeks.

<table>
<thead>
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<th>Age Weeks</th>
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<th>Houses in Complexes</th>
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</tr>
<tr>
<td></td>
<td>#</td>
<td>% Pos</td>
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<tr>
<td>Tot</td>
<td>448</td>
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</tr>
</tbody>
</table>
the contamination of shell eggs with SE. As of August 1994 there were over 160 flocks participating in the program representing over 12 million birds.

Below are the specific program requirements for participation in the PEQAP:

**Pullets**
- Purchase chicks from U.S. Sanitation Monitored *Salmonella enteritidis* negative breeder flocks.
- Obtain samples of chick dropping papers at time of delivery. Sample every 10th chick paper and submit to laboratory for culture for SE.
- Sample and culture the manure at 10 to 15 weeks of age. A culture consists of two samples taken from the manure beneath each row of cages.
- Houses with positive manure or chick samples must be cleaned and disinfected before new chicks can be placed.

**Layers**
- Purchase and place pullets from an SE monitored flock. Pullets from an unknown or SE positive status flock will require that the manure be sampled and cultured 7 to 14 days after placement.
- Sample and culture manure at 29 to 31 weeks of age and again at 44 to 46 weeks of age. A culture of the manure during any test will consist of two samples taken from the manure beneath each row of cages.
- Houses with positive manure samples must be thoroughly cleaned and disinfected between the flocks.

**Eggs**
- Houses with negative manure samples are not required to test eggs.
- Houses with positive manure samples must test 480 eggs every 2 weeks for 4 lots of samples. Eggs are cultured in pools of 20. If the 4 lots of eggs are negative, a sample of 480 eggs must be sampled each month for the life of the flock.
- If any egg pools are positive all eggs must be diverted for pasteurization or hard cooking. To resume sale of eggs as table eggs, 1000 eggs submitted every two weeks for 4 lots of samples must test negative. (If 50% or fewer environmental samples were positive and the flock had only one positive egg pool, the 4 lots of 1000 eggs may be combined into one lot of 4000 eggs.) Following return to the table egg market, 480 eggs must be sampled each month for the life of the flock.
- Egg testing eliminates the need for further environmental testing.

**Force Molted Flocks**
- Test manure at five to seven weeks following return to feed and follow egg testing procedures if the manure is positive.
THE SALMONELLA ENTERITIDIS PILOT PROJECT AND THE PENNSYLVANIA EGG QUALITY ASSURANCE PROGRAM

Rodent Control
Producers must maintain an acceptable rodent control and monitoring program at all times.

Bio-security
All participants must maintain an acceptable biosecurity program at all times.

Refrigeration
Eggs must be kept under refrigeration.

Although the primary emphasis is on quality assurance, program operational and sampling data are collected and computerized for subsequent analysis. Consequently many of the observations and studies conducted during the SEPP can be continued with the PEQAP. In a somewhat similar fashion, this program is also serving as a pilot project for a full-scale egg quality assurance program.

Computer models of the program predict that if all flocks in Pennsylvania participated in the PEQAP, the estimated number of positive eggs would decrease from over half a million to around 200,000 or less per year.
TRANSMISSION OF *SALMONELLA CHOLERAESUIS* TO NAIVE SWINE

Jeffrey T. Gray, Paula J. Fedorka-Cray, Thomas J. Stabel
National Animal Disease Center
Ames, IA 50010
Ted T. Kramer
Veterinary Medical Research Institute
Iowa State University, Ames, IA 50011

Introduction

*Salmonella choleraesuis* is a host adapted, facultative intracellular pathogen that causes swine paratyphoid (1). In 1990 the National Veterinary Services Laboratory reported that swine salmonellosis exceeded salmonellosis from all other animals in Illinois, Indiana, Iowa, Minnesota, and Nebraska (2). *Salmonella choleraesuis* is the most frequent *Salmonella* serotype recovered from swine (2) and was isolated from >95% of swine salmonellosis outbreaks in Iowa in 1989 (3). The National Animal Health Monitoring Survey estimated that swine salmonellosis is responsible for 28 million dollars in annual production losses in Iowa and 100 million in losses nationwide (3).

Although *S. choleraesuis* is the most frequent porcine isolate, it is rarely isolated from swine feeds or non-porcine *Salmonella* reservoirs. The source of *S. choleraesuis* seems to be limited to carrier pigs and facilities previously contaminated with this serotype (1). The carrier state and transmission of *S. typhimurium* in swine has been described (4,5,6), however, similar information pertaining to *S. choleraesuis* in swine has not been available.

It has been shown that intranasal inoculation of *S. choleraesuis* in swine results in more severe clinical disease and dissemination of the organism to more tissues when compared to a gastric challenge (7). The purpose of this experiment was to study the natural transmission of *S. choleraesuis* to naive swine.

Twenty-four naive pigs were commingled with 12 experimentally challenged pigs and clinical response, shedding patterns and tissue distribution of *S. choleraesuis* were observed for 12 weeks post commingling. We hypothesized that natural transmission of *S. choleraesuis* to naive swine would result in a long term carrier state.

Materials and Methods

*Bacterial strains and challenge cultures.* Wild type *S. choleraesuis* X3246 (8), kindly provided by the laboratory of Roy Curtiss III, Washington University, St Louis, MO, was used to challenge a 12 week old pig by intranasal inoculation. Following recovery from the ileocolic lymph node,
the isolate was redesignated 3246pp and stored at -70°C in glycerol. This pig passaged isolate was used as the challenge strain. The strain has a naturally acquired resistance to streptomycin. Challenge cultures were prepared by inoculating 10 ml of LB broth with 100 μL of a -70 frozen stock culture of 3246pp. The culture was grown overnight at 37°C on an orbital shaker at 150 rpm. A 1% inoculum was transferred into fresh LB broth and grown for 3.5 hours at 37°C and 220 rpm. The culture was centrifuged, the pellet was resuspended in 1/2 volume of phosphate buffered saline (PBS) and adjusted to an final concentration of 1x10^8 cfu/ml (OD_{600} = 0.123) in PBS.

Experimental design. At 6 weeks of age 40 Salmonella culture-negative pigs were randomly divided into three groups. Each group was housed in separate isolation facilities and allowed to acclimate to the new environment for 1 week. Pigs were challenged at 7 weeks of age (day 0). Group 1 challenged pigs (n=12) were inoculated intranasally (INT) with 1 ml (0.5 ml in each nostril dropwise on inspiration, alternating nostrils) of 3246pp at 1x10^8 cfu/ml. One day post inoculation (PI) group 2 naive pigs (n=24) were commingled with the challenge pigs. Group 3 (n=4) served as uninoculated controls.

Bacteriologic examinations. Two pigs from the challenge group and 4 pigs from the naive group were necropsied at 1, 2, 4, 6, 9 and 12 weeks PI. One control pig was necropsied at 1, 4, 9 and 12 weeks PI. Fecal pools were obtained by collecting individual feces (10-20 g samples) from >60% of the pigs per group. Two grams from each sample was pooled and cultured. Also, environmental fecal samples were collected from the pen floor and cultured. All tonsil, nasal, rectal swabs, fecal pools and tissues (collected at necropsy) were incubated at 37°C in GN-Hajna (GN) broth with 200 μg/ml streptomycin sulfate for 18 to 24 hours then streaked on brilliant green agar with sulfadiazine and streptomycin sulfate (BGS-S). Additionally, at 18 to 24 hours 100 μl was transferred to Rappaport-Vlasidalis (RV) medium (9), incubated at 37°C for 18 hours, then streaked to BGS-S. All BGS-S plates were incubated 24 hours at 37°C. Colonies having the appearance typical of Salmonella were picked and inoculated into triple sugar iron and lysine iron agar slants. Positive isolates were confirmed as serogroup C by agglutination with Salmonella antiserum group C, O (Difco, Detroit, MI). Representative isolates were serotyped at the National Veterinary Services Laboratory. Quantitative bacteriology was conducted using the 5 tube most probable number (MPN) method (10) with GN, BGS-S and RV media as described above and are reported as the mean of the respective group for each necropsy day. Differences between time points were evaluated by the X^2 test.

Results and Discussion
Pigs in the challenge group elicited a febrile response which peaked at
41.2°C 4 days PI and persisted until day 11 PI. The naive pigs also developed a febrile response which began on day 3 PI, peaked at 41.4°C on day 5 and persisted until day 8 PI. Pigs from both groups had moderate to severe depression and severe diarrhea during the acute infection. Clinical signs in both groups were resolved by day 14 PI. Tonsil, nasal, rectal swabs and group fecal pools collected from individual animals indicated that pigs in the challenge group began shedding *Salmonella choleraesuis* one day PI. Pigs in the naive group began shedding *Salmonella choleraesuis* one day after commingling (day 2 PI; Table 1). Fecal shedding peaked in the challenge group at $4.52 \times 10^3$ cfu/g of feces on day 7 PI. Naive group fecal shedding was much lower and peaked at $3.3 \times 10^4$ cfu/g feces on day 9 PI (Table 2). Fecal shedding could not be detected in either group after week 8 PI (Table 1). *Salmonella choleraesuis* could not be detected on tonsil, nasal or rectal swabs and in fecal samples after week 8 PI.

Necropsy results indicated that both the naive and challenge groups were heavily infected with *Salmonella choleraesuis* at 1 and 2 weeks PI (Table 3). The level of infection dropped steadily for both groups after 2 weeks PI and was markedly reduced in both groups at 12 weeks PI. Following resolution of clinical signs the tissues of predilection for the naturally exposed naive group and the experimentally challenged group were the tonsil, ileocolic junction, ileocolic lymph node, cecum, cecal contents, colon and colonic lymph nodes. These data indicate that naive swine exposed to swine with acute paratyphoid can be infected within 24 hours of exposure. At the time of naive exposure clinical signs were not evident in the challenge group and the level of fecal shedding was 2 logs of *Salmonella choleraesuis* per gram of feces. This indicates that for natural transmission of *Salmonella choleraesuis*, the infective dose may be considerably less than what is commonly used for experimental challenge. In addition, even after a severe clinical outbreak of *Salmonella choleraesuis* as seen in the naive group, the level of *Salmonella choleraesuis* carried in deep tissue and in the environment was markedly reduced. A significant ($P < 0.01$) number of naive pigs were able to clear *Salmonella choleraesuis* between 9 and 12 weeks PI, indicating that not all pigs will become long term carrier animals.

References

4. Fedorka-Cray, P.J., Whipp, S.C., Isaacson, R.E., Nord, N., Lager, K.,
TRANSMISSION OF SALMONELLA CHOLERAESUIS TO NAIVE SWINE


<table>
<thead>
<tr>
<th>Day/Week</th>
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<td>Week 1</td>
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<td>Week 2</td>
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<td>Week 3</td>
<td>+</td>
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<td>Week 4</td>
<td>+</td>
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<td>Week 12</td>
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Table 1. Qualitative Fecal Shedding
(+), (-). Gray, Fedorka-Cray, Stabel.

Table 2. Quantitative Fecal Shedding
Log_{10} cfu of S. choleraesuis/g feces

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Table 3. Total Number of Animals and Tissues Positive at Necropsy

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<tr>
<td>12</td>
<td>0</td>
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* Number of animals positive
  b Number tissues positive/Number tissues processed

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REPORT OF THE COMMITTEE ON SALMONELLA

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Vice Chairman: Dr. K. V. Nagaraja, St. Paul, MN

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This year the Salmonella Committee met for an entire day, with eighty members and guests present. The committee recognized and thanked Dr. Ben Pomeroy for his many years of service as Chairman of the Salmonella Committee. Twenty-three papers and subcommittee reports were presented, indicating the high level of interest in the subject. Five papers were presented by scientists from Europe.

Drs. Bean and Potter reported for CDC on Salmonella serotypes from humans for 1992. A total of 34,000 isolates were identified, with S. typhimurium and S. enteritidis (SE) being the two most frequently isolated. The leading human serotypes are also the most common serotypes isolated from animals. An estimated 2,000,000 human cases of salmonellosis occur each year in the United States. Dr. Saeed and coworkers from Purdue reported that there is a variation in virulence of different strains of SE, which is not easily explained by the existence of certain plasmids and which may be influenced by the host. Dr. Shivaprasad (Califor-
nia) reported that there was a variation in egg transmission between SE strains due to an unknown virulence factor. Dr. Mason reported on the status of the SE Control Program. Thirty-one percent of the SE outbreaks in humans could be traced back through eggs to the farms of origin. So far in 1994, the incidence of human outbreaks of SE is down from the previous four years. He reported that the USDA has a new agency in charge of food safety, and that the SE Task Force has completed its study.

Dr. Gholi of Arkansas described a case report of recontamination of premises of an egg-type breeder flock with SE, which illustrates the role of mice and rats in maintaining SE in an area. Dr. Willoughby and colleagues reported on an outbreak of SE in California layers. These workers found SE phage type 4 in eggs, chickens, cats, skunks, and rodents. Dr. Sisak of the Veterinary Research Institute, Czech Republic, described their favorable experiences over years using a modified live S.typhimurium (Gal E, Meth, Leu, Trypt, strep dependent) for vaccinating chickens. They found that modified live vaccines were more efficacious than an inactivated oil vaccine.

Dr. Lee Ann Thomas of NVSL reported that when user fees for Salmonella serotyping were instituted in September 1993, submissions dropped suddenly to 50% of previous levels. A hue and cry went up about the loss of this important serotyping data, and user fees were rescinded in March 1994 (for all except research and some private studies). Since that time, the number of Salmonella cultures submitted for serotyping has rebounded close to 1993 levels. The USAHA Salmonella Committee voted to commend the USDA for rescinding user fees for serotyping. Dr. K. E. Ferris reported for NVSL on serotypes isolated from animals and related sources during July 1993-June 1994. There were 22,029 Salmonella serotyped. The five most common were S.enteritidis, S.typhimurium, S.heidelberg, S.Kentucky, and S.hadar. S.dublin made the top ten list for all animal isolates for the first time, which is indicative of its continued spread in the U.S. cattle population.

Dr. Corrier and USDA colleagues in Texas and Iowa reported on the beneficial effects in chicks of a lyophilized competitive exclusion culture containing 29 bacterial isolates. Feed conversion was improved in treated chicks, and Salmonella isolations were reduced. The product is patented and should be on the market soon. Dr. Lahellec and French coworkers reported that treatment of young chicks with Baytril and subsequent competitive exclusion markedly decreased the number of Salmonella contaminated carcasses, but failed to eradicate Salmonella. Dr. Welsh of Oklahoma reported that Salmonella isolations and clinical syndromes associated with Salmonella in ratites (ostriches and emus) appear to be increasing in his state. Dr. Bender and coworkers at Minnesota reported on a pilot study of feeding low levels of Salmonella to two cows, and being able to recover the organism from the rumen. Dr. Franco of the National Renderers Association reported that renderers are making serious efforts to achieve zero Salmonella in their products by use of HACCP and improved worker education programs.
REPORT OF THE COMMITTEE

Dr. Hurd and USDA colleagues reported that the National Dairy Heifer Evaluation Project found some differences in Salmonella serotype distribution in dairy heifers compared with NVSL cattle data. Dr. Pezzotti and coworkers at the research institute in Teramo, Italy reported on two outbreaks of SE in sheep. One involved a human outbreak traced back to SE contaminated sheep cheese. SE was isolated from the udder and mammary lymph nodes of two ewes. Similar findings from cattle have been previously reported. The SE was still able to be recovered from cheese two months later.

Dr. B. Smith and coworkers at Davis, California, reported that Salmonella serotesting using phenol-water extracted LPS antigen is highly O (somatic) antigen specific, and useful for epidemiologic studies, vaccine assessment, and detection of carrier cattle. Dr. Van der Heijden of the Netherlands reported that group D LPS could be chemically modified to eliminate ELISA cross reactions with group B, without undue loss of sensitivity. Thus an SE-infected poultry flock could be serologically detected and differentiated from a flock infected with \textit{S.typhimurium} or other group B Salmonella. Dr. House of Davis reported that use of IgG isotypes (IgG, and IgG2) and both porins and LPS as plate antigens, increases the ability of ELISA to distinguish Salmonella carriers from infected recovered and from vaccinated cattle. In many cases, a single serum sample could effectively determine carrier status. Mr. G. Dilling of Davis reported on the remobilization of 107 non-motile group D Salmonella from cattle supplied by Drs. Thomas and Ferris at NVSL. One hundred five were identified as \textit{S.dublin} and two as \textit{S.enteritidis} following re-mobilization.

Dr. Bauerfeind and coworkers at Giessen, Germany evaluated an LPS-ELISA for detecting anti-Salmonella antibodies in horses. They found fecal IgA titers to be more predictive of infection status than serum IgG titers. Drs. Srinand and Robinson and coworkers at Minnesota developed two indirect ELISAs using OMP or LPS as antigen to detect antibodies to \textit{S.choleraesuis} in the sera of pigs. Dr. Kramer and colleagues at Iowa State and NADC developed an antiglobulin ELISA for detecting antibodies to \textit{S.choleraesuis} in pigs.

Four resolutions were proposed and two passed. One of the two resolved that the USAHA support the Salmonella Committee in developing a Symposium on Salmonella Diagnostics to be held in conjunction with the 1995 USAHA/AAVLD Meeting. The other resolution urged that the issue of pre-harvest food safety be promoted through the appropriate USAHA committees. The Salmonella Committee members are very optimistic that the diagnostic tools now becoming available, such as ELISA serology and PCR probes, will make screening for Salmonella control a reality. Comprehensive Salmonella risk reduction programs for poultry and for turkeys are now available, and a program for dairy cattle is being developed by the Salmonella Committee. The Committee urges industry and government agencies to work with researchers to increase awareness of these programs and to promote pre-harvest Salmonella risk reduction programs.

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SALMONELLA SEROTYPES FROM HUMAN SOURCES, JANUARY 1992 THROUGH DECEMBER 1992

Nancy Hargrett Bean, Ph.D.
Morris E. Potter, D.V.M.

Summary

The National Salmonella Surveillance System collects reports of isolates of Salmonella from human sources from every state in the United States and the District of Columbia. The reports are sent regularly by the Public Health Laboratory Directors and State and Territorial Epidemiologists to the Division of Bacterial and Mycotic Diseases (DBMD) in the National Center for Infectious Diseases (NCID). The system, which previously had been paper based has been replaced with an electronic reporting system, the Public Health Laboratory Information System (PHLIS) (1).

PHLIS is a PC-based reporting system for local, county, or state organizations to enter, edit, and analyze data and to transmit data electronically to other state or federal offices. PHLIS is capable of handling any data types (e.g., epidemiologic, laboratory, hospital, special studies, etc.). Currently, information concerning Salmonella isolates is electronically reported each week through PHLIS from 48 State Public Health Laboratories, Guam and the District of Columbia. PHLIS is available without cost and is transportable to other agencies, states, or countries interested in implementing the system for their own needs.

The objectives of the Salmonella surveillance system are to 1) define endemic patterns of salmonellosis, particularly those with interstate ramifications, 2) identify trends in disease transmission, and 3) monitor control efforts. The following report is based on data collected in the year 1992.

Results and Discussion

The total number of Salmonella isolates reported from human sources in 1992 continued to show a slow decline from the peak year of 1987 (an unusually large number of isolates reported in 1985 (56,750) was primarily due to one large S. typhimurium outbreak associated with pasteurized milk (2)). The downward trend in number of reported isolates from human sources since 1987 is as follows: 1987 (44,768), 1988 (43,788), 1989 (41,806), 1990 (41,012), 1991 (40,472), and 1992 (34,520) (Table 1). The percentage of Salmonella isolates reported from western states has increased in the last few years.

In 1992, 20 Salmonella serotypes reported to CDC from human sources constituted most of the total Salmonella reported (Table 2). The ten most frequently reported serotypes accounted for 67% of all reports to CDC. S. typhimurium was the most commonly reported serotype in each of the last
ten years except 1990, when S. enteritidis was the most frequently reported serotype. Between 1991 and 1992, the number of isolates decreased for all of ten most commonly reported serotypes.

Although the proportion of all Salmonella isolates accounted for by S. enteritidis had increased consistently since 1985, the proportion of Salmonella identified as S. enteritidis in 1991 and 1992 did not increase. The general increase in the number of S. enteritidis has been in large part due to the outbreaks associated with grade A shell eggs (3). The control measures that have been instituted by the egg, retail, and restaurant industries and the regulatory agencies may be accounting for the change in the S. enteritidis trend.

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Table 1. Table of region by year

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SALMONELLA SEROTYPES FROM ANIMALS AND RELATED SOURCES REPORTED DURING JULY 1993 - JUNE 1994

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L. A. Thomas, D.V.M., M.S.
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Summary
Serotyping results for 22,029 Salmonella isolates from animal disease cases and epidemiologically related sources are reported for July 1, 1993, through June 30, 1994. The most frequently identified serotypes were Salmonella enteritidis, S. typhimurium, S. heidelberg, S. kentucky, and S. hadar.

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.

Discussion
A total of 22,029 Salmonella isolates from animals and related sources were serotyped. This represents a 39% decrease from the 36,073 isolates received the previous year. Two factors were associated with this decrease; user fees and decreased Salmonella enteritidis (SE) testing activi-
SALMONELLA SEROTYPES FROM ANIMALS

ties. On September 1, 1993, a user fee for Salmonella serotyping was initiated. All isolates, with the exception of those submitted as a result of testing under the National Poultry Improvement Plan (NPIP) or SE control activities, were subject to the $20 per culture user fee. In addition, there were fewer egg-related outbreaks of human SE requiring traceback testing, and surveillance activities of the Pennsylvania Pilot Project decreased. The NVSL reassessed the user fee charges for Salmonella serotyping as a result of resolutions from the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and the U.S. Animal Health Association (USAHA) and decided to discontinue the charges beginning March 15, 1994, for isolates received with the required information.

Although the total number of isolates submitted decreased, the relative percentage of isolates from cattle and swine was similar to that received last year, 12% and 13%, respectively. The relative percentage of chicken isolates increased from 23% to 37% of all isolates received for serotyping. The percentage of environmental isolates with no source species identified decreased from 28.5% last year to 13.5% this year. Eleven percent (2,503) of all isolates received were not completely serotyped but were identified as "Not SE" (Table 3). This compares with 23% in this category last year.

Salmonellae were isolated from animals and related sources from 46 States, the District of Columbia, and Puerto Rico (Tables 1 and 2). Two-hundred and fifty-four different serotypes were identified (Tables 3 and 6). The 10 most common serotypes (Table 12) accounted for 59% of the total isolates identified. Salmonella cholerasuis var. kunzendorf was not included in the ten most frequently identified serotypes (Table 12) for the first time since we began reporting results to the USAHA in 1975. A possible reason for this could be that laboratories, to avoid a user fee, identified this serotype rather than sending it to the NVSL for confirmation. Salmonella cholerasuis var. kunzendorf is a bioserotype and can be identified by serogrouping and biochemical tests. Salmonella dublin was included in the 10 most frequently identified serotypes for the first time since 1990. Isolates were received from 27 states. The total number identified was only 2 less than the total last year.

For the first time, S. brandenburg was included in Table 12. The majority of isolates of this serotype were from turkeys (82%) in North Carolina and West Virginia (74%) [Tables 1, 2, and 3]. Only 12% of the isolates from turkeys were identified as being associated with a primary or secondary infection (Table 7), while nearly all (22 of 23) of those from swine were listed in that category (Table 10). This does not necessarily imply that S. brandenburg is more pathogenic in swine than in turkeys because 75% of all isolates from swine were from clinical cases, whereas only 12% of the total submissions from turkeys were classified as clinical isolates.

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FERRIS, THOMAS

References
### Table 1. Distribution of Salmonella Serotypes in Animals

| Serotype | AL | AR | CT | DC | FL | GA | IL | IN | KY | LA | MD | ME | MS | MO | NE | NH | NJ | NY | OH | PA | SC | TN | VA | WI | WY | MN | IA | OR | WA | SD | NE | VT | ME | HI | AK | ID | CA | NV | AZ | NM | CO | OH | MI | IN | IL | MO | KS | TX | LA | MS | AL | NC | SC | GA | FL | VA | WI | MI | MI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI | WI 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| Totals     | 570      |

(A) Table omits the following isolates:
- From HI - 2 Atahualpa, 1 Medellin
- From VT - 1 Bovine, 1 Typhimurium
(B) Var. bartlettii
(C) Var. copenhagenensis
| State | AZ | CA | CO | ID | KS | ME | MI | MN | ND | NE | NV | NY | OH | OR | PA | RI | SC | TX | UT | VA | WA | WV | WY |
|-------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Arizona | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| California | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Colorado | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Idaho | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kansas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Maine | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Michigan | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Minnesota | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Montana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nebraska | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nevada | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| New Mexico | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| New York | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| North Carolina | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ohio | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oregon | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pennsylvania | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhode Island | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| South Carolina | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Texas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Utah | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Virginia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Washington | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| West Virginia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Wyoming | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Total:** 448
| SHIGA TYPE | AZ | CA | CO | ID | IA | KS | MI | MO | MT | NE | NH | NJ | NY | OH | OK | OR | PA | RI | SC | SD | TN | TX | UT | VA | VT | WA | WI | WY | TOTALS |
|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
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| RHODE ISLAND | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TENNESSEE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| LEO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTALS | 60 | 3002 | 108 | 35 | 306 | 126 | 550 | 648 | 13 | 202 | 36 | 95 | 137 | 45 | 2211 | 40 | 82 |

(A) TABLE Omits the following isolates:
From NV - 1 CHOLERAUSIS (KUNZERHOFF), 1 DUBLIN, 1 TYPHMIURUM, 1 TYPHMIURUM (COPENHAGEN)
From OR - 1 BERN, 1 TYPHMIURUM, 1 TYPHMIURUM (COPENHAGEN)

(B) VAR. KUNZERHOFF

(C) VAR. COPENHAGEN
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(B) VAR. RUNZEHORF
(C) VAR. COPENHAGEN
**TABLE 4. DISTRIBUTION OF ARIZONA SEROTYPES BY STATE FROM 07/93 THROUGH 06/94 - EASTERN STATES**

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**Total** | 1 | 2 | 2 | 2 | 10 | 1 | 10 | 14 | 2 | 17 | 6 | 4 | 7 | 5
**SALMONELLA SEROTYPES FROM ANIMALS**

**Table 5. Distribution of Arizona Serotypes by State from 07/93 through 06/94 - Western State**

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TABLE 7. TURKEY—MOST FREQUENTLY IDENTIFIED SEROTYPES FROM 07/93 THROUGH 06/94

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<th>SURV/RESEARCH</th>
<th>ENVIRONMENT</th>
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### TABLE 8. CHICKEN--MOST FREQUENTLY IDENTIFIED SEROTYPES FROM 07/93 THROUGH 06/94

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**TABLE 12.** Salmonella Serotypes Identified Most Frequently from July 1, 1993 through June 30, 1994 with Comparison Data for 5 Years (All Sources)

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<td>ENTERITIDIS</td>
<td>3780* (1)**</td>
<td>7148 (1)</td>
<td>3675 (1)</td>
<td>4824 (1)</td>
<td>1499 (3)</td>
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<td>TYPHIMURIUM</td>
<td>2685 (2)***</td>
<td>3696 (2)</td>
<td>3321 (2)</td>
<td>3137 (2)</td>
<td>2550 (2)</td>
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<td>HEIDELBERG</td>
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<td>KENTUCKY</td>
<td>1183 (4)</td>
<td>789 (8)</td>
<td>812 (8)</td>
<td>634 (11)</td>
<td>908 (3)</td>
<td>726 (5)</td>
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<td>HADRAR</td>
<td>963 (5)</td>
<td>1375 (4)</td>
<td>1308 (4)</td>
<td>1576 (4)</td>
<td>873 (6)</td>
<td>1031 (4)</td>
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<td>AGORA</td>
<td>692 (6)</td>
<td>865 (6)</td>
<td>826 (7)</td>
<td>714 (9)</td>
<td>647 (10)</td>
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<td>READING</td>
<td>692 (6)</td>
<td>700 (9)</td>
<td>456 (14)</td>
<td>802 (7)</td>
<td>870 (7)</td>
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<td>BRANDENBURG</td>
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<td>107 (37)</td>
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<td>MONTEVIDEO</td>
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<tr>
<td>DUBLIN</td>
<td>415 (10)</td>
<td>417 (12)</td>
<td>340 (11)</td>
<td>574 (13)</td>
<td>660 (9)</td>
<td>564 (11)</td>
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</tbody>
</table>

* Number of times serotype was identified

** Rank beginning with the most common

*** Includes *S. typhimurium* and *S. typhimurium* var *copenhagen*
REPORT OF THE COMMITTEE ON SHEEP AND GOATS

Chairman: Dr. Cleon V. Kimberling, Ft. Collins, CO
Vice Chairman: Dr. Warren C. Foote, Kanab, UT

Dr. Arthur A. Andersen, IA; Dr. Marie S. Bulgin, ID; Dr. Andres De La Concha, TX; Dr. Nancy E. East, CA; Dr. James E. Fox, GA; Dr. Chester A. Gipson, FL; Dr. R. David Glauer, OH; Dr. John S. Glenn, CA; Dr. John R. Gorham, WA; Mr. Joe N. Huff, CO; Dr. Michael M. Jochim, CO; Dr. LaRue Johnson, CO; Dr. William E. Ketter, MD; Dr. Jimmy Kwang, NE; Dr. Linda L. Logan-Henfrey, Kenya; Prof. C. John Maré, AZ; Dr. Michael R. Marshall, UT; Dr. Charles A. Mebus, NY; Dr. Bennie I. Osburn, CA; Dr. Charles Palmer, CA; Dr. Robert K. Pelant, AR; Dr. Robert A. Robinson, MN; Mr. Paul Rodgers, CO; Dr. M. D. Salman, CO; Dr. John A. Schmitz, NE; Dr. J. Glenn Songer, AZ; Dr. William L. Thomas, OH; Mr. Olin H. Timm, CA; Dr. Peter H. Timm, CA; Mrs. Michele C. Turner, TX; Dr. Percy R. Turner, TX; Dr. Robert J. Velure, ND; Dr. Howard W. Whitford, TX.

The Sheep and Goat Committee met October 31, 1994, Grand Rapids, Michigan an Educational Program was presented:

Current status of the sheep industry, populations shifts, and research

P. Rodgers (ASI)

Quality audit, quality assurance/food safety of the sheep industry

W. Cunningham (CSU)

Caseous lymphadenitis/pasteurellosis research and development

Kim Brogden (NADL)

Ovine progressive pneumonia research and diagnostic development

Gary Ross (MARC)

Drug use in small ruminants

G.A. Bert Mithcell (FDA)

Biological guidelines for small ruminants

A. Robinson (U of M)

Scrapie update and importation of sheep and goat germ plasm

D. Harpster (APHIS)

Scrapie / long incubation or scrapie resistant genotype

K. O'Rourke (USDA ARS PULMAN)
Business Meeting:
Three Resolutions were introduced

1. To encourage USDA to fund research on embryos as it pertains to scrapie transmission. The resolution was passed by the committee.

2. That USAHA oppose FDA'S Proposed Rule Docket #93N-0467 and encourage FDA'S continued cooperation in monitoring surveillance programs that are now in place.

3. The control of sheep and goats that are produced from embryos originating from countries which are not scrapie free. The resolution was presented by Mr. Phillip Coffin, President of the newly formed North American Livestock Health Association. The committee felt the provisions of the current import law and the regulation being adopted for the voluntary scrapie certification program to handle offspring from imported embryos was adequate. The resolution was unanimously defeated.


Consumer demand for the red meats (including lamb) has shifted considerably lower since the late 1970s. In terms of real dollars, sheep are worth approximately 50 percent of what they were in 1980. Retail prices continue to be relatively constant. The resulting "forced disinvestment" by producers and the processors has had many industry wide carry over effects, including reductions and repositioning of the supply service sectors which provide such products as capital, education, research, flock health care and management, feed and equipment sales, transportation, etc.

Numbers of sheep in the U.S. will likely bottom out over the next one to two years and the industry will be restructured with the supply and service sectors realigned in some manner to meet changed needs.

The market is fairly predictable under the current marketing and production system and overall economic conditions. Any of these factors alone or in combinations can make significant changes in the outlook for the sheep industry. Substantial opportunities exist for lamb to break-out of its current trading range and pricing pattern, if the industry chooses to make it happen. This must include an investment in changing the consumer demand picture for lamb and implementing a structure that will bring a reasonable return back to those essential segments of the production and marketing system who contribute to change.

This investment must include:

A. Quality assurance - few defects, long shelf-life, product consistency.
B. New products - single portion sized, minimal preparation time, lean, visual appeal both in and out of the package, consistency in taste
REPORT OF THE COMMITTEE

and flexibility in preparation.

C. Production, processing and marketing system changes - specified genetics and nutrition for specific markets/products, improved efficiency, more accurate evaluation of quality, more accurate and more efficient pricing system at each level, risk management.

D. Different products from the supply and service sectors—greater access to science based information at the producer level, emphasis on preventive flock health management and integrated, economical flock care systems based on records and quantitative risk analysis.

E. The capital and the time to implement these changes simultaneously and see them through.

Quality Audit, Quality Assurance/Food Safety. Wayne Cunningham (Colorado State University)

Recently, an article in THE ECONOMIST (March 1994) stated, "There is, surprisingly, little or no evidence that modern doctors, pills or surgery have improved people's overall state of health. The increase in American's average life expectancy from 63 years in 1940 to 76 today has been ascribed to increased wealth, better sanitation, nutrition, housing, and the widespread introduction of the refrigerator than to modern medicine." Whether the statement is valid or not, it does make the point that prevention and avoidance of exposure to food borne disease is the best approach to prevent food borne illness. It is estimated that food borne disease costs the U.S. 6.5 billion dollars annually in lost wages and increased medical expenses. As professional animal health experts, we must educate producers in the techniques necessary to produce safe, high quality animal products.

Audit:

Lambs

Visits were made to plants across The U.S. These plants represented 85% of all lambs slaughtered under FSIS inspection. Residues were found in less than 2 lambs per 100,000 slaughtered. These were traced to antibiotics in feed.

Pathogen Reduction - There is little information available relative to pathogenic microbial contamination of sheep carcasses. This is an area currently being studied by a team at Colorado State University. Current sanitation issues (e.g., wet manure laden lambs), cost the industry $9.50 per lamb contaminated.

Physical Damage - Bruises and injection site tissue damage adversely effect the economic value of slaughter lambs. Twenty-seven percent of all lambs slaughtered under FSIS inspection have some form of bruis-
ING. Trim-out of bruises accounts for a loss of $2.00 per lamb affected. Pelt damage can decrease the value of the pelt by as much as 50% or up to $5.00 per pelt.

Diseases and Parasites - Pneumonia is the most significant disease causing total or partial condemnation in slaughter lambs. Fifty percent of Midwest lambs slaughtered have some form of loss due to pneumonia. Parasites are significant cause of liver condemnations. Fifty percent of all lamb livers are condemned.

**Adult Sheep**
Residues have not been identified as a problem in the adult sheep slaughtered and monitored under the FSIS. Over forty percent of all ewes slaughtered in the U.S. have some amount of condemnation as result of caseous lymphadenitis.

**Quality Assurance**
Quality assurance programs can play an important role in reducing consumer concerns about the livestock products they consume.

**Caseous Lymphadenitis/ Pasteurellosis - research and development. Kim Brogden (National Animal Disease Center, Ames, la.)**
The efficacy of experimental vaccines containing the synthetic adjuvant, muramyl dipeptide (MDP) and Corynebacterium pseudotuberculosis or Pasteurella haemolytica capsular polysaccharide was determined in lambs. Lambs vaccinated with a bacterin containing C. pseudotuberculosis (1 mg whole cells and 50 ug MDP/dose 10% light mineral oil) developed antibody titer and were protected against both experimental infection and natural caseous lymphadenitis. No adverse local or systemic reactions were seen during the vaccination period. Lambs vaccinated with a subunit vaccine containing capsular polysaccharide (CF) of P. haemolytica serotype A1 (1.0 mg CP with 50 ug of MDP/dose in 10% light mineral oil) developed antibody titer characterized by an early IgM response. Lambs were protected from pulmonary consolidation and concentrations of P. haemolytica in lung lesions. Use of MDP in these vaccines for sheep could help reduce losses associated with caseous lymphadenitis and pneumonic pasteurellosis.

**Ovine Progressive Pneumonia - Research and Diagnostics Development. Gary Ross (U.S. Meat Animal Research Center ARS Clay Center, Nebraska).**
Ovine Progressive Pneumonia (OPP) is caused by a retrovirus infection of the subfamily lentivinae which means “slow virus”. It can occur in all breeds of sheep and is distributed throughout the United States but occurs in a non-uniform distribution with high prevalence flocks and low prevalence or negative flocks being present in all geographic areas. The disease occurs in sheep 2-3 years of age or older and is characterized by any or all
of the following: chronic weight loss, respiratory secretions among older sheep in intensive management systems. Diagnosis of non-clinically infected animals is by serological testing for antibodies to the virus. This has been predominately done with an agar gel immunodiffusion (AGID) test. However, the technology exists to develop and use recombinant protein enzyme-linked immunosorbency assay (ELISA) tests which should enhance sensitivity of testing methods and potentially reduce testing and control costs. Future research will be directed toward the development of highly sensitive and specific diagnostic tests which are fast and low cost. These will be used to characterize modes of transmission, develop cost effective control programs, and measure the true economic effect of this disease.

Drug Use In Small Ruminants. G. A.(Bert) Mithcell (FDA/CVM). UNAPPROVED USES.

General Rule.-- Section 512(a) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(a) is amended by adding the following new paragraphs at the end: "(4)(A) Except as provided in subparagraph (B), if approval of an application filed under subsection (b) is in effect with respect to a particular use or intended use of a new animal drug, the drug shall not be deemed unsafe for the purposes of paragraph (1) and shall be exempt from the requirements of section 502(f) with respect to a different use or intended use of the drug, other than a use in or on animal feed, if such use or intended use-- *(i) is by or on the lawful written or oral order of a licensed veterinarian within the context of a veterinarian-client-patient relationship, as defined by the Secretary; and

Biologic Guidelines for Small Ruminants. Ashley Robinson (University of Minnesota).

Guidelines for the use of biologic in animals are issued periodically by the Council on Biologic and Therapeutic Agents (COBTA). The guidelines are intended to document currently accepted protocols for vaccinations based on the best available scientific information. A survey of vaccination practices recommended by members of the American Association of Small Ruminant Practitioners (AASRP) conducted by Drs Ashley Robinson and Cindy Wolf of the College of Veterinary Medicine, University of Minnesota served as the basis of the guideline. The contributions of the other members of the AASRP who assisted in preparation of these guidelines are also gratefully acknowledged. The veterinary practitioner must realize that licensed biologic should be used in accordance with label directions. However, because the number of biologic licensed for use in small ruminant species continues to be limited, products licensed for use in other species (particularly cattle) frequently are considered to be effective in sheep, goats, and llamas.
Scrapie Update. Dan Harpster (USDA/APHIS/VS)

As of October 24, 1994, there were 70 flocks enrolled in the VSFCP, 6 source and 81 infected flocks. The National Scrapie Oversight Committee has had several conference calls and two meetings during 1993-1994. The Committee has made recommendations regarding:

1. Introduction of germ plasm from lower or nonenrolled flocks to higher flocks with no adverse affect on the higher flock’s certification status. The semen portion of this recommendation was accepted while the embryo portion was not due to inconsistencies of research results on embryos;
2. Encouraging the use of electronic identification;
3. Distribution of Program Information to the public;
4. The Commercial Category;
5. Flock Plan Components; and

Scrapie: Update on Scrapie Susceptibility Genes. Katherine O’Rourke (USDA/ARS Pullman, Wash.)

The cause of scrapie is not known but the sheep prion protein is involved in the disease process. It has now been reported that the prion protein gene may regulate susceptibility to the disease in animals exposed to the scrapie agent. In a genetic study of the prion gene from more than 40 carried the form of the gene known as “QQ171”. This form of the gene was represented in approximately half of the normal animals studied, suggesting that QQ171 is a susceptibility allele. Expanded studies including nearly 500 Suffolk sheep with experimental exposure to scrapie are being conducted to determine whether animals with other forms of the prion gene are carriers of the disease or are truly resistant animals.
The cause of scrapie is not known but the association between scrapie and the prion protein remains strong. The prion protein is a normal protein which assumes an abnormal conformation in scrapie-affected animals. The scrapie-associated form, PrP-Sc, may actually be the agent which transfers the disease to other sheep.

Prion allotypes in sheep scrapie: The prion gene occurs in several slightly different forms, or allotypes, differing only at one or two amino acids. The two amino acids which appear to control scrapie incubation times are at positions 136 and 171. The position 136 changes have been reported in sheep in the U.K. (1) but appear to be rare in Suffolks in the U.S. and the U.K. In a study published this spring (2), researchers from the University of California and APHIS have proposed that the amino acid at position 171 may predispose Suffolk sheep to scrapie if they are exposed. Position 171 can encode the amino acids glutamine, abbreviated “Q”, or arginine, “R”. Because sheep inherit one copy of the prion gene from each parent, an animal can be QQ171, RR171 or QR171.

Is there a genetically susceptible sheep? In the U.S. study (2), nearly half the animals surveyed were QR171 but all the scrapie-affected sheep sampled were QQ171. This observation is consistent with unpublished data from other laboratories. We can conclude that QQ171 sheep may be susceptible to scrapie if exposed to the agent under the right conditions. However, most QQ171 animals are scrapie-free.

These studies once again raise the possibility, proposed decades ago, of breeding for scrapie resistance. Although the new data on prion types is encouraging, several important questions must be answered before a selective breeding scheme could be developed.

Is susceptibility different from incubation time? Scrapie susceptibility genes in experimental animals control the length of the incubation period, not the eventual development of scrapie. Under experimental conditions, even long incubation animals eventually develop the disease if they survive long enough. An incubation period of more than 7 years may be necessary before we can equate a long incubation period with resistance in sheep.

Are QR171 animals “carriers”? The scrapie agent probably replicates in the lymph nodes of animals throughout their lives, even though damage
to the brain becomes evident after age 3. The data on scrapie susceptibility is a measure of the agent in the brain. We do not know if QR171 animals harbor scrapie infectivity in their lymph nodes or if they can transmit the disease. Studies on the carrier state in genetically defined animals have been initiated.

Are RR171 animals resistant to scrapie? In one human prion disease, people accidently exposed to the infectious agent were susceptible to disease if their maternal and paternal prion genotypes were identical. It did not matter which amino acid was carried on the genes, as long as both genes carried the same prion type. The number of RR171 sheep in the U.S. study (2) was too low to analyze susceptibility. Larger numbers of flocks are being surveyed to estimate the incidence of RR171 sheep in U.S. Suffolks. Further, experimental inoculation of RR171 Suffolks must be done to determine whether these sheep are truly resistant or simply have a long incubation time.

Until we have these additional data, we can conclude that QQ171 sheep are susceptible to scrapie if exposed, but we cannot draw any conclusions about QR171 or RR171 animals.

Other considerations: Flock owners who diversify to include goats or llamas should be aware that goats are natural hosts of scrapie. The susceptibility of llamas to scrapie is not known but segregation of animals during the high risk lambing season may be recommended by the flock veterinarian.

References
Introduction

Recently, an article in _THE ECONOMIST_ (March 19th-25th, 1994) stated, “There is, surprisingly, little or no evidence that modern doctors, pills or surgery have improved people's overall state of health. The increase in American's average life expectancy from 63 years in 1940 to 76 today has been ascribed to increased wealth, better sanitation, nutrition, housing, and the widespread introduction of the refrigerator than to modern medicine.” Whether this statement is valid or not, it does make the point that prevention and avoidance of exposure to food borne disease is the best approach to prevent food borne illness. It is estimated that food borne disease costs the United States 6.5 billion dollars annually in lost wages and increased medical expenses. As professional animal health experts, we must educate producers in the techniques necessary to produce safe, high quality animal products.

The dairy, poultry, and all red meat industries except lamb, have in place producer level quality assurance (QA) programs. QA programs vary from voluntary to regulatory in nature. Each of the various meat industries' QA programs currently in place was developed in response to specific consumer concerns about human health risks (whether actual or perceived) associated with the consumption of livestock products. In each case, producer participation in QA programs has helped restore consumer confidence, as evidenced by regained market share.

The sheep industry has been fortunate in that, to date, sheep products have not been linked to any food borne disease outbreak, and only in rare instances have lamb samples been found to contain violative residues. One reason for lamb's clean record is that the vast majority of lambs have traditionally been raised on western ranges. In this type of production system lambs are rarely exposed to confinement, vaccination, or drug therapy. In addition, traditional consumers of lamb prepare most meat cuts “well done”, and very little commercial ground lamb product is consumed compared to ground beef and ground pork products.

Sheep production practices are, however, changing. The increased pressures faced by range sheep producers utilizing public lands have resulted in a dramatic decline in extensive range operations. While the number of large range operations is decreasing, the number of small farm flock operations, owned and managed by producers with little or no agricultural background, is increasing. Sheep raised under more intensive systems are at greater risk of exposure to drugs, vaccines, and pathogenic microorganisms. This increased exposure can result in increased quality
short falls. The challenge for the sheep industry is to be certain that producers of sheep are keenly aware they are food producers; that quality sheep products begin with the producer; and that it is imperative for producers to address consumer concerns and focus on consumer needs.

Without a producer QA program in place, the American Sheep Industry (ASI) was concerned that sheep producers and the entire sheep industry were at risk of being perceived by consumers as lacking in human health concerns. Taking a proactive stance, ASI contacted Colorado State University (CSU) about developing a sheep QA program. The CSU team accepted ASI’s challenge. A USDA-ES competitive grant was awarded, and along with ASI support funding, the CSU team began developing the sheep QA program. Since the sheep industry was the last of the red meat industries to develop a QA program, it had the advantage of being able to draw upon the experience and expertise of existing livestock QA programs.

Both of the beef and pork QA programs initially addressed specific problems in their respective industries. Through these programs, the beef and pork industries have made significant progress toward re-establishing consumer confidence in their products. CSU Meat Scientists have developed an assessment process termed an audit to help identify quality short-falls in livestock production systems. The success of this approach has led to the use of the QA program as a mechanism for addressing other quality issues. Industries that employ the audit process are able to identify and gain an understanding of where quality short-falls are occurring in the production chain and to use that information to develop strategies to correct those short-falls.

Since the sheep industry has not experienced the critical eye of the consumer or consumer groups, there was no predefined area of focus relative to a QA program. The CSU sheep QA team initiated developing the sheep QA program by designing and performing a comprehensive sheep audit. The audit process included meat, pelts, wool, lanolin, milk, and processes (i.e., slaughter, transportation, feedlot, and production systems). Information gained from the audit has been utilized to develop the producer quality assurance program, “Producing High Quality Consumer Products From Sheep”. Educational materials are currently being developed to accompany the release of the producer program.

Audit

Lambs

The audit process for lamb was initiated at the lamb packing plant level. Since the slaughter process represents the narrowest point in the production-to-consumption chain, the CSU team was able to quantify and qualify the quality defects occurring as result of production and processing. Eight years of Food Safety and Inspection Service (FSIS) cause of
condemnation data was analyzed and summarized prior to the actual on-
sight visits and face-to-face interviews conducted with packers. The lamb
packing plants visited were located across the U.S.. These plants
represented in excess of 85% of all lambs slaughtered under FSIS
inspection.

**Residues** - Lambs produced commercially rarely have violative
chemical residues detected by the FSIS residue monitoring program.
(However, lambs raised for show purposes often times have a wide variety
of illegal residues, e.g., clenbuterol, anabolic steroids and antibiotics).
When commercially raised lambs are identified with residue problems it is
typically a result of antibiotics fed in finishing rations. The overall national
percentage of lambs condemned due to violative residues is .0017; less
than 2 lambs per 100,000 lambs slaughtered.

To insure that producers continue to produce lamb free of residues, and
to address the residue problem currently existing in the show lamb portion
of the industry, the producer QA program promotes and educates producers
about the importance of establishing and maintaining a valid veterinary-
client-patient relationship. The program also seeks to educate producers
about the importance of following all drug label directions, adhering to
proper withdrawal times (especially with extra label drugs), and following
proper "drug usage" protocols (e.g., treatment records, identifying treated
animals, etc.).

**Pathogen Reduction** - There is little information available relative to
pathogenic microbial contamination of sheep carcasses. The FSIS beef
study completed in 1993 characterized the incidence of pathogenic
microorganisms on beef carcasses. Pathogen studies for pork and poultry
are in place and are to continue this year. A similar microbiological study is
also currently being conducted by the CSU QA team for lamb. However, no
information is available for release at this time. Although no human case
has been linked to the consumption of lamb, a study conducted in Wisconsin
indicates that the incidence of *E. coli* 0157:H7 in lamb may be similar to that
of beef. That is not to say that lamb carcasses are not at risk of microbial
contamination during processing. In fact, according to an on-going
microbial contamination study at CSU, lamb carcasses can become
severely contaminated with microorganisms during processing from a
variety of sources. Preliminary data indicates that the length of the wool,
manure contamination, and wetness of the pelt are all factors that can
directly contribute to the amount of microbial contamination that occurs
during the slaughter process. Pre-evisceration washing of carcasses can
serve as a disseminating mechanism for microbes. Carcass contamination
during depelting is generally confined to localized areas on the carcass.
However, washing at low pressures, coupled with inadequate transit times
through the washing cabinet, often results in increased total carcass
microbial contamination. Fecal material from the anus and trotters are also
sources of contamination during the pre-evisceration wash. In addition, handling and required trimming of visual contamination by FSIS inspectors many times only serves to increase total carcass contamination. Increased fecal contamination of carcasses directly affects the pathogen contamination risk. Surface contamination of lamb carcasses with both pathogenic and nonpathogenic microorganisms is not necessarily related to human disease, but can be related to increased spoilage and decreased product shelf life. The fact that lamb has the shortest shelf life of all the red meats, provides further evidence that excessive carcass microbial contamination is occurring during the lamb slaughter and/or fabrication process. Consumer education and responsibility in the preparation and handling of all meat products is an important final safeguard against food borne illness.

Currently sanitation issues (e.g., wet manure laden lambs), cost the industry $9.50 per lamb contaminated. Intervention strategies that minimize sanitation problems include shearing lambs prior to slaughter, bedding lambs on dry straw or shavings, regular pen cleaning, and providing dry bedding and covered pens 24 hours prior to slaughter. Producers can help reduce the risk of the spread of pathogens through proper handling of sick animals. Minimizing preslaughter stress can also play an important role in reducing the initial pathogen load by reducing the opportunity for pathogens to colonize, multiply and be shed by animals harboring these organisms in their intestinal tracts.

Physical Damage - Bruises and injection site tissue damage adversely effect the economic value of slaughter lambs. Twenty-seven percent of all lambs slaughtered under FSIS inspection have some form of bruising. Over 90% of these lambs have bruises which were inflicted during the forty-eight hours prior to slaughter, (bright bruise). Bright bruises are the result of poor handling practices during loading, transportation, unloading, and movement to the packing plant kill point. Trim-out of bruises accounts for a loss of $2.00 per lamb affected. Without exception, when carcass bruising occurs pelt damage occurs as well. Pelt damage can decrease the value of the pelt by as much as 50%; or up to $5.00 per pelt.

Injection sites can be a problem when injections are given improperly, administered in inappropriate sites, administered when lambs are wet, and when excessively irritating material is injected. Lambs at greatest risk for injection site abscesses or scarring are those lambs raised in intensive production systems that require more frequent immunizations and treatment by an injection route. Proper injection techniques such as using sterile syringes and sharp clean needles, removing drugs and vaccines from containers with a needle specifically intended for that purpose, administering injectables subcutaneously whenever the product is labeled for subcutaneous injection, and using the “tent” technique, can help reduce injection site tissue damage. Administering routine vaccinations only when
lambs are dry will reduce the likelihood of carrying wool and skin surface microorganisms into the injection site. Using the neck region whenever possible will also minimize the impact of injection site reactions on high value cuts and on the pelt.

**Diseases and Parasites** - Pneumonia is the most significant disease causing total or partial condemnation in slaughter lambs. Fifty percent of Midwest lambs slaughtered have some form of loss due to pneumonia. The northwest states also experience a very high incidence of pneumonia. When these lambs are transported to feedlots, increased levels of stress during shipping can exacerbate a quiescent pulmonary lesion, resulting in chronic cases, and sometimes death. Preventing pneumonia at all stages of a lamb’s life will help reduce the negative economic impact of this disease. Regionally, other diseases adversely affect lambs (e.g., sarcocystis, cysticercosis, and arthritis).

Parasites are a significant cause of liver condemnations. Fifty percent of all lamb livers are condemned. Of these, parasites and migratory tract scars left by parasites are the major reasons for condemnation.

**Adult Sheep**

Less than one third of all cull ewes and other adult sheep in the U.S. are currently being slaughtered in this country. However, it is interesting to note that residues have not been identified as a problem in the adult sheep slaughtered and monitored under the FSIS. The most significant disease causing condemnation in this group is caseous lymphadenitis. Over forty percent of all ewes slaughtered in the U.S. have some amount of condemnation as result of caseous lymphadenitis.

An emerging dairy sheep enterprise is also raising concerns about drug residues in sheep milk. Until recently, all sheep milk in this country was used for the production of cheese. Cheese production will not tolerate any antibiotic residues. This has prompted dairy sheep producers to remove any ewe requiring antibiotic therapy from the milking string for the remainder of her current lactation. This is a very admirable approach, and certainly is the safest, as accurate withdrawal times have yet to be established for lactating ewes.

Zoonotic diseases, such as tuberculosis and brucellosis, raise additional concerns for the emerging sheep dairy industry. Little has been done to define the incidence or assess the risk of transmission of the diseases from sheep to the human population. As the industry moves into the production of fresh milk products, and as it gains acceptance under the dairy PLO [[write out]], sheep dairy producers will require help and guidance in addressing these issues.

**Government Agencies**

All food animal producers and industries will have to address changes
in government policies which will dictate how food animals are to be produced and handled from the farm to the consumer's plate. Animal and Plant Inspection Service (APHIS) is concerned about reducing pathogens carried by living food animals and subsequently entering the human food supply (Pre-Harvest Pathogen Reduction). FSIS is redesigning its current organoleptic based inspection system, to incorporate microbial and risk assessment procedures. Regulatory agencies will increasingly become involved in reducing the risk of illness from red meats in an attempt to protect consumers. The Federal Drug Administration currently relies on livestock industry QA programs to help educate producers who may unknowingly and unintentionally market animals for slaughter that exceeded violative residue levels. Producer QA programs should help producers make the transitions mandated by government agencies.

Summary & Comments:
Quality assurance programs can play an important role in reducing consumer concerns about the livestock products they consume. Consumer confidence is the driving force in keeping sheep production a viable industry. Voluntary programs can reduce the impact of new regulations resulting from government agencies' attempts to protect the consuming public. Veterinarians are the logical and a credible link between producers, other segments of the industry, and the government.
DEVELOPMENT OF VACCINES CONTAINING MURAMYL DIPEPTIDE FOR THE PREVENTION OF CASEOUS LYMPHADENITIS AND PNEUMONIC PASTEURELLOSIS IN SHEEP

Kim A. Brogden* and Francoise Audibert


*Corresponding author.

Abstract

The efficacy of experimental vaccines containing the synthetic adjuvant, muramyl dipeptide (MDP) and Corynebacterium pseudotuberculosis or Pasteurella haemolytica capsular polysaccharide was determined in lambs. Lambs vaccinated with a bacterin containing C. pseudotuberculosis (1 mg whole cells and 50 μg MDP/dose in 10% light mineral oil) developed antibody titers and were protected against both experimental infection and natural caseous lymphadenitis. No adverse local or systemic reactions were seen during the vaccination period. Lambs vaccinated with a subunit vaccine containing capsular polysaccharide (CP) of P. haemolytica serotype A1 (1.0 mg CP with 50 μg of MDP/dose in 10% light mineral oil) developed antibody titers characterized by an early IgM response. Lambs were protected from experimental infection and had significantly reduced areas of pulmonary consolidation and concentrations of P. haemolytica in lung lesions. Use of MDP in these vaccines for sheep could help reduce losses associated with caseous lymphadenitis and pneumonic pasteurellosis.

Introduction

Vaccination of sheep against bacterial infectious disease ensures better health of the animal resulting in increased productivity to the producer. However, in spite of current advances in microbiology (i.e., identification of protective epitopes on many veterinary bacterial pathogens, identification of new adjuvants, and improvement of vaccine formulations), many vaccines are still ineffective in controlling disease in sheep. This may be due to the following reasons. First, vaccines for sheep must be easy to produce and inexpensive to manufacture. This often prohibits the use of subunit vaccines involving many purification steps. Therefore, conventional whole cell or crude culture supernatant vaccines predominate. Second, vaccines must contain effective adjuvants. Conventional adjuvants can induce undesirable side effects and often result in sub-optimum efficacy. Finally, vaccines must protect against numerous serotypes of the organism inducing disease. For some organisms (i.e., Corynebacterium pseudotuberculosis) this is not a problem but for other organisms (i.e., Pasteurella haemolytica)
vaccines should be multivalent and protect against serotypes A1, A2, and A6.

A logical approach would be to use new synthetic adjuvants in conventional vaccine formulations. This would not only give "new life" to the vaccine by boosting its efficacy as well as potentiate the immune response to poorly immunogenic vaccine constituents but also significantly reduce adverse systemic or localized injection side effects. In addition, manufacturing processes and cost would not be compromised.

In the past few years, adjuvant technology has increased dramatically. New adjuvants have been identified and chemically defined, have molecular structure-function relationships in the type of immune response they stimulate, and can be synthesized in bulk, making their use in vaccines cost effective. Two examples include muramyl dipeptide [2-acetamido-2-deoxy-3-O-(D-2-propionyl-L-alanyl-D-isoglutamine)-D-glucopyranose; MDP] and a lipophilic derivative, MDP-sn-glyceryl-dipalmitoyl [3-O-(N-acetylmuramyl-L-alanyl-d-isoglutaminyl)-1,2-di-O-palmitoyl-sn-glycero1; MDP-GDP]. MDP is the minimal structure essential for the adjuvant effect of mycobacteria in Freund's complete adjuvant (Warren et al. 1986; Audibert and Lise 1993) and may be better than present adjuvants for potentiating conventional vaccines. In this paper, we summarize work to develop vaccines for the prevention of caseous lymphadenitis and pneumatic pasteurellosis in sheep using the adjuvant analogs MDP or MDP-GDP.

Protection of lambs by Corynebacterium pseudotuberculosis whole cell vaccines and MDP

Abscesses can be found on the heads, necks and occasionally in the visceral cavities of sheep. These abscesses result from infections in the peripheral lymph nodes, thoracic lymph nodes, and occasionally the lung due to infected shearing nicks or natural exposure. Corynebacterium pseudotuberculosis is the most common organism isolated.

Vaccination with C. pseudotuberculosis reduces the incidence of caseous lymphadenitis (CLA) in young lambs. Development of an effective vaccine has followed two distinct paths. The first strategy has emphasized the use of a toxoid prepared from C. pseudotuberculosis phospholipase D (Brown et al. 1986). This toxin is a sphingomyelin-specific, phosphatidylycholine phospholipidohydrolase that helps the organism invade and colonize host tissue. Vaccination with toxoid halts the spread of the organism and induces protection. Still, there are additional immunogens associated with the organism. For example, attenuated mutants of C. pseudotuberculosis, without the phospholipase D gene, elicit a strong humoral and cell-mediated immune response and protect sheep from wild-type challenge (Hodgson et al. 1992).

The second strategy of vaccine development has emphasized the use of whole cell bacterins (Cameron 1982; Cameron et al. 1972), chemically extracted whole cell bacterins (Brogden et al. 1985), or cell wall fractions
DEVELOPMENT OF VACCINES CONTAINING MURAMYL DIPEPTIDE

(Cameron 1982; Brogden et al. 1985; Cameron et al. 1969). Vaccination with bacterins also halts the spread of the organism and induces protection. Efficacy can be increased by adding an adjuvant (Brogden et al. 1990).

The latter has been the approach of our laboratory. A whole cell antigen (WC) is prepared from C. pseudotuberculosis ATCC 19410 and washed to remove lipid; once in 50% acetone, once in 100% acetone, twice in ethyl ether and air dried (Figure 1). WC (1 mg/ml) is mixed with 50 ug MDP and emulsified in 10% mineral oil. In laboratory work, this formulation induced high prechallenge antibody titers (Figure 2) and protective immunity against experimental infection (Brogden et al. 1990) (Table 1). No pulmonary or vaccination site abscesses were seen in vaccinated lambs.

The efficacy of this bacterin was determined under field conditions in lambs from a purebred university sheep flock (Brogden, et al. 1994a). Serum antibody titers to C. pseudotuberculosis rose sharply one month after the initial vaccination and remained significantly higher in vaccinated animals throughout the trial period. There was also substantial protection after 18 months in lambs against clinical caseous lymphadenitis. Subcutaneous abscesses were seen in 5% of vaccinated lambs and in 23% nonvaccinated lambs.

Protection induced by Pasteurella haemolytica capsular polysaccharide and MDP

Pasteurella haemolytica contains two biotypes, A and T, and 16 capsular serotypes. Pasteurella haemolytica biotype A (serotypes 1, 2, 5-9, 11-14, and 16) causes two distinct disease syndromes in sheep: septicaemia in young lambs and enzootic pneumonia, the principal disease, in all ages of sheep (Gilmour 1980; Gilmour et al. 1980). Vaccination reduces P. haemolytica pneumonia. But the exact nature of the immunogen is not known.

The P. haemolytica capsular polysaccharide (CP) is a possible vaccine immunogen to consider. Generally, CP represents the initial point of contact between the invading pathogen and the host. Therefore, an immune response to CP is desirable to activate antibody-mediated killing and phagocytic clearance mechanisms important in protecting the lung from bacterial infection and colonization. Highly purified CP from P. haemolytica strain P-1075 (serotype A1), purified from culture supernatants by acetone and ethanol precipitation (Adlam et al. 1984), consists of \(-\text{3)}\)-O-(2-acetamido-2-deoxy-4-O-acetyl-B-D-mannopyranosyluronic acid)-(1\text{\textperiodcentered})-O-(2-acetamido-2-deoxy-B-D-mannopyranosone)-(1\text{\textperiodcentered}). CP (1 mg/ml) is mixed with 50 ug MDP or MDP-GDP and emulsified in 10% mineral oil. In laboratory work, CP + MDP induced a rapid IgM titer by day 7 and remained elevated above the titers of lambs vaccinated with only CP (Figure 3a). IgG titers, however, fluctuated with no specific trend (Figure 3b). CP + MDP-GDP induced a similar response; IgM and IgG titers of lambs remained elevated above the mean titers of the nonvaccinated control lambs.
and lambs vaccinated with CP.

With an ovine adenovirus-\textit{P. haemolytica} challenge model (Lehmkuhl et al. 1989) control lambs and lambs vaccinated with only CP had gross evidence of varying severity of combined viral and bacterial pneumonia, characterized by parenchymal consolidation and variable necrosis, hemorrhage, and fibrinous pleuritis (Brogden et al. 1994b) (Table 2).

In one experiment, lambs vaccinated with CP + 50 \textmu g MDP had lesser areas of consolidation attributed primarily to the adenovirus infection and lesions did not contain \textit{P. haemolytica} (Table 2). In another experiment, lambs vaccinated with CP + MDP or CP + MDP-GDP, also had small areas of consolidation attributed to both the adenovirus and slight bacterial infection but contained lower concentrations of \textit{P. haemolytica} than the other groups of lambs (Table 2).

Both vaccines induced minimal if any lesions at the injection site. The 2 ml dose resulted in small, volume related nodules in all vaccinated groups that resolved by day 21. No other adverse effects of vaccination (i.e., immediate anaphylaxis, etc.) were seen in any lamb during either initial or booster vaccination or at challenge exposure.

Although there have been some successes (Gilmour et al. 1979), CP alone as a vaccine immunogen has not been seriously considered. Usually, anti-CP antibodies are produced in low quantities, often lack specificity, and are not bactericidal. In this study, vaccines containing CP + MDP or MDP-GDP in 10\% oil, induced a strong serologic response in lambs characterized by high titers of IgM antibodies in 7 days. Protective immunity was induced. However, other \textit{P. haemolytica} serotypes such as A2 and A6 also induce pneumonia. In this study, we examined the ability of muramyl dipeptides to increase the humoral and protective response of lambs to serotype A1 CP. Later, similar studies would look at the efficacy of CP from serotypes A2 and A6.

\textbf{Summary}

Synthetic adjuvants in current veterinary vaccine formulations may improve vaccine efficacy without dramatically affecting the production costs associated with vaccine manufacture. In this paper, we showed that MDP can be used to potentiate the immune response to conventional vaccines (i.e., WC of \textit{C. pseudotuberculosis}) and induce satisfactory immune responses to poorly immunogenic vaccine constituents (i.e., CP of \textit{P. haemolytica} serotype A1) without adverse systemic or localized injection side effects. Such advances should reduce the losses associated with bacterial infectious disease in sheep.

\textbf{References}

DEVELOPMENT OF VACCINES CONTAINING MURAMYL DIPEPTIDE


480


Table 1

Protection induced in lambs by Corynebacterium pseudotuberculosis whole cell (WC) antigen with muramyl dipeptide (MDP).a

<table>
<thead>
<tr>
<th>vaccine</th>
<th>No. abscesses in infected lambs</th>
<th>Concentration in infected lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>25.0</td>
<td>4.5</td>
</tr>
<tr>
<td>WC</td>
<td>23.0</td>
<td>1.3</td>
</tr>
<tr>
<td>WC + 50 ug MDP</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*aBrogden et al. 1990.
bMean number of abscesses per entire lung surface.
cLogarithmic mean concentration of C. pseudotuberculosis per gram of lung.

Table 2

Protection induced in lambs by Pasteurella haemolytica serotype A1 capsular polysaccharide (CP) and muramyl dipeptide (MDP) or MDP-sn-glyceryl-dipalmitoyl (MDP-GDP).a

<table>
<thead>
<tr>
<th>experiment</th>
<th>% total lung consolidation</th>
<th>bacterial isolation</th>
<th>concentration CFU/gm lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 none</td>
<td>19.7b</td>
<td>5/5</td>
<td>2.4 x 10^7c</td>
</tr>
<tr>
<td>CP</td>
<td>18.0</td>
<td>4/5</td>
<td>3.6 x 10^7</td>
</tr>
<tr>
<td>CP + MDP (50 ug)</td>
<td>10.4</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>2 none</td>
<td>10.9</td>
<td>4/5</td>
<td>2.9 x 10^6</td>
</tr>
<tr>
<td>CP</td>
<td>9.0</td>
<td>5/5</td>
<td>3.4 x 10^6</td>
</tr>
<tr>
<td>CP + MDP (50 ug)</td>
<td>5.8</td>
<td>3/5</td>
<td>9.9 x 10^1</td>
</tr>
<tr>
<td>CP + MDP-GDP (50 ug)</td>
<td>5.4</td>
<td>3/5</td>
<td>5.2 x 10^3</td>
</tr>
</tbody>
</table>

*aBrogden et al. 1994b.
bMean consolidation.
cMean CFU.
DEVELOPMENT OF VACCINES CONTAINING MURAMYL DIPEPTIDE

Figure 1. Morphology of *C. pseudotuberculosis* after a) growth on Tween-albumin agar showing with a thick outer lipid layer, heavy cell wall, and intact cytoplasmic membrane containing a nucleoid, intracellular vacuoles, and mesosomes within a dense cytoplasm and after dehydration in acetone and delipidated in ether. Cells were aggregated because of fused outer lipid layers. The cytoplasm was homogenous and distinct nucleoids, vacuoles, and mesosomes could not be seen. In some cells, the cytoplasmic membrane had pulled away from the cell wall. Bar = 0.5 μm

![Image of cell morphology](image1)

Figure 2. Antibody response to *C. pseudotuberculosis* WC and WC + MDP vaccines determined with a microagglutination assay (Menzies and Muckle 1989). This assay uses *C. pseudotuberculosis* grown in broth containing 0.003% triphenyltetrazolium chloride as the antigen (Brogden et al. 1990).

![Image of antibody response](image2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>Mean (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg WC</td>
<td>0</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>1 mg WC + 50 μg MDP</td>
<td>0</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

**DAYS**

0 5 10 15 20 25 30 35 40 45

**COMPLEMENTARY TESTER**
Figure 3. Serum titers of lambs vaccinated with *Pasteurella haemolytica* CP and CP + MDP vaccines; a. IgM titers, b. IgG titers. Serum antibody responses to *P. haemolytica* CP were determined with an enzyme-linked immunosorbent assay to CP immobilized onto styrene plates (Brogden et al. 1994b).
Program Background
The Voluntary Scrapie Flock Certification Program (VSFCP) started on October 1, 1992. The first two years of the program have been used to educate producers about the program’s functions and merits and to enroll flocks.

The National Scrapie Oversight Committee, which provides input to the Deputy Administrator for the direction of the program, has had several teleconferences during 1994 along with two meetings. The main topics of discussion were the germ plasm and identification issues.

Committee Actions
The Committee has recommended to the Deputy Administrator that semen and embryos from lower or nonenrolled flocks be allowed to be used in higher level flocks with no negative impact on the recipient flock’s certification status.

Semen and Embryos
The Deputy Administrator has accepted this recommendation for semen but, due to conflicting results on embryo studies, has not accepted the recommendation for embryos. Semen from lower or nonenrolled flocks will be allowed to be used in higher level flocks with no negative impact on the recipient flock’s certification status. The use of embryos from lower or nonenrolled flocks in higher enrolled flocks will continue to lower the recipient flock’s certification status to that of the donor flock.

Electronic Identification
Electronic identification (EI) is recognized as an official means of identification with the following stipulations:
1. A reader will be provided by the owner when it is needed during inspections, issuing of health certificates and at sales;
2. The implants are compatible with the International Standards when they become available;
3. The Food Safety and Inspection Service is notified by the owner when implanted animals go to Federally inspected slaughter plants; and
4. Implanted animals are tattooed with the seller’s premises tattoo code when sold to flocks not using EI.

Other issues discussed by the Oversight Committee were:
Distribution of Program Information

Lists of Infected, Source and Enrolled flocks are available through Veterinary Services' Area Offices and the Hyattsville Headquarters Office. Lists of Enrolled flocks are being sent to some sheep magazines and the National Oversight Committee Members quarterly.

The "800" telephone line in Fort Collins, Colorado, for the 24 hour toll free access to program information, is on hold. The estimated cost of initial setup for the system exceeded $60,000.00, so further evaluation of such a system is being conducted.

Commercial Category

The Commercial Category of the VSFCP became effective on July 1, 1994. This part of VSFCP is separate and independent from the original program and emphasizes ram identification, recordkeeping and inspections of cull breeding stock.

Flock Plans

Flock plans for Infected or Source flocks with high risk animals were reviewed. Those Infected or Source flocks from which high risk animals were identified are required to follow the seven points of Title 9, Code of Federal Regulations Part 79.2. These requirements are to be included in the flock plan.

Exhibition Guidelines

The Committee developed a set of suggested exhibition guidelines for the VSFCP. The guidelines recognize that the scientific knowledge regarding the lateral transmission of scrapie is incomplete.

Actions such as penning enrolled sheep separately from nonenrolled sheep, defining limited contacts and commingling and preventing contact with lambing ewes at an exhibition are included in the guidelines. The guidelines are available through Area Offices, State Scrapie Certification Boards or the Hyattsville Office.

The VSFCP continues to be shaped to meet the needs of the sheep and goat industry and to better address the scrapie issue.

Proposed Import Amendments

The proposed amendments to the import regulations affect Title 9, Code of Federal Regulations parts 92, for live animals, and 98, for semen and embryos. The proposals are currently being prepared for publication in the Federal Register.

These proposals are being developed to allow sheep and goat germ plasm from foreign countries to enter the U.S. by using the VSFCP. The previous five year quarantine policy will still be an option along with the VSFCP. Further details will be available when the proposals are published in the Federal Register which should be in the near future.
DRUG USE IN SMALL RUMINANTS
ANIMAL DRUG AMENDMENTS ACT OF 1994
G.A. Mitchell, D.V.M.
Director, Office of Surveillance and Compliance
Center for Veterinary Medicine/FDA
at USAHA Sheep and Goats Committee
on October 31, 1994 in Grand Rapids, MI

H.R. 5056

SECTION 1. SHORT TITLE.
This Act may be cited as the “Animal Drug Amendments of 1994”.

SECTION 2. UNAPPROVED USES
(a) GENERAL RULE. —Section 512(a) of the Federal Food, Drug, and
Cosmetic Act (21 U.S.C. 360b(a) is amended by adding the following new
paragraphs at the end:
“(4)(A) Except as provided in subparagraph (B), if an approval of an
application filed under subsection (b) is in effect with respect to a particular
use or intended use of a new animal drug, the drug shall not be deemed
unsafe for the purposes of paragraph (1) and shall be exempt from the
requirements of section 502(f) with respect to a different use or intended
use of the drug, other than a use in or on animal feed, if such use or inten
tended use—
“(i) is by or on the lawful written or oral order of a licensed veteri
narian within the context of a veterinarian-client-patient relation
ship, as defined by the Secretary; and
“(ii) is in compliance with regulations promulgated by the Secretary
that establish the conditions for such different use or intended use.
Regulations under clause (ii) may prohibit particular uses of an
animal drug and shall not permit such different use of an animal
drug if the labeling of another animal drug which contains the same
active ingredient and which is in the same dosage form and con
centration provides for such different use.

*4(B) If the Secretary finds that there is a reasonable probability that a
use of an animal drug authorized under subparagraph (A) may present a
risk to the public health, the Secretary may—
“(i) establish a safe level of a residue of an animal drug when it is
used for such different use authorized by subparagraph (A); and
“(ii) require the development of a practical, analytical method for
the detection of residues of the drug above the safe level estab
lished under clause (i).

The use of an animal drug which results in residues exceeding a safe
level established under clause (i) shall be considered an unsafe use of such
MITCHELL

drug under paragraph (1). Safe levels may be established under clause (i) either by regulation or order.

"4(C) The Secretary may by general regulation provide access to the records of veterinarians to ascertain any use or intended use authorized under subparagraph (A) that the Secretary has determined may present a risk to the public health.

"4(D) If the Secretary finds, after affording an opportunity for public comment, that a use of an animal drug authorized under subparagraph (A) presents a risk to the public health or that an analytical method required under subparagraph (B) has not been developed and submitted to the Secretary, the Secretary may, by order, prohibit any such use.

"(5) If the approval of an application filed under section 505 is in effect, the drug under such application shall not be deemed unsafe for purposes of paragraph (1) and shall be exempt from the requirements of section 502(f) with respect to a use or intended use of the drug in animals if such use or intended use—

"5(A) is by or on the lawful written or oral order of a licensed veterinarian within the context of a veterinarian-client-patient relationship, as defined by the Secretary; and

"5(B) is in compliance with regulation promulgated by the Secretary that establish the conditions for the use or intended use of the drug in animals."

(b) OTHER AMENDMENTS.—

(1) SECTION 301.—Section 301 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 331) is amended—

(A) in paragraph (e), by inserting "512(a)(4)(C)," before "512(j),";

(B) by adding at the end of the following:

"(u) The violation of section 512(a)(4)(A), 512(a)(4)(D), or 512(a)(5)."

(2) SECTION 512(e).—Section 512(e) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(e) is amended in subparagraph (A), by inserting before the semicolon the following: "or the condition of use authorized under subsection (a)(4)(A)"

(3) SECTION 512(l).—Section 512(l)(1) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(l)(1) is amended by inserting after "relating to experience" the following: ", including experience with uses authorized under subsection (a)(4)(A),"

(c) REGULATIONS.—Not later than 2 years after the date of the enactment of this Act, the Secretary of Health and Human Services shall promulgate regulation to implement paragraphs (4)(A) and (5) of section 512(a) of the Federal Food, Drug, and Cosmetic Act (as amended by subsection (a)).

(d) EFFECTIVE DATE.—The amendments made by this section shall take effect upon the adoption of final regulations under subsection (c).
Introduction

Ovine Progressive Pneumonia (OPP) is seen in sheep, usually 2-3 years of age or older. The first sign of illness is usually a progressive weight loss in the presence of a normal appetite. This can be followed some time later by the early signs of a respiratory problem, a cough, and nasal discharge. As the disease reaches its later stages, marked difficulty in breathing becomes apparent. During this stage, the sheep is especially susceptible to bacterial pneumonia. This form of the disease will inevitably result in the death of the animal. The virus can also affect the mammary gland causing firmness of the udder and decreased milk production. The joints can also become infected resulting in joint distension and lameness. Flocks with high prevalence of the disease tend to have higher flock replacement rates and decreased pounds of lamb produced per ewe in the flock.

Causative Agent

OPP is caused by infection with a retrovirus. This virus is in the subfamily lentivirinae which means "slow virus". This virus usually infects the animal early in life, but does not cause observable signs of disease for several years. A "slow virus" causes the infected animal to produce an immune response against it; however, the virus persists by infecting and hiding within the cells of the immune system and undergoing slight structural changes that foil the immune system.

Transmission

Since the virus infects cells of the immune system, the virus can infect any organ system that contains these cells. For practical purposes, the organs that have the greatest impact on the spread of the virus in the flock are the mammary gland and the respiratory tract. The primary method of spread is through the colostrum and milk of the ewe to her nursing offspring. It also appears that the virus can be spread through respiratory secretions among adult animals, especially in closely confined production systems.

Susceptibility

Though there appears to be varying susceptibility among different breeds of sheep and differences in the infectivity of the several strains of the virus, all sheep are susceptible to the OPP virus and once infected are unable to
rid themselves of it. No vaccines are available to prevent the disease or raise the animal's resistance to the virus.

**Breaking the Chain of Infection**

At this time, the only way to reduce the prevalence of the disease in a flock and control the infection is to prevent the passage of virus laden substances from infected to noninfected sheep. Depending on the percentage of the flock infected, this can be accomplished by preventing newborn lambs from nursing infected ewes (flocks with high levels of infection), or culling ewes that are positive for OPP from the flock prior to breeding (flocks with low levels of infection).

**Diagnosis**

A positive diagnosis of OPP in the live animal must be made with laboratory tests on serum. An agar gel immunodiffusion (AGID) test has been used most for this purpose, but the test has a relatively low sensitivity that results in a significant number of infected animals being identified by the test as negative. Newer, inexpensive recombinant protein based ELISA tests utilizing the P25 core protein and the GP40 transmembrane protein have higher sensitivities and have specificities comparable to the AGID. These tests should enable control programs to be developed by the producer, veterinarian, and cooperating diagnostic laboratory. A diagnosis of OPP in the dead animal should be made by a veterinarian familiar with the disease and supported by a veterinary diagnostic laboratory, if appropriate.

**Research and Development Priorities**

1. The manufacturer of a commercial OPP ELISA test or OPP recombinant antigen(s) with appropriate positive and negative controls for interlaboratory consistency.
2. Develop the means to diagnose the infection by detection of circulating virus (antigen capture ELISA or polymerase chain reactor (PCR)) to support control programs based on ELISA diagnostics.
3. Use these tests to further define the modes of transmission, control strategies and the economic impact of the disease in a production environment.
4. Determine the biochemical factors that cause the virus infected monocyte to initiate virus replication.
5. Investigate the usefulness of vaccination and/or genetic resistance to control the disease.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY AND OTHER AVIAN SPECIES

Chairman: Dr. Stanley H. Kleven, Athens, GA
Vice Chairman: Dr. Robert J. Eckroade, Kennett Square, PA

Dr. John K. Atwell, NC; Mr. William C. Baisley, GA; Dr. Colin Baxter-Jones, WV; Dr. Charles W. Beard, GA; Dr. Leroy M. Coffman, OR; Dr. Morris S. Cover, MD; Dr. Michael David, MD; Dr. Mark A. Dekich, MD; Dr. Thomas G. Dickson, GA; Dr. Nicholas M. Dorko, Jr., CT; Dr. B. C. Easterday, WI; Dr. Oscar J. Fletcher, NC; Dr. David Frame, UT; Dr. Milton Friend, Wi; Dr. Leonard W. Fussell, AR; Dr. G. Yan Ghazikhanian, CA; Dr. Hashim M. Ghori, AR; Dr. Earl E. Grass, CA; Dr. C. M. Groocock, MD; Dr. Leland C. Grumbles, TX; Dr. D. A. Halvorson, MN; Dr. Karen D. Hicks-Alldredge, TX; Dr. Frederic J. Hoerr, AL; Mr. Robert L. Hogue, IN; Dr. G. Tom Holder, MD; Dr. William D. Hueston, CO; Dr. Daryl C. Johnson, GA; Dr. I. Howard Kahan, FL; Dr. Glenn E. Kolb, WI; Dr. Mahesh C. Kumar, MN; Dr. Hiram N. Lasher, DE; Dr. Joan Leonard, KS; Dr. David J. Ligda, IN; Dr. Edward T. Mallinson, MD; Dr. Richard H. McCapes, CA; Dr. Janis K. McMillen, KS; Dr. Jack A. Meister, CT; Dr. C. U. Meteyer, WI; Mr. Marshall Meyers, DC; Mr. Thomas R. Mickel, GA; Dr. Ram Mohan, OH; Dr. Ahmed Mutalib, MS; Dr. K. V. Nagaraja, MN; Dr. Edwin M. Odor, DE; Dr. R. E. Omohundro, TX; Dr. John S. Orsborn, Jr., CA; Dr. Archibald B. Park, MD; Dr. J. E. Pearson, IA; Dr. Larry J. Peters, OK; Dr. B. S. Pomeroy, MN; Dr. Peter E. Poss, MN; Dr. G. Donald Ritter, MD; Dr. John P. Sanders, Jr., FL; Dr. H. L. Shivaprasad, CA; Dr. V. Sivanandan, MN; Dr. Richard D. Slemons, OH; Dr. H. Wesley Towers, DE; Dr. Deoki N. Tripathy, IL; Dr. Max A. Van Buskirk, PA; Dr. Stanley A. Vezey, GA; Dr. Gary L. Waters, IL; Dr. Robert G. Webster, TN; Dr. David H. Willoughby, CA; Dr. Richard L. Witter, MI, Dr. T. H. Woods, MO.

The committee met on November 1 and 2, 1994 with a total of 95 members and guests attending.

Drs. R.E. McCapes, Harold Chute and Bill Baisley presented eulogies for Ray Bankowski, Everett Bryant, and Mike Rosenstein, dear friends and colleagues who passed away during the past year. Dr. Bankowski served as Chair of the Committee from 1973-1987.

I. Diseases of Importance and Related Issues

A. Poultry Industry-Supported Disease Research Priorities

Dr. C. W. Beard, Vice President of the SEPEA in charge of research presented the following summary of industry supported research.

As of September 1, 1994, there were 80 active research projects funded by SOUTHEASTERN totaling $2,440,256.
TRANSMISSIBLE DISEASES OF POULTRY AND OTHER AVIAN SPECIES

Since the beginning of the program, funds have gone to 40 research institutions in the subject areas of food safety, poultry health, husbandry, waste disposal and food science. North Carolina State University has received the greatest share of the funds followed by the Universities of Georgia and Arkansas.

The number of proposals received at the semi-annual competitions have increased to 78 for August 1994. Seventeen proposals were funded as a result of this competition for a total of $547,556.

Research proposals are evaluated and scored by a Research Advisory Committee which has 15 members, 14 of whom are employed in the industry. The emphasis of the program is to fund research that is directed toward the resolution of important industry problems. A list of industry-based research needs is available to those interested in seeking funds.

B. Current Poultry Industry Health Issues

1. Broiler Industry Subcommittee - Dr. Tom Holder, Allen's Hatchery, presented the following summary of current health issues of concern to the broiler industry.
   a. Infectious Bronchitis - This disease is ever changing due to the nature of the virus. Two years ago, at this meeting, a resolution was passed recommending the establishment of a national typing laboratory. What's the status of this very important project?
   b. Marek's Disease - This disease continues to plague our industry. Virulence changes, strains change and vaccines fail to protect as they once did. Support for research in this area needs to continue.
   c. Mycoplasma - This organism is reemerging in different forms and degrees of virulence. Research needs to continue in all areas of this disease to help the industry understand how to best deal with this long standing problem.
   d. Avian Influenza - This is a disease that needs constant attention. It is always a threat to our commercial industry. Eradication is not likely because of the wild waterfowl infections. Control is the key. It is my understanding that technology exists to develop a genetically engineered vaccine that will allow short lived birds to be protected. The broiler industry would like to see a vaccine developed, stored and used under USDA supervision when an AI outbreak gets out of hand. A technical advisory committee could help advise USDA in this endeavor.
   e. Inspection Regulations - FSIS is in a state of confusion. Certain groups within the agency are wanting HACCP, PEP, and pathogen reduction. All of these initiatives are certainly not without great cost to the industry. The agency needs to be focused on one program and pilot test to make sure the changes do what
is intended. An industry advisory group is recommended to work with FSIS on future proposed changes.

f. Preharvest Food Safety - This is a hot topic presently in USDA. It is consumer driven and not science based. We as an industry, are aware of the fact that reducing pathogenic bacteria helps the overall cost. We are constantly improving growing conditions to improve performance. Will the preharvest food safety initiative decrease deaths, reduce food borne pathogens, lower the incidence of food poisoning or give the consumer a better quality product? Are other alternatives available that will give equal or better results than the preharvest food safety initiative?

2. Table Egg Industry Subcommittee – Dr. Gary Waters, DeKalb Research, presented the following summary of current health issues of concern to the egg industry.

Salmonella enteritidis serotype enteritidis remains the number one concern of the table-egg industry due to the public health concerns. The number of outbreaks related to eggs are sharply reduced (only two) according to the September 13 APHIS report. There are however nine pending cases.

The continued evolution of Marek's Disease virus that circumvents the protection provided by currently available vaccines is a serious threat. Current molecular biology contributions have been directed at delivery mechanisms rather than the more serious issue of antigenic efficacy.

Coli-bacillosis due to E. coli that is resistant to all legally available antibiotics is rapidly becoming a broad scope problem.

The endemic nature of Mg and Ms infections in 1,000,000 plus bird complexes that are not responsive to normal control measurers and threaten breeder operations and other industries is a serious threat. This is compounded by the lack of availability of quality screening antigens.

Exotic sources of Avian Influenza and water fowl reservoirs of VVND/NVND threaten continuation of international embargoes and potential state quarantines that destroy export activities.

Lastly but not least is the current fear associated with reticuloendotheliosis contaminated poultry biologics.

Egg industry pathology considerations have evolved from mortality and production loss issues to those of armageddon scope industry destructions.

Planning control strategies as opposed to reacting to outbreaks is critical.

3. Turkey Industry Subcommittee – Dr. G. Y. Ghazikhanian, Nicholas Turkeys, presented the following summary of current health issues of concern to the turkey industry.

Several members of the United States turkey breeders and integrators were contacted to investigate the current status of turkey health in their regions from mid-1993 to mid-1994.
Once again, it was pleasing to hear that there was no serious industry disruptive diseases which occurred in turkeys. Diseases and health disorders reported by the industry members were of the common type, some with a higher incidence in one region than in others. However, the overall prevalence of various diseases were minor.

In this presentation, diseases and health disorders in turkeys are presented under three major regions in the U.S.A., East, Midwest and West/Southwest.

**Eastern Region (NC/SC/VA/PA/MI/OH)**
- Early poult enteritis with questionable etiology (disease/feed/stress).
- Spiking mortality in 2-4 week old poult during the late spring and summer was still present in some integrators more so than in others. The etiology still remains to be unknown. A task force is charged to study the epidemiology of the syndrome.
- Field Newcastle disease challenge in meat-type turkeys resulted in excessive mortality and condemnations due to complications with B. avium and colibacillosis.
- Sporadic fowl cholera outbreaks in meat and breeder types.
- Sporadic outbreaks of field *M. gallisepticum* in meat turkeys.
- Sporadic outbreaks of F strain *M. gallisepticum* in meat turkeys.
- Outbreak of *M. synoviae* in meat turkeys adjacent to fields spread on with fresh chicken manure.
- Salmonellosis in poult originating from infected breeders.
- Encephalomalacia due to Vitamin E deficient diets.
- Outbreaks of an odd type pneumonia in turkey breeders and some meat type resulted in a significant acute and chronic mortality. Depending on the examining laboratory, either *Pasteurella gallinarum* or *Omitobacterium rhinotracheali* or *Flavobacterium* agents were suspected to be the etiological cause.
- The incidence of breast buttons and blisters and the associated downgrading was, again, high in the summer of 1994.
- Osteomyelitis was, again, a matter of concern to processors.

**Midwest Region (MN/IA/MI/AR/IN)**
- Poult enteritis with questionable etiology (disease/feed/stress).
- Spiking mortality in one small grow-out region. The etiology remains to be unknown. However, corona virus antigen in affected intestinal tissues was detected by FA technique.
- Massive aspergillosis outbreak in poult originated from a hatchery faced with a recycling of *A. fumigates* in their environment.
- Field challenge with mild strains of Newcastle virus in range turkeys occurred. Vaccination of 4-week old meat type turkeys with oil emulsion commercial vaccine prevented the heavy males from
REPORT OF THE COMMITTEE

experiencing high levels of condemnations.

- Increased mortality and condemnations were experienced in meat turkeys due to recycling of Newcastle virus and *B. avium* in confinement turkey premises. Colibacillosis often complicated the problem.
- Non-clinical H7N1 influenza was detected in meat type turkeys during a routine serologic monitoring in processing plant. The very same serotype was also detected serologically in live meat turkeys on farms in the region.
- PMV-3 was diagnosed in a breeder flock.
- Coban toxicity (40-140 ppm) in non-exposed breeders killed 50% of a flock.
- *Salmonella arizonae* outbreak in meat flocks originating from positive commercial breeders.
- Erysipelas in commercial turkeys.
- Breast buttons and blisters were present in a high incidence in the summer months.
- Clinical leg abnormalities experienced in turkeys after 12-14 weeks of age. Type of turkey and the feed quality was suspected in the etiology of the lameness.
- Osteomyelitis was a matter of concern.

West/Southwest Region (CA/WA/CO/TX)

- Early poult enteritis and non-specific flushing in 8-12 week old turkeys resulted in poor performance.
- Viral hepatitis/bordetellosis/colibacillosis complex caused moderate levels of mortality and reduced performance.
- Necrotic enteritis subsequent to mild coccidiosis was experienced.
- Outbreaks of a slow spreading and low pathogenic *M. gallisepticum* in meat turkeys caused low levels of condemnation. Commercial live 6/85 strain of MG vaccine was used in some of the outbreaks to modify the course of condemnations.
- PMV-3 outbreak in a breeder flock resulted in 25% drop in egg production.
- Clinical leg abnormalities, irrespective of breed, was experienced due to push for increased rate of early growth in turkeys was experienced. Modification of the diet and change in breed alleviated the problem.
- Low level of ionophore toxicity in meat turkeys occurred during the summer heat.
- High incidence of drop crop was noticed in the summer.
- The incidence of breast blisters in males was higher in some companies than in the others during the summer. Leg problems exacerbated the problem.
Osteomyelitis in males was a matter of concern in the processing plants.

4. Ratite Industry – Dr. Karen Hicks-Alldredge, Sweetwater Veterinary Hospital, presented the following summary of current health issues of concern to the ratite industry.

The term "ratite" is Latin for "raft-like keel." Although all ratite birds have a raft-like keel, are flightless and originate from the southern hemisphere, there are innumerable anatomical, physiological, disease susceptibility, reproductive and product related differences that are well documented in the formal literature. These birds belong to different taxonomic orders, and cannot be generalized as one "type" of animal by any stretch of the educated imagination.

Species specificity of potential pathogens has been clearly defined for several organisms. Eastern and Western equine encephalomyelitis, for example affect adult emus causing profuse hemorrhagic diarrhea and acute death. Ostriches can be housed in the same environment and test positive for the virus while showing no clinical signs. The Birna virus isolated from ostrich in Texas, Florida, and California may be an ostrich specific virus based on preliminary tests.

Organisms such as *Erysipelothrix rhusiopathiae* affecting emu and *Serpulina hyodysenteriae* affecting rheas, may affect these species due to differences in management practices for producers of the various species. *Salmonella typhimurium* is a fairly common isolate from emu and rhea and is much less common in ostrich. However, many people that raise emu and rhea feed catfish food to the birds which may increase exposure. *Salmonella enteritis* phage type 4 has been isolated from an emu in the Netherlands.

A listing of potentially pathogenic agents isolated from ostrich, emu, and rhea follows:

**Viruses**

1. Avian Influenza
2. Myxovirus-like
3. Newcastle Disease (Paramyxovirus-1)
4. Paramyxovirus-2
5. Coronavirus
6. Adenovirus
7. Picornavirus
8. Birnavirus
9. WEE
10. EEE
11. Reovirus
12. Astrovirus
REPORT OF THE COMMITTEE

13. Poxvirus
14. 35-40 nm (Calici ?)
15. 15-18 (Circovirus?)
16. Paramyxovirus-3 (NVSL)
17. Borna disease
18. Crimean-Congo Hemorrhagic Fever Virus
19. Wesselbron Virus
20. Spongiform Encephalopathy

Bacteria
1. *Escherichia coli*
2. *Klebsiella spp.*
3. *Salmonella hadar, montevideo, typhimurium, and enteritidis*
5. *Alpha Streptococcus*
6. *Clostridium spp.*
7. *Enterobacter spp cloaceae*
8. *Enterobacter spp agglomerans*
10. *Acinetobacter spp.*
12. *Edwardsiella tarda*
13. *Aeromonas spp.*
14. *Bacillus anthracis*
15. *Staphylococcus spp.*
16. *Mycoplasma cloacae*
17. *Acholeplasma laidlawii*
18. *Mycobacterium avium*
19. *Campylobacter spp.*
20. *Corynebacterium spp.*
22. *Chlamydia spp.*
23. *Bordetella spp.*
25. *Morganella morganii*
26. *Alcaligenes denitrificans*
27. *Flavobacterium spp.*
28. *Actinomyces spp.*
29. *Altenomonas putrifaciens*
30. *Serratia spp.*
31. *Citrobacter freundii*

Fungal
1. *Candida albicans*
2. *Aspergillus spp.*

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5. *Scopulariopsis* spp.
11. Unidentified fungi

Parasites
1. *Libostrongylus douglassi*
2. *Codiostomum struthionis*
3. *Deletrocephalus dimidiatu*
4. *Cyanthostoma varigatum*
5. *Houttynia struthionis*
6. *Esthiopterum struthionis*
7. *Philothalamus gralli*
8. *Isospora-coccidia*
9. *Giardia*
10. *Histomonas*
11. *Serpulina hyodysentery*
12. *Capillaria spp*
13. *Trichomonas spp*

The primary diseases of ratites continue to be improper management and nutrition.

5. Avian import/export activities Drs. James E. Pearson and Keith Hand, USDA, APHIS, VS, gave the following status report.

Avian Import Activities, Fiscal Year 1994:

A. Poultry and Hatching Eggs

There were 2,725,542 poultry, including day-old chicks, and 10,048,120 hatching eggs imported into the United States during fiscal year (FY) 1994.

B. Commercial Birds

As in recent years, the importation of commercial birds continues to be at much lower levels than in the mid 1980s (Table 1). Fiscal year 1993 was subjected to importation quotas on many species of birds by the Department of Interior. This year the quota is no longer in effect, and the importation of most species of birds is prohibited or restricted. This resulted in the importation of many of the nonprohibited species such as finches and other song birds. There were 131,184 commercial birds quarantined with a total of 110,570 birds released at the end of the quarantine period. Disposition of
a shipment containing 10,503 birds is pending due to the isolation of avian influenza.

C. Pet Bird Program

Pet birds continue to be imported and quarantined at Rock Tavern, New York; Miami, Florida; Los Angeles, California; Honolulu, Hawaii; and Mission, Texas. There were 1,520 birds imported and quarantined during FY 1994. All pet birds are tested for velogenic viscerotropic Newcastle disease virus, and no virus was isolated.

D. Smuggled Birds

A total of 973 birds were smuggled, confiscated, or seized at U.S. borders. Such birds are quarantined at the U.S. Department of Agriculture (USDA) quarantine facilities and, after completing the minimum 45-day quarantine, they are sold at public auctions as permitted by a Memorandum of Understanding between the Departments of Agriculture, Interior, Justice, and Treasury.

E. Ratite Importation

There are 108 foreign ostrich farms in 11 countries approved by the USDA to export ratites and ratite hatching eggs to the United States. Namibia in southern Africa continues to be the largest supplier. So far, the only ratite hatching eggs to be imported have been ostrich.

A total of 55,439 ostrich eggs were placed in privately owned bird quarantine facilities. Of these imported eggs, 16,509 (29.8 percent) were released at the end of quarantine. One shipment of ostrich eggs was refused entry in Miami, Florida, because they arrived without the required permits.

During FY 1994, seven rheas were imported, quarantined, and released. A total of 692 ostrich chicks were quarantined at the New York Animal Import Center (NYAIC) and the Miami Animal Import Center. Of these, 643 (92.9 percent) were released from quarantine. In addition, 1,145 emus were placed in quarantine. Of these, two lots at the Hawaii Animal Import Center totaling 315 were refused entry due to the isolation of Salmonella enteritidis (SE) phage type 4, and 775 were released from quarantine. Another lot at NYAIC containing 26 emus was refused entry because the birds were also found to be infected with SE phage type 4. One additional lot at the NYAIC containing 12 cassowaries and 18 emus was refused entry after an AIV was isolated.

The total number of ratites and ratite hatching eggs imported into the United States had declined slightly. However, the percentage of live chicks released after hatching increased from 22 percent in FY 1993 to 29.8 percent in FY 1994. The decline of live exotics imported could be related to the drastic decline in the value of these birds more than any other factor.
F. Virus Isolations from Import Birds

During this fiscal year there was only one isolation of velogenic Newcastle disease virus (VNDV) from import birds. This isolation was from a pintail finch which was in a lot of birds imported from China into a commercial quarantine station in California. Due to the restrictions on bird importations, especially psittacine species, there will probably be very few isolations of VNDV from import birds in the future.

Avian influenza virus (AIV) was isolated from four lots of import birds (Table 2). Avian influenza virus subtype H7N1 was isolated from passerine birds that originated from Indonesia and China. The H7N1 isolates from both groups of birds were not pathogenic for susceptible experimental chickens. However, the isolate from Pekin robins imported from China had an amino acid sequence at the hemagglutination (H) cleavage site compatible with that in highly pathogenic (HP) AIV. This was the first AIV isolate made at the National Veterinary Services Laboratories since sequencing was started in 1992 that has had a sequence that was compatible with HPAIV. The owner has been ordered to destroy the birds or remove them from the United States. The sequence of the isolate from the Indonesian birds was not compatible with highly pathogenic AIV, and the birds were released. An emu from The Netherlands yielded AIV H5N9. The isolate was not pathogenic for experimental chickens, and the amino acid sequence at the H cleavage site was consistent with that of nonpathogenic AIV H5. However, due to the restrictions on movement of emus positive for H5 virus in the United States, the birds were refused entry and returned to The Netherlands.

The other virus isolations from import birds are summarized in Table 2. With the decrease in import birds, there has been a decrease in other virus isolations. The lentogenic Newcastle disease virus isolate from a yellow nape parrot was a confiscated bird in the USDA quarantine station. There were no VNDV isolations from smuggled or domestic birds in FY 1994.


<table>
<thead>
<tr>
<th></th>
<th>FY 1992</th>
<th>FY 1993</th>
<th>FY 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry and day-old chicks</td>
<td>6,723,351</td>
<td>6,282,363</td>
<td>2,725,542</td>
</tr>
<tr>
<td>Poultry hatching eggs</td>
<td>20,928,075</td>
<td>17,593,184</td>
<td>10,048,120</td>
</tr>
<tr>
<td>Commercial birds</td>
<td>271,913</td>
<td>133,435</td>
<td>110,570</td>
</tr>
<tr>
<td>Ratites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ostrich eggs</td>
<td>1,193</td>
<td>15,556</td>
<td>16,509</td>
</tr>
<tr>
<td>ostrich chicks</td>
<td>1,825</td>
<td>1,322</td>
<td>643</td>
</tr>
<tr>
<td>Emus</td>
<td>0</td>
<td>1,238</td>
<td>776</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>27,926,357</strong></td>
<td><strong>24,027,098</strong></td>
<td><strong>12,902,160</strong></td>
</tr>
</tbody>
</table>
### Table 2. Virus isolations from import birds: October 1993 through September 1994.

<table>
<thead>
<tr>
<th>Species of birds</th>
<th>Country of origin</th>
<th>Virus</th>
<th>Month/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pintail finch</td>
<td>China</td>
<td>VNDV+</td>
<td>October 1993</td>
</tr>
<tr>
<td>Canaries, finches,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pekin robin</td>
<td>Tanzania</td>
<td>AIV+</td>
<td>December 1993</td>
</tr>
<tr>
<td>Emu</td>
<td>The Netherlands</td>
<td>AIV (H5N9)</td>
<td>February 1994</td>
</tr>
<tr>
<td>Softbill, blue and white flycatcher</td>
<td>Indonesia</td>
<td>AIV (H7N1)</td>
<td>March 1994</td>
</tr>
<tr>
<td>Pekin robins and Magpie robin</td>
<td>China</td>
<td>AIV (H7N1)</td>
<td>July 1994, August 1994</td>
</tr>
<tr>
<td>Parrot</td>
<td>Tanzania</td>
<td>PMV-2</td>
<td>October 1993</td>
</tr>
<tr>
<td>Finch</td>
<td>China/Tanzania</td>
<td>PMV-2</td>
<td>November 1993</td>
</tr>
<tr>
<td>Finch</td>
<td>Indonesia</td>
<td>PMV-2</td>
<td>December 1993</td>
</tr>
<tr>
<td>Finch</td>
<td>Mali</td>
<td>PMV-2</td>
<td>April 1994</td>
</tr>
<tr>
<td>Finch</td>
<td>China/Tanzania</td>
<td>L-NDV</td>
<td>November 1993</td>
</tr>
<tr>
<td>Yellow nape parrot</td>
<td>Unknown</td>
<td>L-NDV</td>
<td>April 1994</td>
</tr>
<tr>
<td>Pigeon</td>
<td>Germany</td>
<td>PPMV</td>
<td>December 1993</td>
</tr>
</tbody>
</table>

*VNDV=Velogenic Newcastle disease virus
†AIV=Avian Influenza virus
‡PMV-2=Paramyxovirus 2
§L-NDV=Lentogenic Newcastle disease virus
⁰PPMV=Pigeon paramyxovirus 1

### C. Disease Status Reports

1. NVSL Diagnostic Bacteriology Lab – Dr. Lee Ann Thomas, USDA APHIS NVSL, presented the following report on activities of the NVSL Diagnostic Bacteriology Laboratory.

Although there was a general decrease in the surveillance and trace back testing for *Salmonella enteritidis* (SE) in commercial egg layers, this past year a great deal of interest was generated by the identification of SE phage type 4 in commercial egg layers. As a result of this finding, samples were submitted to the National Veterinary Services Laboratories (NVSL) for testing. Samples were obtained from chickens (n = 206), environment (n = 60), a cat, a skunk, mice (n = 23), and birds (n = 2). *Salmonella enteritidis* PT4 was isolated and identified from chicken (n = 9), cat, and skunk samples. Additional isolates from mice and eggs that were submitted by California were also positive for SE PT4. Additional SE PT4 isolates have been identified as surveillance of this facility continues.

*Salmonella enteritidis* PT4 was isolated and identified from commercial birds in USDA quarantine facilities. Three shipments of emus,
two that originated in France and one in Belgium, were found to be positive. Isolations were made from both environmental and cloacal swabs. These shipments were refused entry into the United States and were returned to their point of origin. A total of 1,319 samples were received from quarantine facilities. Of these, 243 (19%) were positive for salmonellae. *Salmonella typhimurium* continues to be the most common serotype. It was isolated and identified from 91 samples. A total of 44 serotypes were identified. Of these, 10 serotypes were identified for the first time since the inception of this surveillance in 1990.

As previously mentioned, SE surveillance and traceback testing decreased this fiscal year. A total of 1,648 samples were submitted as compared to 7,359 from fiscal year 1993. A summary of results follows:

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>No. of Samples</th>
<th>No. Positive for SE (%)</th>
<th>No. Positive for Other Salmonellae (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues</td>
<td>806</td>
<td>24 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Environmental</td>
<td>637</td>
<td>71 (11%)</td>
<td>245 (38%)</td>
</tr>
<tr>
<td>Mice</td>
<td>205</td>
<td>48 (23%)</td>
<td>19 (9%)</td>
</tr>
</tbody>
</table>

A total of 257 *Pasteurella multocida* isolates from poultry were submitted to the NVSL for somatic typing and/or DNA fingerprinting. The DNA fingerprint profile of 37 isolates (14.4%) were identical to profiles of attenuated vaccines (CU, M-9, and PM-1). The profiles of 15 isolates were identical to the profile of reference strain P-1059 (type 3). The fingerprint profiles of 207 isolates (79.9%) did not match a somatic reference strain, attenuated or killed vaccine. The most common somatic types were identified as: 3,4 (34.7%), 3 (16.6%), and 1 (11.2%).

2. Newcastle Disease Investigations & Outbreaks – Dr. C. Groocock APHIS VS & J. Pearson APHIS NVSL indicated that there were no significant outbreaks of NDV during the past year that do not appear elsewhere in this report.

3. Turkey Rhinotracheitis and Swollen Head Syndrome – Dr. James Pearson, USDA APHIS NVSL gave the following report.

In fiscal year 1994, 821 samples from 11 states were tested for TRT and SHS of chickens. All the samples from the United States were negative. However, 48 samples were received from chickens from Panama with evidence of swollen heads. Fourteen of the birds had antibody against TRT\SHS. The NVSL has developed the capability to test for the two antigenically different TRT\SHS viruses that have been reported.

II. FDA Issues

Dr. George Mitchell, FDA-CVM provided the following update on issues
Selenium

In two separate legislative actions, Congress and the President have responded to the FDA's stay of the 1987 amendments to the selenium food additive regulation. In FDA Appropriations legislation, signed by President Clinton on September 30, 1994, an amendment was included that suspended that stay until December 31, 1995. The second action, which was included in the Federal Crop Insurance Reform Act signed on October 13, 1994, states that the FDA shall not implement or enforce the stay unless the Commissioner of the FDA finds that selenium supplementation at 0.3 ppm in complete diets is not essential to maintain animal health, is not safe to animals consuming the additive or humans consuming edible portions of selenium-supplement animals, is not effective to promote normal growth and reproduction, and that the manufacture and use of supplemental selenium cannot be reasonably controlled by adherence to current good manufacturing practice requirements.

As a result of these actions, FDA's stay of the 1987 amendments is no longer in effect. Animal feeds may be supplemented with the levels of selenium stated in the 1987 amendments. These levels are listed in Title 21, Part 573.920 of the Code of Federal Regulations. The maximum supplementation level in complete feed for chickens, swine, turkeys, sheep, cattle, and ducks is 0.3 part per million. The levels of feed supplements for limit feeding and in salt-mineral mixtures for free-choice feeding for sheep and beef cattle return to those provided for by the 1987 amendments. In addition, the osmotic selenium bolus, approved for use in beef and dairy cattle in 1989, can also be used as a source of selenium. The bolus provides 3 mg of selenium per day. The legislative actions also remove the requirement that premix manufacturers analyze each batch of selenium premix.

The Agency will take appropriate action to revoke all references to the stay resulting from the Federal Register announcement of September 13, 1993. The actions will make it clear that the levels of selenium permitted to be added to feed are those set out in the 1987 amendments to the selenium food additive regulation.

Animal Drug Amendments Act of 1994

The legislation was initially intended to codify FDA's Compliance Policy Guide on Extra Label Use of Animal Drugs, Section 7125.06, in order to validate the veterinarian/client/patient relationship (also to avoid liability and high insurance premiums). CPG 7125.06 states that CVM will not ordinarily pursue regulatory action for extra label use of approved drugs in animals, if certain criteria are met. One criteria is that the determination for
such use must be based upon a valid veterinarian/client relationship. Other criteria include that extra label use should be used only in certain serious situations where the traditional therapy is not working (there is no such restriction in this legislation), or where no approved therapy exists (a restriction this legislation does incorporate, but only for animal drugs). The CPG also provides a list of certain drugs that are prohibited for extra label use whether or not the above mentioned criteria are met. It is expected that those drugs, currently prohibited from extra label use under CPG 7125.06, will retain that status through regulations to be promulgated by the Agency under the new law.

This legislation includes several important safeguards: if the Secretary finds there is a reasonable probability that a use may present a risk to public health, the Secretary may establish a safe level for a residue for such off-label use, by regulation or order, and require the development of analytical methods for the detection of residues. If the Secretary finds, after affording an opportunity for public comment, that a use presents a risk to public health or if no analytical method is developed the Secretary may, by order, prohibit such use. The Secretary may also by general regulation provide access to records of veterinarians to ascertain any use of intended use that the Secretary has determined may present a risk to public health.

Regulations are required not later than 2 years after the enactment of this Act. The Act becomes effective with the adoption of the regulation.

## Hazard Analysis Critical Control Points (HACCP)

HACCP is the subject of a lot of discussion in producer organizations and among Federal Agencies. I will comment on the activity at the Federal level.

- **HACCP for seafood** - proposed final rule (FDA) FR 1/28
- **HACCP for landfood** - ANPR (FDA) FR 8/4
- **HACCP for FSIS slaughter plants** (FSIS)
  - promised by the end of year
- **HACCP for live animals** (APHIS)
- **Pathogen Reduction Act**
- **HACCP for live animals** (GAO)
  - GAO report 9/28/94

"While improvements to the National Residue Program could incrementally increase its effectiveness, GAO believes that fundamental changes to the basic regulatory approach now used are needed. GAO believes that a risk-based approach, established by industry with FSIS' assistance and oversight, and operated by industry with FSIS' monitoring, would be a more effective alternative. However, such a fundamental change requires congressional approval before it can be implemented.

An industry-operated, risk-based system that integrates residue prevention, detection, and quality control from the farm through the slaughter-
house, established with FSIS' assistance and oversight, would be more effective than the current federal program. Therefore, the Congress may wish to direct FSIS to adopt such an approach while maintaining an oversight role to monitor the effectiveness of industry programs. To ensure that FSIS could effectively carry out these responsibilities, the Congress may also wish to provide FSIS with additional access to industry records and enforcement authority." (GAO NRP Report 9/94).

III. Pre Harvest Food Safety

The following overview of USDA APHIS animal production (preharvest) pathogen reduction food safety and quality assurance initiatives was presented by Dr. Bonnie Buntain, USDA-APHIS.

USDA APHIS Perspective in Quality Assurance Programs

Consumers nationally and internationally will continue to demand safe and wholesome meat, poultry and egg products at a reasonable cost. A number of industry sponsored Quality Assurance Programs (QAPs) already contribute to a safer and higher quality food supply. These QAPs are part of good total quality management practices which help meet market demands. End product testing for serious human health pathogens in high risk products may help reduce risks, but it cannot ensure total quality and safety of all products. To that goal, commodity groups, food industries, researchers, State and Federal agencies, educators, practicing veterinarians and other stakeholders must collaborate to add value to those products produced by a QAP. Certification of QAPs may help ensure that good production practices are being followed.

Animal and Plant Health Inspection Service (APHIS) believes that improved decision-making is based on science. Identification by research of critical control points for good production practices will result in appropriate intervention strategies and QAPs which improve marketability. APHIS can serve to enhance prevention systems and facilitate the development of certifying strategies and processes for commodity QAPs to meet regulatory and consumer demands.

Background

Historically, USDA, APHIS, has utilized its Veterinary Services (VS) personnel at many critical control points to conduct animal health surveillance, disease control, and eradication missions. Today, VS personnel consists of veterinarians and animal health technicians with significant experience in livestock identification and traceback, animal movement, animal and human disease outbreak and hazard investigation, monitoring and surveillance, epidemiology, preventive medicine, risk and economic analyses, information technology management, communication and technology transfer, diagnostic laboratory support, emerging issues analyses, applied re-
TRANSMISSIBLE DISEASES OF POULTRY
AND OTHER AVIAN SPECIES

search and methods development, and public health programs.

The USDA, APHIS, VS, Centers for Epidemiology and Animal Health (CEAH) maintain a cadre of analytical epidemiologists, economists, statisticians, and computer specialists who can support the field operations in the design and implementation of large-scale epidemiologic projects that are national in scope.

The APHIS VS National Veterinary Services Laboratories (NVSL) provide disease diagnostic services and play a pivotal role in conducting applied research and serve as a reference laboratory for quality, sensitivity and specificity of microbiological and disease diagnostic testing nationwide. Currently, NVSL has state of the art diagnostic capabilities for human pathogens of animal origin, such as E. coli O157:H7 and Salmonella enteritidis.

USDA APHIS recognizes that food safety is the shared responsibility by all participants in the food chain. The successful implementation of national food safety and quality assurance programs depend upon cooperation, collaboration and coordination among Federal and State agencies, the food industries, animal production commodities, and consumers. Veterinarians, with their comprehensive professional training, play a key role in implementing such programs from farm to table.

USDA Animal Production Pathogen Reduction Programs in FY 1994

1. USDA Pathogen Reduction Task Force

The USDA Pathogen Reduction Task Force (PRTF) was commissioned by Agriculture Secretary Mike Espy in late 1993 to provide leadership, coordination and oversight of the Department's programs to ensure a safe and wholesome food supply. The task force, chaired by acting assistant secretary for Marketing and Inspection, Patricia Jensen, had the responsibility for coordination all Departmental activities associated with pathogen reduction. In addition, the task force has representatives from the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC).

The first task was to establish technical subcommittees to focus on research priorities in the farm-to-table continuum. The Subcommittees were: Live Animal Production, Slaughter and Processing, and Food Preparation and Consumption. Opportunities for interagency collaboration and coordination along the continuum were enhanced.

The Live Animal Production (Preharvest) research priorities included ecology and epidemiology, microbial physiology, pathogenicity, virulence and genetics, Hazard Analysis Critical Control Points (HACCP), economic and risk analyses, technology transfer and risk communication, and traceback technologies. Other than traceback technologies, all of the above categories were common to each subcommittee's priorities. Key researchable questions were identified under each category for each subcommittee. The final report has not been released to date.
2. USDA APHIS' Animal Production (Preharvest) Pathogen Reduction, Quality Assurance and Food Safety Initiatives

National Food Safety Agenda: The APHIS perspective was presented in this document (APHIS 10-05-002) published in March, 1994. It stresses a coordinated effort and describes APHIS' core strengths and services.

*Escherichia coli* O157:H7 Issues and Ramifications: Published also in March, 1994, by APHIS, VS, CEAH represents an interagency cooperative effort with Food Safety Inspection Service (FSIS) and Extension Service. The document helps define the role of cattle as a source of this human pathogen in food products, particularly ground beef. It summarizes why there is such interest in *E. coli* O157:H7, what is know about it in cattle, patterns of production and consumption, and potential future directions. (A copy may be obtained by contacting CEAH at 555 South Howes, Fort Collins, CO 80521).

**Food Safety Contingency Funds Allocation FY 1994:**
- Enhancing NVSL's ability to respond to *E. coli* outbreak, surveillance, monitoring and field studies' demands
- Enhancing Preharvest/Animal Production HACCP
  - Animal Production Technical Analysis Group
  - Critical Control Points Pacific NW
  - Dairy Cattle Surveillance CA
  - Dairy Cattle Surveillance WI
  - Cattle-on-Feed Pretest
- Developing an Emergency Response and Enhancing the Food Safety Field Epidemiology Delivery System
  - Emergency Investigations
  - Policy Development Workshop
  - APHIS Regional Food Safety Workshops
  - Communications and FAX-Back Systems
  - Food Safety Fact Sheets
- Enhancing Coordination/Collaboration Among Preharvest/Animal Production Stake-holders

Total $724,000

**Animal Production Technical Analysis Group (AP-TAG):**

Started by FSIS Track II support in 1993, and continued by APHIS in 1994, the AP-TAG was independently contracted out. The Chair chose 17 members representing experts in all production areas from public and private sectors. The sub-groups were: Ruminant Production (beef, dairy, veal, lamb/sheep, and horses); Pork Production; Poultry Production; and Risk/Health Impact. The AP-TAG report identifies hazards and controls and the risk and health impact of foodborne pathogens. (APHIS and FSIS)

*E. coli* O157:H7 Methodology, Ecology and Epidemiology Studies:

Evaluation of Automated Identification Equipment Compared to Tradi-
tional Laboratory Diagnostic Methods for \textit{E. coli} O157:H7 (NVSL, Ames, Iowa)


- Development of the University of California's ELISA Serological Test for \textit{E. coli} O157:H7 (VS and UCD)

- Use of Pulsed-Field Genomic Typing to Establish the Origin, Succession, and Dissemination of Strains of \textit{E. coli} O157:H7 (VS and University of Wisconsin-Madison)

Field Investigations of \textit{E. coli} O157:H7 Outbreaks (APHIS and FSIS)

- NAHMS National Dairy Heifer Evaluation Project (NDHEP) and Follow-Up Study: the first national prevalence study of \textit{E. coli} O157:H7 in cattle (CEAH, Ft. Collins, CO)

- Current NAHMS Cattle on Field (COFE) Project: testing fecal samples from feedlots nationwide larger than 1,000 head and comparing laboratory media, transport, isolation, enrichment, and identification methodologies (CEAH).

- Field Monitoring and Survey of Cattle Operations in Wisconsin for the Presence of \textit{E. coli} O157:H7 and Subsequent Follow-up on Positive Operations (Longitudinal Study, CEAH).

- How Livestock and Poultry Monitoring and Surveillance Data is Essential in Tracking Human Health Issues of Zoonotic Concern (APHIS and CDC)

- Tri-State (Washington, Oregon and Idaho) Prevalence Study of Dairy Herds (Heifers and Culled Cows) for \textit{E. coli} O157:H7 and the Effects of Pasture Slurry Application

- Raw Milk and \textit{E. coli} O157:H7 in Oregon Dairy Herds (VS and State Public Health)

Salmonella in Poultry Studies:

- Rodent Density and Prevalence of \textit{Salmonella enteritidis} (SE) in Layer Houses (CEAH)

- Comparison of NVSL, Human Health (CDC), and NAHMS Surveillance Data on Serotype Distribution of Salmonella Isolates Obtained from Active and Passive Surveillance (CEAH)

- SE Pilot Project in Pennsylvania (SE Program, VS)

Swine Studies:

- HACCP Concepts for On-Farm Pathogen Reduction (VS)

- Risk Factors and Critical Control Points for Trichinella in Swine Herds

Quality Assurance Program (QAP) Support:

- California Cull Dairy Cow QAP: an interagency collaboration to reduce the incidence of drug residues in cull dairy cows and to evaluate it as a
model for other food safety programs (VS).

California Egg QAP: an integrated voluntary program designed to confirm the safety and further reduce the risks of foodborne pathogens in shell eggs using quality control procedures of HACCP (VS).

California Dairy QA Project: will aid in the identification of critical control points and intervention strategies. A training program for VS and State veterinarians in HACCP, QAP and research technology/methodology, animal identification and tracking, sample collection/handling, health records, computer and manual record systems and field training is underway (VS and University of California-Davis)

California Multidisciplinary Cooperative Team Evaluation of Antibiotic Residue Assays to Determine Test Sensitivity and Specificity in Urine, Milk, and Tissue Samples (USDA FSIS and APHIS, CA State, UCDE, FDA CVM, and Industry Organizations)

Training Workshops:

VS conducted five regional and area food safety/quality assurance workshops nationwide. These grassroots efforts created program enthusiasm and facilitated the involvement of APHIS VS in interactive and interdisciplinary teams of local stakeholders to assist implementation of QA and food safety programs.

Dr. Peter E. Poss, Willmar, Minnesota, presented the following overview of the industry perception of pre harvest food safety & trace back:

FSIS and APHIS are involved in a new cooperative initiative toward improving food safety and have extended their efforts to include live animal production. Pre harvest Food Safety, the concept of involving the farming producer in all aspects of food safety is new. Monitoring and regulatory action by FSIS and FDA has caused the producer to take on the responsibility for preventing contamination of the food supply with the drugs and chemicals they utilize or may expose their animals to during production. However, the responsibility for providing human pathogen free or safe meat and animal products is a real and understandable concern to the livestock industry.

Physical and chemical contamination can be considered a fairly straight forward preventable problem. Physical and chemical contaminants may accumulate with repeated exposure but do not multiply or reproduce in livestock. The producer has control of the agents involved through testing and monitoring and the management of inputs, to assure that contamination does not and has not occurred. Physical contaminants have not been of major concern in recent years with modern livestock production systems. Chemical contamination has come under control by the industry over the past decade due to government monitoring and trace back which created accountability in a system where the producer controls the inputs.

Microbiological contamination poses a much different problem. The
question begging an answer is: is it possible for the producer to change the microbiological flora of livestock produced for food, so that food safety risk is eliminated or even reduced? Pathogens, in very small numbers, can be introduced at a multitude of different points during the production of livestock and then can infect and multiply to create disease and or food safety problems. Current technology and production expertise does not provide the producer with control over all the points of introduction or the total ability to prevent infection or shedding. Since any one introduction can result in a problem, prevention in this living biological system must be all or none.

The Hazard Analysis Critical Control Points (HACCP) system of assuring compliance is cited as the method to use in assuring food safety. In production of live animals, it has disadvantages. The principals can be applied to food animal chemical and physical food safety. Applying the HACCP system to the control of human pathogens in livestock production is a very different situation and raises questions. A successful HACCP program requires a process or system with defined procedures that will result in a known quality end product. We don't have the capability or the knowledge required to modify live production systems to assure no pathogens will be present at market time. We can identify a multitude of control points, where pathogens may be introduced or where factors may influence infection or shedding, and any one of them could be the critical one (control point that will assure safe food).

Using Quality Control Points or Food Safety Control Points with the goal of reduction and minimizing pathogens may be more appropriate and more acceptable to a quality conscious industry and ultimately the consuming public. Hazard is a very negative and undesirable term to associate with food when communicating with the consumer in the market place.

Livestock producers strive to maintain healthy disease free livestock in order to compete and to be economically viable. Management procedures required to prevent or minimize the introduction and contamination of food animal production systems with animal pathogens should be appropriate with human pathogens as well.

Good and best management practices (GMP's & BMP'S) have been developed by the various livestock industries for production of healthy disease free livestock. Many voluntary and government assisted disease control and eradication programs are established and working. These documents and programs need continual updating. It is an ongoing living process. Governmental agencies alone are not going to come up with new workable management and disease control practices for the industry that will be adopted. The effort must be cooperative. Research, both public and privately funded is essential to continually improve the efficiency of livestock production and the quality of the products produced. The improvements and the programs that are effective will be adopted by the industry very quickly via the information grapevine in our very competitive world of business.
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FSIS has full time on site organoleptic and procedural inspection in processing plants to enforce compliance with minimum food safety standards. A multitude of sites make this impossible in live production.

Trace back is suggested by FSIS and APHIS as a tool to improve food safety in live production. It connotes the testing of animals and animal products during processing or distribution where there is a positive identification system in place allowing identification of the source live animal production unit should a food safety contaminant be detected. The agency involved would then attempt to determine the cause and the methods needed to eliminate the problem. This system makes the producer nervous with a number of very real concerns. First of all, the accuracy and validity of results is critical and a retest procedure for verification is essential. Second, government regulatory personnel are not well grounded and up to date in industry biosecurity and management programs (which are dynamic) and do not have credibility with the industry. Third, public release of negative food quality information can cause serious economic disruption of the producer/processor or integrator and timing and sensitivity of reporting is critical. Fourth, liability from producer to processor to consumer is a serious concern and may involve business interruption losses as well as human suffering. All of these concerns indicate the necessity of a cooperative effort between government and industry.

Monitoring and testing the end product will not in itself insure food safety. It is an important tool, however making sure the current best production practices required to assure food safety are done, is more important. The industry in total should be stimulated to do what they know needs to be done and what is required to do to minimize the risk of pathogen contamination of live animals and animal food products. A regulatory trace back, verification and information system developed with industry input, could be a major factor in accomplishing this goal.

The practical goal for microbiological food safety in livestock production must be reduction or minimization and it would seem then that pasteurization of animal products during processing is necessary to reduce the pathogen level even closer to zero, and assure food safety in the market place. Additionally, since animal products may not be sterile with respect to pathogens and spoilage organisms, consumer education in the proper handling and distribution of food is also necessary.

IV. Turkey Osteomyelitis Complex

Dr. Peter E. Poss, Willmar, MN, presented the following report on the turkey osteomyelitis complex:

Inspection procedures for TOC were developed by FSIS and implemented in the fall of 1988. The procedure was developed to detect and remove lesions of osteomyelitis (OM) from wholesome turkey and turkey products. The industry protested on the basis that the procedure was flawed. Both the industry and USDA have funded research to study OM and the
TOC inspection procedure however, the procedure has not been changed and the conflict is ongoing.

The procedure is initiated with each new lot of birds being processed. Ten OM biased or suspect birds having swollen articulations and/or green liver discoloration, are selected by the FSIS inspectors and examined for lesions of OM. These birds are examined by making a minimum of 10 exploratory cuts which includes opening stifle, hip, and shoulder joints, the soft tissue on the lateral aspect of the stifle joints and a cut across the proximal end of both tibias. The lot is designated OM positive if any evidence of OM is detected in any one of the birds and then all suspect birds must be removed from the line and examined similarly. This is a salvage procedure requiring a minimum of eight (8) cuts as described above with the exception of the cut to open the end of the tibias. The carcass may be salvaged by adequate removal of the affected tissue or may be condemned if lesions are extensive and salvage is not possible.

The conflict may be summarized with the following arguments and perceptions:

**USDA**
1. Synovitis in turkeys that is not caused by a mycoplasma infection, is the result of bacterial OM.
2. Green liver discoloration is a common sign of staphylococcal infection in turkeys and the infection often results in OM.
3. *Staphylococcus hyicus* is a common isolate from OM lesions in turkeys and has been shown to cause emesis in humans.
4. OM is a public health and/or wholesomeness issue and every lesion that can be detected must be removed during processing.
5. Turkey thigh bones have the greatest incidence of OM lesions and are included in comminuted meat.

**Industry**
1. Green liver discoloration is caused by many things and is a poor indicator of OM.
2. Many bacterial agents cause OM in turkeys, particularly *Escherichia coli* which is the most common cause of mortality in turkeys.
3. Removing OM lesions to remove staphylococcal organisms as a human food safety issue is a weak argument.
4. Tibial dyschondroplasia and fractures with hemorrhage in the proximal end of the tibia are common lesions in turkeys and easily confused with OM.
5. Obvious synovitis and OM lesions can be detected and salvaged on the line under regular synovitis trim procedures. The green liver indicator is not necessary.
6. The small lesions in the bone that are detected and those that are missed are not significant to human health and product quality or...
wholesomeness.
7. Cutting up three to six turkeys to find one with synovitis or OM lesions does not justify the cost and losses incurred by the processing plant.

V. Avian Influenza
A. Dr. B. S. Pomeroy, University of Minnesota, presented the following report of the avian influenza subcommittee:

This report includes data from questionnaires sent to State Veterinarians and laboratory results reported by NVSL-USDAsAPHIS- VS.

Individual States
Responses to the questionnaires were received from 34 states. No corrections were suggested in Table 1 (pages 353-355, 1993 USAHA Proceedings) Avian Influenza Serotypes isolated from Turkeys, Chickens and other domestic fowl in the United States or based on serology (1964-1993).

Turkeys
According to state reports and the NVSL laboratory report, the incidents of AI in turkeys were at a low level and were based on serology with no isolations of the virus. The reports from the states and NVSL indicated AI was diagnosed serologically in four states, Minnesota, North Carolina, North Dakota and New Hampshire.

Minnesota
Minnesota has continued its extensive monitoring programs of turkey and broiler flocks in 1994. Positive AGPT samples were submitted to NVSL. In the 1993 influenza season, the first flock was identified in August, 1993 and the last flock in December. Four flocks on four different farms totaling 60,000 birds were identified serologically with H1N1, H4N6, H5N9 and H9N2.

The first flock of the 1994 avian influenza season was identified in June. Six flocks on four farms involving 170,000 birds have been identified to date with H7N1. For FY1994 nine flocks on seven farms totaling 200,000 birds were identified. The following serotypes were identified: H1N1, H4N6, H7N1, and H9N2.

New Hampshire
Turkey flock was identified with H6N2.

North Carolina
H1N1 was identified serologically in five laboratory submissions.

North Dakota
Three flocks from two farms were processed in Minnesota in October and November, 1993 and were found serologically positive with H5N3.
Two additional flocks from one farm were identified with H10N7 in July, 1994.

**Virginia**
Two flocks involving 20,000 birds were identified with H1N1.

**Chickens**
No commercial chicken flocks were reported infected with avian influenza.

**Back Yard Flocks**
**Maryland**
Increased serological surveillance of back yard flocks revealed three flocks with H5N2 antibodies with no virus isolation from the fowl on these premises as well as sentinel chickens.

**Live-Birds Market (NVSL)**
Veterinary Services surveyed live bird markets and back yard flocks in eight states on the eastern seaboard and only two states revealed positive live-bird markets. Dr. S. Trock (VS) will present a detailed report.

**New Jersey**
H7N2 was isolated from three markets and H7N3 from one market. Other subtypes identified were H1N1, H3N8, and H11N2.

**New York**
Several serotypes were identified from 13 live bird markets: H1N1, H2N2, H3N8, H3N9, and H7N2.

The subtypes H7N2 and H7N3 (NJ, NY) were determined to be nonpathogenic to chickens at NVSL and molecular characterization was similar to that in nonpathogenic subtypes.

**Florida**
Florida submitted a detailed report of AI monitoring of dealers and sellers in Dade County and samples collected at fairs and exhibition and game bird flocks.

Avian influenza was identified on two premises serologically (H10) and virus isolation was made from sentinel birds on one premise, H4N6. It was considered nonpathogenic by bird inoculation at NVSL. The premises were depopulated, cleaned and disinfected. No serological evidence of AI was found in birds outside Dade County.

**Other Fowl (NVSL)**
**Arkansas**
H7N3 was isolated from quail on a game farm. The isolate was considered nonpathogenic by chicken inoculation tests and molecular charac-
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terization.

California
H4N6 was identified serologically in quail.

Maryland
In November, 1993 three subtypes of AI were isolated from pheasants and waterfowl on a game farm, H3N2, H5N2, H11N2 (pheasants) and H3N2, H5N2, H5N8 (waterfowl). The National Veterinary Services Laboratory found the H5N2 isolations nonpathogenic but had the potential to become more virulent through genetic mutation. Because of the potential danger to commercial broiler operations, the game farm was depopulated at a cost of $253,366. Surveillance of commercial broiler operations in the area revealed negative results.

Pennsylvania
H1N1 antibody was detected from guinea fowl serum in March, 1994 and H6N8 from goose and H9 from duck.

Texas
Texas reported several thousand serological tests (4710) on a variety of birds; ostrich 693, 1 positive; emu 3,040, 10 positives; rhea 155, 13 positives; swan 1, 1 positive; ducks 10, 1 positive; other fowl were negative including 148 penguins and 17 prairie chickens.

Wisconsin
Samples were submitted from duck (H1), goose (H11), and swan with incomplete results.

Ratites
Dr. J. Pearson (NVSL) will present a detailed report on serological findings of samples from emus, rheas, and ostriches received from 16 states. Serotypes identified H1N1, H2N2, H3N2, H4N2, H4N6, H4N8, H5N2, H6N8, H7N1, H7N3, H9N2, H10N1, and H10N4.

Comparatives Studies of Serological Tests
A report presented at the 66th Annual Northeastern Conference on Avian Diseases (1994) by Snyder, Robison and Stein compared the sensitivity of AGPT and indirect ELISA. The serum samples in the surveillance study were from commercial broiler operations, backyard flocks and H5N2 positive game farm (pheasants) and wild and captive waterfowl. The ELISA test was more sensitive and identified two of the premises of positive backyard fowl before AGPT. On the game farm (pheasants) there was good agreement between AGPT and ELISA. Wild and captive waterfowl on a pond on the farm, with the exception of one goose, were all negative by AGPT where
as a considerable number of ELISA positive samples were found and confirmed by H and N tests at NVSL.

The National Veterinary Services Laboratory has the capability to characterize avian influenza viruses at a molecular level.

Mr. Dennis Senne (Diagnostic Virology Laboratory) completed an extensive training program at St. Jude Children's Research Hospital in molecular pathotyping of H5 and H7 AI viruses. This technology will be available at NVSL and the molecular pathotyping may reduce the need to use live birds for pathotyping AI viruses in the future.

**Pathogenicity Tests**

All H5 and H7 isolates were tested in chickens at NVSL and all were considered non-pathogenic.

**Use of Avian Influenza Vaccine - Turkeys**

Minnesota reported the use of H1 and H6 vaccines in market and breeder flocks. North Carolina and Ohio reported the use of H1 vaccines in breeder flocks.

**Summary**

Avian influenza was identified in the following species and states.

**Turkeys**

- Minnesota: H1N1, H4N6, H7N1, H9N2
- North Carolina: H1N1
- North Dakota: H5N3, H10N7
- New Hampshire: H6N2

**Chickens**

No commercial flocks

**Live Poultry Markets**

- New Jersey: H1N1, H3N8, H7N2, H7N3, H11N2
- New York: H1N1, H2N2, H3N8, H3N9, H4N6, H4N8, H7N2
- Florida: H4N6, H10

**Backyard Flocks**

- Maryland: H5N2

**Other Fowl**

- **Quail**
  - Arkansas: H7N3
  - California: H4N6

- **Pheasants**
  - Maryland: H3N2, H5N2, H11N2

- **Ducks, Geese**
  - Maryland: H3N2, H5N2, H5N8
  - Pennsylvania: H6N8, H9
**Guinea fowl**
- Pennsylvania: H1N1

**Duck, Goose, Swan**
- Wisconsin: H1, H11

**Table 1. Avian influenza serotypes isolated from turkeys, chickens and other domestic fowl in the U.S. or based on serology (1964-1994).**

<table>
<thead>
<tr>
<th>State</th>
<th>Year First Identified</th>
<th>Hemagglutinin Antigens Identified</th>
</tr>
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<tbody>
<tr>
<td>Turkeys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>California</td>
<td>1964</td>
<td>H1, H5, H6, H9</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1965</td>
<td>H6</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1965</td>
<td>H1, H2, H5, H6, H9</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1966</td>
<td>H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H13; 1994: H1, H4, H7, H9</td>
</tr>
<tr>
<td>Washington</td>
<td>1967</td>
<td>H6</td>
</tr>
<tr>
<td>Oregon</td>
<td>1970</td>
<td>H6, H7</td>
</tr>
<tr>
<td>Iowa</td>
<td>1971</td>
<td>H1, H2, H4, H5, H6</td>
</tr>
<tr>
<td>Colorado</td>
<td>1972</td>
<td>H1, H5, H7, H9</td>
</tr>
<tr>
<td>Ohio</td>
<td>1975</td>
<td>H1</td>
</tr>
<tr>
<td>South Dakota</td>
<td>1978</td>
<td>H1</td>
</tr>
<tr>
<td>Texas</td>
<td>1979</td>
<td>H5, H7, H9</td>
</tr>
<tr>
<td>Indiana</td>
<td>1980</td>
<td>H1, H2, H4, H10</td>
</tr>
<tr>
<td>Missouri</td>
<td>1980</td>
<td>H1</td>
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<td>Kansas</td>
<td>1980</td>
<td>H1</td>
</tr>
<tr>
<td>Arkansas</td>
<td>1981</td>
<td>H1</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1982</td>
<td>H1, H4; 1994: H1</td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H1, H2, H4, H5, H10; 1994: H1</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>H1, H5</td>
</tr>
<tr>
<td>Michigan</td>
<td>1985</td>
<td>H1, H9</td>
</tr>
<tr>
<td>Utah</td>
<td>1985</td>
<td>H6, H4, H10</td>
</tr>
<tr>
<td>Nebraska</td>
<td>1988</td>
<td>H1</td>
</tr>
<tr>
<td>New York</td>
<td>1988</td>
<td>H9</td>
</tr>
<tr>
<td>Illinois</td>
<td>1991</td>
<td>H1</td>
</tr>
<tr>
<td>Florida</td>
<td>1991</td>
<td>H9</td>
</tr>
<tr>
<td>Maryland</td>
<td>1993</td>
<td>H5</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>1994</td>
<td>H6</td>
</tr>
</tbody>
</table>

**Chickens**
- Alabama: 1975, H4

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**TRANSMISSIBLE DISEASES OF POULTRY AND OTHER AVIAN SPECIES**

<table>
<thead>
<tr>
<th>State</th>
<th>Year(s)</th>
<th>Virus(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>1978, 88</td>
<td>H6, H9</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983, 86</td>
<td>H1, H2, H5</td>
</tr>
<tr>
<td>Maryland</td>
<td>1983, 84</td>
<td>H5, H9</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1983, 86</td>
<td>H5</td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H2, H4, H5, H7</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>New York</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>Ohio</td>
<td>1991</td>
<td>H1, H2</td>
</tr>
<tr>
<td>Michigan</td>
<td>1992</td>
<td>H1, H6</td>
</tr>
<tr>
<td>Delaware</td>
<td>1993</td>
<td>H5</td>
</tr>
</tbody>
</table>

**Chickens - Live Market**

<table>
<thead>
<tr>
<th>State</th>
<th>Year(s)</th>
<th>Virus(s)</th>
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</thead>
<tbody>
<tr>
<td>District of Columbia</td>
<td>1980</td>
<td>H1, H5</td>
</tr>
<tr>
<td>Connecticut</td>
<td>1986</td>
<td>H2, H5</td>
</tr>
<tr>
<td>Florida</td>
<td>1986</td>
<td>H5; 1994: H4, H10</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1989</td>
<td>Turkey H9</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>Delaware</td>
<td>1990</td>
<td>Duck H2, H5</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1991</td>
<td>Guinea Fowl H2, H5, H6</td>
</tr>
<tr>
<td></td>
<td>1992</td>
<td>Pheasant H12</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1993</td>
<td>H5</td>
</tr>
</tbody>
</table>

**Chickens - Dealer / Backyard Flocks**

<table>
<thead>
<tr>
<th>State</th>
<th>Year(s)</th>
<th>Virus(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maryland</td>
<td>1983</td>
<td>H5</td>
</tr>
<tr>
<td>Ohio</td>
<td>1986</td>
<td>H5</td>
</tr>
<tr>
<td>Georgia</td>
<td>1987</td>
<td>H5</td>
</tr>
<tr>
<td>Maryland</td>
<td>1993</td>
<td>H1, H3, N8, H4</td>
</tr>
<tr>
<td>Maryland</td>
<td>1994</td>
<td>H5</td>
</tr>
</tbody>
</table>

**Other Species**

<table>
<thead>
<tr>
<th>State</th>
<th>Year(s)</th>
<th>Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania</td>
<td>1969</td>
<td>Ducks H1, H3, H5, H10</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1974</td>
<td>Geese NA</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>Guinea Fowl NA</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>Pheasants H3, H7, H8</td>
</tr>
<tr>
<td>New York</td>
<td>1978</td>
<td>Ducks H3, H4, H5, H6, H11</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>Guinea Fowl, Quail H5</td>
</tr>
<tr>
<td>Maryland</td>
<td>1984</td>
<td>Ducks, Guinea Fowl H3, H5</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>Chukar H5</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>Ducks H4</td>
</tr>
<tr>
<td>Washington</td>
<td>1985</td>
<td>Pheasant H9</td>
</tr>
</tbody>
</table>

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Virginia 1985  Ducks, Swans, Geese H2, H5, H7  
Oregon 1986  Quail, H5  
Pennsylvania 1986  Guinea Fowl H1, H6, H11; 1994: H1  
1986  Guinea Fowl, Chukar H5  
Georgia (Dealer) 1987  Guinea Fowl H5  
Maryland 1987  Ducks, Geese H9  
Wisconsin 1988  Pheasant H9  
Pennsylvania 1988  Geese H1, H6, H11, H12  
North Carolina 1988  Ducks H6  
Connecticut 1990  Pheasant H4  
New Hampshire 1990  Pheasant H10  
California 1990  Quail H1, H4; 1994: H4  
Maryland 1991  Quail H6 or H1, H5, H6  
Arkansas 1992  Quail H5, H6, H9, H10; 1994: H7  
New Jersey 1993  Guinea Fowl H5, Duck H3  
Pennsylvania 1993  Pheasant H5  
New York 1993  Pheasant, Guinea fowl, Duck H5  
Michigan 1993  Ducks, Geese H5, H11  
Ohio 1993  Muscovy duck H1  
Wisconsin 1993  Duck H10  
Maryland 1994  Pheasants H3, H5, H11  
Maryland 1994  Duck, Geese H3, H5  

Ratites  
Texas 1993  Rheas, Emus H5, H7  
North Carolina 1993  Rheas H7  
9 States 1993  Serological evidence  
16 States 1994  Serological evidence  

NA= Not Available  

Table 2. Presence of avian influenza virus (AIV) or AIV-specific antibodies in gallinaceous birds other than those in live-bird markets. (NVSL)  

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Subtypes(s)</th>
<th>Isolation / Serology</th>
<th>Mo./Yr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>Quail</td>
<td>H7N3</td>
<td>Isolation</td>
<td>03/94</td>
</tr>
<tr>
<td>California</td>
<td>Quail</td>
<td>H7N3</td>
<td>Serology</td>
<td>03/94; 04/94</td>
</tr>
<tr>
<td>Maryland</td>
<td>Pheasant</td>
<td>H11N2</td>
<td>Isolation</td>
<td>11/93</td>
</tr>
<tr>
<td></td>
<td>Pheasant</td>
<td>H3N2</td>
<td>Serology</td>
<td>11/93</td>
</tr>
<tr>
<td></td>
<td>Pheasant</td>
<td>H5, H11N2</td>
<td>Serology</td>
<td>11/93</td>
</tr>
<tr>
<td></td>
<td>Pheasant</td>
<td>H5N2</td>
<td>Serology</td>
<td>11/93</td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>H5N2</td>
<td>Serology</td>
<td>12/93</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Turkey</td>
<td>H1N1</td>
<td>Serology</td>
<td>12/93</td>
</tr>
</tbody>
</table>
TRANSMISSIBLE DISEASES OF POULTRY
AND OTHER AVIAN SPECIES

Turkey  H4N6  Serology  11/93
Turkey  H7N1  Serology  06/93; 07/94
Turkey  H9N2  Serology  10/93

North Carolina
Chicken  N8  Serology  05/94
Turkey  H1  Serology  10/93; 01/94; 02/94
Turkey  H1N1  Serology  01/94; 03/94

North Dakota
Turkey  H10N7  Serology  07/94
Turkey  H5N3  Serology  10/93; 12/93

New Hampshire
Turkey  H6N2  Serology  12/93

Pennsylvania
Guinea Fowl  H1N1  Serology  03/94

Virginia
Turkey  H1N1  Serology  12/93

Table 3. Presence of avian influenza virus (AIV) or AIV-specific antibodies in waterfowl other than those in live-bird markets. (NVSL)

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Subtypes(s)</th>
<th>Isolation / Mo / Yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>Geese</td>
<td>N2, N3</td>
<td>Serology 07/94</td>
</tr>
<tr>
<td>Maryland</td>
<td>Duck</td>
<td>H1, 3, 5, 10, N2, 8</td>
<td>Serology 03/94</td>
</tr>
<tr>
<td></td>
<td>Duck</td>
<td>H1, 4, 5, 6, N2, 3, 7, 8, 9</td>
<td>Serology 01/94</td>
</tr>
<tr>
<td></td>
<td>Duck</td>
<td>H3N2</td>
<td>Isolation 11/93</td>
</tr>
<tr>
<td></td>
<td>Ducks,</td>
<td>H5N2, H5N8</td>
<td>Serology 11/93</td>
</tr>
<tr>
<td></td>
<td>Geese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Goose</td>
<td>H6N8</td>
<td>Serology 11/93</td>
</tr>
<tr>
<td></td>
<td>Duck</td>
<td>H9</td>
<td>Serology 03/94</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>Duck</td>
<td>H1</td>
<td>Serology 08/94</td>
</tr>
<tr>
<td></td>
<td>Goose</td>
<td>H11, H2, 7, 9</td>
<td>Serology 07/94</td>
</tr>
<tr>
<td></td>
<td>Swan</td>
<td>N1, 9</td>
<td>Serology 05/94</td>
</tr>
</tbody>
</table>

B: Dr. James E. Pearson, USDA, APHIS, VS, NVSL, gave the following status report:

Avian Influenza in Ratites:
In June and July 1993, AIV subtypes H5N2 and H7N1 were isolated from emus and rheas in Texas and North Carolina. The isolates were not pathogenic for experimentally inoculated chickens and turkeys. The amino acid sequence at the cleavage site of the hemagglutinin was not compatible with highly pathogenic avian influenza. These isolations resulted in 30 states requiring that ratites be tested for influenza antibody prior to entry. Antibody has been detected in samples from ratites in a total of 21 states, and antibody against AIV subtypes H5 or H7 has been detected in 13 states.
REPORT OF THE COMMITTEE

Antibody against all the hemagglutinin subtypes except H13 and H14 and all nine neuraminidase subtypes were found. The only avian influenza viruses isolated since June 1993 were subtypes H4N6 and H10N4 from emus and rheas from Massachusetts in September 1994. The results of the June to September 1993 testing of ratites were reported last year to this committee. Between October 1993 and September 1994, AIV was isolated from or AIV-specific antibodies were detected in ostriches, emus, and rheas (ratites) from 17 states (Table 1).


<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Antibodies</th>
<th>Month/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>Unknown</td>
<td>H9N2</td>
<td>December 1993</td>
</tr>
<tr>
<td>Illinois</td>
<td>Rhea</td>
<td>H5N2, H3</td>
<td>March 1994</td>
</tr>
<tr>
<td>Indiana</td>
<td>Emu</td>
<td>H1, H3, H6,</td>
<td>March 1994 April 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H8, N4, N8</td>
<td></td>
</tr>
<tr>
<td>Kentucky</td>
<td>Emu</td>
<td>H1N1</td>
<td>April 1994</td>
</tr>
<tr>
<td></td>
<td>Ostrich</td>
<td>H2N2, H5N2,</td>
<td>November 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N2</td>
<td>December 1993</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>Rhea &amp; emu</td>
<td>H10N1, H4,</td>
<td>September 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H7H10N4, H4N6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(isol.)</td>
<td></td>
</tr>
<tr>
<td>Missouri</td>
<td>Emu</td>
<td>H9</td>
<td>February 1994</td>
</tr>
<tr>
<td>Montana</td>
<td>Rhea</td>
<td>H5N2</td>
<td>October 1993</td>
</tr>
<tr>
<td></td>
<td>Rhea</td>
<td>H3, H4, H5, H9</td>
<td>March 1994</td>
</tr>
<tr>
<td></td>
<td>Rhea</td>
<td>H1, H3, H4, H5,</td>
<td>April 1994</td>
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<td></td>
<td></td>
<td>H8, H9, N1-4,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>and N6-9</td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>Rhea</td>
<td>H5, H9, N1, N2</td>
<td>March 1994</td>
</tr>
<tr>
<td>North Dakota</td>
<td>Rhea</td>
<td>H5N2</td>
<td>February 1994 March 1994</td>
</tr>
<tr>
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<td>H6N8</td>
<td>January 1994</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>Emu</td>
<td>H3N2</td>
<td>October 1993</td>
</tr>
<tr>
<td></td>
<td>Rhea</td>
<td>H4N6</td>
<td>October 1993</td>
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<td></td>
<td>Rhea</td>
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<td>October 1993</td>
</tr>
<tr>
<td></td>
<td>Rhea</td>
<td>H9N2</td>
<td>November 1993</td>
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<tr>
<td>Oregon</td>
<td>Rhea</td>
<td>H4, H9, N2,</td>
<td>November 1993</td>
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<td></td>
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<td>N6, N7, N8, N9</td>
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<tr>
<td></td>
<td>Emu</td>
<td>H7N3</td>
<td>August 1994 September 1994</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Rhea</td>
<td>H4N2</td>
<td>December 1993</td>
</tr>
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</table>
C. Dr. Susan Trock, USDA APHIS presented the following report on avian influenza H7N3 in the Northeast.

Because of our experience during the 1983 and 1984 avian influenza (AI) outbreak in Pennsylvania, we have been conducting surveillance for AI in the live bird markets in the Northeast. Surveillance consists of sampling birds and environment for virus during the fall and winter months. Since the virus is rapidly killed in sunlight and warm/hot weather, while survival time is increased in cold and dark weather, the fall and winter were selected in order to bias the sampling in favor of recovering the virus if it is present.

In the fall of 1983, we tested all live bird markets, auction markets, bird terminals, dealers and haulers and did not recover any H5 or H7 influenza viruses.

During February 1994, sampling was again conducted in the live bird markets in the Northeast. Seven (7) live bird markets in New York and three (3) live bird markets in New Jersey had H7N9 virus isolated. One (1) other live bird market in New Jersey had H7N3 virus isolated. The National Veterinary Services Laboratories and St. Jude's Hospital further characterized the virus and reported it to be non-pathogenic for chickens. Nonetheless, all positive markets were emptied of birds, cleaned, disinfected, and sampled again after restocking with new birds.

Trace backs from the positive markets were conducted. States supplying live birds directly to these markets include: Maryland, Pennsylvania, New Jersey, New York, North Carolina, Maine, New Hampshire, and Connecticut. In addition, Pennsylvania increased surveillance and sampling in auction markets handling live birds. Surveillance, in the form of flock sampling, was also established for four large contract growers representing approximately 144 farm premises. No virus or serologic positives were identified at either farm, dealer, auction, or hauler sources.

In October we began sampling in the live bird markets for the fall round of surveillance. To date, no H7 or H9 virus has been recovered.

D. Dr. C. W. Beard, SEPEA presented the following update on avian influenza in Mexico. Dr. Eduardo Riviera-Cruz of Mexico city updated the group on current developments in Mexico regarding AI:

The chicken industry has experienced a problem with avian influenza, primarily in the center of the country, since late 1993. The government
officials received the first viral isolates in early 1994 which have been typed as H5N2. Although considerable and variable losses have been attributed to the virus, the USDA/APHS National Veterinary Services Laboratory in Ames, Iowa evaluated the isolates by chicken inoculation and no clinical disease was observed.

Determination of the nucleic sequences at the hemagglutinin cleavage site in Dr. Webster's laboratory indicates that the Mexican viruses are similar to other nonpathogenic H5 isolates.

The poultry industry and the government officials are still in the discussion phase on the appropriate directions for dealing with the disease. There are those who wish to use the vaccines and there are those (principally in the AI-free states) that are pushing for eradication/control without vaccines. This is a unique situation because H5 influenza viruses have not been known to circulate in chickens for such an extended time period.

Positive serology extends to many states including some that are very near the border of the United States. One complicating factor that will doubtless impact the success of any control program relates to the large portion of the broiler production that is sold through live-bird markets in and around Mexico City.

There are several U.S. broiler companies that have joint-venture arrangements with Mexican broiler companies.

D. The following recommendations were made regarding avian influenza:

Criteria for Determining That an AI Virus Isolation Causing an Outbreak Must Be Considered for Eradication

1. Any influenza virus that is lethal for six, seven, or eight of eight 4- to 6-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1:10 dilution of a bacteria-free, infectious allantoic fluid.
2. Any H5 of H7 virus that does not meet the criteria in item #1, but has an amino acid sequence at the hemagglutinin cleavage site that is compatible with highly pathogenic avian influenza viruses.
3. Any influenza virus that is not an H5 or H7 subtype which kills one to five chickens and grows in cell culture in the absence of trypsin.

VI. Salmonella enteritidis Update

Dr. J. Mason, APHIS-VS, presented the following update on the SE control program:

<table>
<thead>
<tr>
<th></th>
<th>Human SE outbreaks</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Egg-Implicated</td>
</tr>
<tr>
<td>1990</td>
<td>70</td>
<td>22</td>
</tr>
<tr>
<td>1991</td>
<td>68</td>
<td>13</td>
</tr>
<tr>
<td>1992</td>
<td>60</td>
<td>26</td>
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</table>
TRANSMISSIBLE DISEASES OF POULTRY
AND OTHER AVIAN SPECIES

<table>
<thead>
<tr>
<th></th>
<th>1993</th>
<th>1994*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>63</td>
<td>26</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>5</td>
<td>87 (30%)</td>
</tr>
</tbody>
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*Through 10/27/94

For the 87 egg-related outbreaks:

In 40 a single source flock could not be determined.

Tracebacks from 47 outbreaks:

- 5 egg traces are in progress.
- 6 flocks had been depopulated prior to test.
- 3 were negative for SE on test.
- 33 flocks were positive for SE on test.

Eggs diverted for pasteurization from the SE-positive flocks – 1.3 billion eggs.

VII. National Poultry Improvement Plan

Mr. Andrew Rhorer, USDA APHIS NPIP presented the following report on the activities of NPIP:

Pullorum-Typhoid Status:

In calendar year 1993, there were 40 isolations/outbreaks of *Salmonella pullorum* reported to the Poultry Improvement Staff. During calendar year 1994 from January to October 1st, there were 23 isolations of *Salmonella pullorum*. These isolations were reported by 13 states. One state reported 50% of the isolations. There have been no isolations of *Salmonella gallinarum* since 1988.

One hatchery and its supply flocks were responsible for 45 isolations in 1993-94. Investigations were completed on approximately 250 shipments from the suspect hatchery. All suspect flocks that were capable of being traced from the source hatchery were serologically tested. All reactors to the serological tests were submitted for further testing to authorized laboratories or destroyed.

There were 21 culture positive flocks in 1993 and 8 in 1994 that were rapid whole blood plate test positive and tube agglutination test (TAT) negative.

In calendar year 1993, the 40 isolations of *Salmonella pullorum* were all Standard strain. In calendar year 1994, the 23 isolations of *Salmonella pullorum* were all Standard strain. There were no Variant or Intermediate isolates in 1993 or 1994.

In 1993-94 there were 51 isolations in bantam chickens, 6 in standard chickens, 2 in game chickens and 3 in mixed breeds.
The number of birds in *Salmonella pullorum* positive flocks were as follows:

<table>
<thead>
<tr>
<th>Number of birds</th>
<th>Flocks</th>
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<tbody>
<tr>
<td>&lt;5 birds</td>
<td>7</td>
</tr>
<tr>
<td>&gt;5 &lt;15 birds</td>
<td>3</td>
</tr>
<tr>
<td>&gt;15 &lt;25 birds</td>
<td>6</td>
</tr>
<tr>
<td>&gt;25 &lt;50 birds</td>
<td>27</td>
</tr>
<tr>
<td>&gt;50 &lt;75 birds</td>
<td>12</td>
</tr>
<tr>
<td>&gt;75 &lt;100 birds</td>
<td>5</td>
</tr>
<tr>
<td>&gt;100 &lt;200 birds</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>63</strong></td>
</tr>
</tbody>
</table>

National Poultry Improvement Plan

The Biennial Conference of the National Poultry Improvement Plan (NPIP) was held in Nashville, Tennessee, in June 1994. Several changes in the provisions of the NPIP were approved by the voting delegates from the participating states. These changes are as follows:

1. A new U.S. *S. enteritidis* Clean classification for primary meat-type breeding chickens was approved.
2. Protocol for bacteriological examination of baby chicks was established.
3. Various changes in sample sizes were approved for Official Mycoplasma serological tests.
4. A federally licensed SE ELISA was accepted as a NPIP approved serological test.
5. A colony lift assay was added as part of the NPIP approved bacteriological examination protocol.
6. Established the maximum number of serum plate positive samples for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma meleagridis* that will be examined using the hemagglutination inhibition (HI) and or the serum plate dilution (SPD) test.
7. Require a representative sample of male birds from meat-type chickens and waterfowl, exhibition poultry and game birds be serologically samples for pullorum-typhoid.

Several resolutions were approved at the Biennial Conference of the NPIP. Some of these are as follows:

1. To conduct three regional wet laboratory salmonella isolation workshops.
2. To request the Southeastern Poultry and Egg Association to assist in establishment of a Mycoplasma quality assurance laboratory in Athens, Georgia under the direction of Dr. Stanley Kleven.
3. To allow no data derived from required testing or associated with specific participants or their product be revealed to other agencies
VIII. USAHA Feed Safety Committee
Dr. M.S. Cover, chair of the USAHA Committee on Feed Safety gave the following update on Feed Safety Committee activities:

The Feed Safety Committee has been very active during 1994 and much progress has been accomplished. Each of the four subcommittees have met to prepare recommendations for the control of salmonella. The transportation committee by the use of a questionnaire has developed a framework for HACCP plan to be used in the transportation industry. This includes the assessment of hazards, identification of control points, establishment of critical limits and procedure for monitoring. The microbiology subcommittee has met on several occasions to refine the recommendations for sampling including number and size of samples, processing procedures and statistical processing for evaluation of results. This group always has included expertise in statistical evaluation of the process. This is a very important part of the feed control program and a key to its success. The live production subcommittee in the poultry area has submitted specific recommendations which would accomplish our goal in swine production progress is being made in the form of HACCP type procedures. The feed and feed ingredient subcommittee met with members of the American Feed Manufacturers Association in order to coordinate plans and procedures in salmonella control.

FDA/CVM has been very helpful and cooperative in all of our efforts providing key suggestions and guidelines. The feed industry has contributed by their efforts in refining practices and policies to improve the quality of their products.

Of particular interest in this endeavor is that many feed manufacturing and grow-out operations are now using HACCP type programs. I consider this as progress. As more of these programs are activated, those who have been and are now reluctant, hesitant and uncooperative, will of necessity need follow into the overall format of HACCP programming.

IX. Salmonella Committee
Dr. K. V. Nagaraja, Co-Chair of the USAHA Salmonella Committee presented the following report:

Drs. Bean and Potter reported for CDC on salmonella serotypes from humans for 1992. A total of 34,000 isolates were identified with S. typhimurium and S. enteritidis (SE) being the 2 most frequently isolated.

Ten most frequently reported serotypes of salmonella were S. typhimurium, S. enteritidis, S. heidelberg, S. newport, S. infantis, S. agona,

Dr. Saeed and co-workers from Purdue University reported that there is a variation in virulence of different strains of SE which is not easily explained by the existence of certain plasmids and which may be influenced by the host.

Dr. Shivaprasad from California reported that there was a variation in egg transmission between SE strains.

Dr. Mason from the SE task force presented one overview of the SE Control Program. So far, in 1994, the incidence of human outbreaks of SE is down from the previous 4 years. He reported that the USDA has a new agency in charge of food safety.

Dr. Ghorri of Arkansas described a case report of re-contamination of the premises of an egg type breeder flock from SE which illustrates the role of mice and rats in maintaining SE in the premises.

Dr. Willoughby and colleagues reported on an outbreak of SE plaque type 4 in a commercial layer flock in southern California. They found SE PT 4 in egg chickens, cats, skunks, and rodents.

Dr. Frank Sisok from the Veterinary Research Institute, Czechoslovakia Republic, described their favorable experiences over the years using a modified live S. typhimurium (Gal E-, Meth-, Leu-, Trypt-, Streptomycin dependent) for vaccinating chickens.

Dr. Corrier and USDA colleagues in Texas and Iowa reported on the beneficial effects in chicks of a competitive exclusion culture containing 29 bacterial isolates. Feed conversion was improved in treated chicks and salmonella isolations were reduced.

Dr. Laheller and French co-workers reported that treatment of young chicks with Baytril and subsequent competitive exclusion markedly decreased the number of salmonella contaminated carcasses, but it did not eradicate salmonella.

Dr. Welsh from Oklahoma reported that salmonella isolations and clinical syndromes associated with salmonella in ratites (ostriches and emus) appear to be increasing in his state.

X. Migratory Bird Health and Related Issues
Dr. J. R. Fischer, SCWDS, University of Georgia, gave the following report on behalf of Drs. T. J. Roffe and G. A. Kidd, National Wildlife Health Center, USDI:

Migratory Bird Disease Surveillance 1993-1994

This report summarizes wildlife mortality reported to the National Biological Survey National Wildlife Health Center (NWHC) from October 1993 through September 1994. A total of 195 mortality events occurred this year, which is below the 5 year average of 212. The total mortality for the 195 events is estimated at 156,000 birds, primarily migratory species such as ducks and geese. Last year estimated mortality was 102,000. The 1993-1994 increase is primarily a result of increased botulism and avian cholera.
TRANSMISSIBLE DISEASES OF POULTRY
AND OTHER AVIAN SPECIES

mortality.

In addition to this confirmed avian mortality, Colorado Division of Wild-
life has reported a suspicion of widespread salmonellosis mortality in pas-
serines. Although mortality appears to have occurred along the Front Range
and may involve thousands of birds, further information is not available at
this writing.

Avian Cholera

Avian cholera was confirmed from 17 sites this year compared to 12
and 20 in the last two years, respectively. The largest dieoff occurred at
Chesapeake Bay which has not experienced avian cholera since 1978. Over
23 species of waterbirds were involved, primarily oldsquaw (70%), buffle-
head and scooters. Mortality exceeded 35,000 birds. The 1994 outbreak
occurred during weather conditions similar to the 1978 epizootic. Cold
weather in the north delayed migration and caused unusually high bird den-
sities in the Bay area.

Four areas in Texas reported avian cholera mortality this year. The
largest mortality occurred in the Texas Panhandle Area at Cactus Lake and
Rita Blanca Lake. One thousand mallards and Canada geese were picked
up from a wintering population of over 100,000 birds at Rita Blanca Lake.
Six hundred geese and dabbling ducks were reported dead at Cactus Lake,
however, up to one third of the total mortality may be attributed to gunshot
trauma. The Rice Belt was the only Texas area with avian cholera last year
and it again occurred in 1994. Over 800 snow and white-fronted geese
were collected in a single week with total mortality just over 1,000.

Avian cholera mortality exceeding 1,500 was a record loss for Bosque
del Apache NWR in Central New Mexico. Primarily snow geese and over
100 sandhill cranes were picked up including the first whooping crane diag-
nosed with this disease. At Lac qui Parle, Minnesota, geese began dying
shortly after their arrival from Oak Hammock Marsh in Manitoba, Canada.
Lac qui Parle staff had been on the lookout for mortality because over
1,800 ducks and geese had died at Oak Hammock Marsh prior to their
departure. Over 700 Canada geese and mallards died at Lac qui Parle.
Fortunately this was the last area along the migration route to report avian
cholera. Another site with reoccurring endemic avian cholera, the Rainwa-
ter Basin Nebraska, did not have the disease this year.

In the Pacific Flyway, Klamath Basin in Northern California, reported
mortality of an estimated 1,900 geese. At its peak, over 100 birds were
picked up each day. Redwood National Park, also in California, reported
avian cholera mortality on a small body of water in the park. Of the 2,700
birds that died, American coots were the primary species affected. Sacra-
mento NWR in central California lost an estimated 2,600 geese and ducks
from a population of over 300,000 geese and over 1 million ducks.

Botulism

Forty-two botulism outbreaks were confirmed in 1993/94, almost twice

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REPORT OF THE COMMITTEE

the frequency of the last two years (27 and 24 respectively). Numerous flooded areas combined with another wet year may have contributed to toxin formation. The severest mortality in the U.S. occurred in North Dakota in places that had little or no water last year. Over 27,500 waterfowl (primarily pintail, shovelers and teal) died during the six North Dakota outbreaks, 23,000 of which died on Lake Venturia. Lake Venturia and nearby Dry and Bader Lakes had sites with 10 feet of water which were in hay production in previous years.

In the Pacific Flyway, Klamath Basin NWR, California has on-going botulism mortality (as of October 15). Over 10,000 ducks and geese have died. Palm Desert Water Treatment Plant in the southern part of California lost an estimated 870 ducks (mostly mallards) to botulism. However, the treatment ponds contained a large amount of oleander clippings from surrounding bushes which may have contributed to the mortality. Oleander produces a digitalis-like water soluble toxin but this toxin was not detected. California Department of Fish and Game - Wildlife Investigation Laboratory (CDFG) reported mortality of 3,000 ducks and coots at the Fresno Wastewater Treatment Plant.

Malheur NWR, Oregon, lost over 4,500 birds, mostly teal and mallards, to botulism. One site averaged over 100 birds picked up per day during peak mortality. This area has had only one botulism outbreak (1992 - 2,400 birds) in the last five years. In Utah, Bear River Migratory Bird Refuge and adjacent Farmington Bay Wildlife Management Area had mortalities of 750 and 18,000 respectively. Both areas are on the periphery of the Great Salt Lake and are endemic botulism areas.

Atchafalaya Delta Waterfowl Management Area in southern Louisiana lost an estimated 1,000 ducks (mostly teal) and shorebirds. The areas affected were new spoil areas which had recently flooded during heavy rains. The Southeastern Cooperative Wildlife Disease Study (SCWDS) reported mortality in gulls from Lake Lena and Lake Ariana, Polk County, Florida during late December and early February. An estimated 1,500 gulls died.

Duck Plague

Six duck plague outbreaks occurred this year identical to last years total. Unlike last year, however, a major duck plague epizootic occurred that involved wild migratory waterfowl. This event represents the only known large scale duck plague outbreak in wild migratory waterfowl since the 1973 epizootic at Lake Andes, South Dakota. Approximately 1,400 ducks and geese died. Most mortality involved black and mallard ducks. Six-hundred-forty necropsies were conducted and lesions typical of duck plague were found in 597. Many of the ducks that died appeared to be resident ducks fed by local residents. State, provincial and federal biologists from the United States and Canada developed and implemented a disease contingency plan. This situation was also unique in that previous plague outbreaks (with the exception of Lake Andes) typically involve fewer than 100 birds. The
1973 and 1994 duck plague epizootics point to the potential for significant mortality in wild migratory waterfowl from this virus.

Duck plague was also confirmed in muscovies from Virginia Beach, Virginia. The ducks were found at Lynn Haven Inlet, a creek that runs through a housing area. In Perry County, Pennsylvania, Summerdale State Diagnostic Lab reported an estimated 30 muscovies dead at a youth camp 7-10 days following the donation of 2 new mallards. The mallards were unaffected and were quarantined.

Texas A&M reported duck plague in late March from three suburbs in Dallas: Addison, Carollton and Farmer's Branch. Mortality occurred in city park muscovies. Following virus isolation the NWHC and State made quarantine and disinfection recommendations. All three areas followed through on the recommendations, however the fate of the quarantined birds once again became a debated issue. In the end, one suburb euthanatized and incinerated the affected birds, one suburb gave the birds to a wildlife group for permanent quarantine and the third, Farmer's Branch, decided to release the birds back onto the site. Subsequently, birds continued to die at Farmer's Branch from duck plague until mid-May.

CDFG reported two duck plague outbreaks this year. In Napa, 14 of 14 muscovy ducks died on a private 1/4 acre pond. At Santa Rosa, a private landowner had 4 of 4 muscovy ducks die from a private collection. No other species died although 3 of 7 domestic mallards had positive titers. All remaining birds were quarantined.

**Toxicosis**

Poisoning was confirmed or suspected in 36 epizootics this year, similar to last year's 34. This year's total includes four lead poisoning dieoffs. Five of the 36 are potential or ongoing legal cases. As has been the typical pattern, most (29) of the dieoffs involved small numbers of animals (<50).

Non-specific cholinesterase-inhibiting poisons (organophosphates and carbamates) were diagnosed in several outbreaks this year. These included the largest toxin-related mortality this year in St. Augustine, Florida where an estimated 225 gulls died near a landfill. A carbamate compound was suspected based on cholinesterase testing but the source was not identified. Similarly unidentified organophosphates were suspected as the cause of mortality in Dover-Foxcroft, Maine (50 grackles at a bird feeder), Brunswick County, Virginia (gulls in dam raceway and landfill), and Monroe County, Michigan (50 herring and ring-billed gulls at a landfill).

Diazinon was confirmed as the cause of death of an estimated 100 mallards and domestic pigeons from a park in Moline, Illinois, and 50 mallards in Shakopee, Minnesota. Recently ingested grains were suspected to have been the carrier of the toxins but the source could not be identified. In Warren County and in Muskingum, Ohio diazinon poisoning in waterfowl was suspected to be associated with improper application of the chemical to lawns.
Wildlife mortality may occur on golf courses following pesticide application. Usually this is due to organophosphorus compounds, as occurred in mallards in Murray, Utah, or carbamates. However, at a Park City, Utah, golf course zinc phosphide applied to wheat berries to control rodents was implicated in the death of 29 mallards on one occasion and a Canada goose several months later.

In Muscoda, Wisconsin, 18 cedar waxwings were found dead or dying in a backyard juniper tree. A pan of stagnant water containing a flea collar was found below the tree. Diagnostic testing revealed chlorpyrifos, a pesticide used in flea collars, as the causative agent.

Mortality due to lead poisoning was relatively low this year. Five die-offs occurred in California, Illinois, and Minnesota. Most experienced losses of less than 30 birds.

**Miscellaneous**

An estimated 100 tundra swans died on Mattamuskeet NWR, NC. Visceral gout was diagnosed as the cause of death.

Over 2,100 eared grebes, ruddy ducks, gulls and other waterbirds died at Salton Sea NWR in southern California. This outbreak is similar to the one which occurred in 1992. Avian cholera was responsible for a small part of the mortality, however the primary cause remains undetermined. As with the previous outbreak, many of the grebes were observed to exhibit unusual behavior including excessive preening, congregating and drinking at freshwater tributaries, and moving onto land. The involvement of biotoxins is also being investigated.

Morbidity and mortality of house finches has been reported throughout a 9 state area in the mid-Atlantic. Affected finches have swollen conjunctivae. Mycoplasma has been isolated from some birds by researchers in Georgia and North Carolina. Another report to this committee will be covering the details of this outbreak.

Nineteen bald eagles were found dead in the vicinity of a whale carcass near Prince of Whales Island, Alaska. All eagles were emaciated and covered with viscous whale oil. The feeding eagles may have became oiled from the blubber resulting in hypothermia and death. Follow-up with sources in Alaska indicate similar situations have occurred in the past.

Steatitis, which is rare in wild birds, caused the death of herons from Point Loma nesting colony in California. Steatitis has been seen in herons from Point Loma the past several years. Speculation as to the etiology of the disease includes rancid bait fish and/or contaminants.

Dr. John R. Fischer, SCWDS, University of Georgia, presented the following report:

**Conjunctivitis in House Finches**

A previously unrecognized conjunctivitis in house finches (*Carpodacus mexicanus*) has been reported in Connecticut, Delaware, Maryland, Mas-
Massachusetts, New Jersey, New York, North Carolina, Pennsylvania, Virginia, and West Virginia. The first reports were received in February 1994 from Maryland and Virginia. Since that time, hundreds of sick birds, virtually all house finches, have been observed at feeders or submitted to wildlife rehabilitators. The most recent reports are from New York and Connecticut. The disease outbreak is simultaneously being investigated by the Southeastern Cooperative Wildlife Disease Study (SCWDS), the National Wildlife Health Center (NWHC), wildlife agencies in numerous states, and veterinary diagnostic laboratories in MD, VA, NC, and GA.

Clinical signs and gross lesions have ranged from mild to severe unilateral or bilateral conjunctival swelling with serous to mucopurulent drainage and nasal exudate. Microscopically, lesions consisted of chronic lymphoplasmacytic conjunctivitis, rhinitis, and sinusitis. The cause of the conjunctivitis tentatively has been identified by culture and polymerase chain reaction as *Mycoplasma gallisepticum* (MG). Although MG is a well-known pathogen of domestic poultry, it previously has not been documented as a cause of disease in songbirds.

At this time, the disease seems important only for house finches, but the effects on house finch populations are unknown. House finches flock to bird feeders in large numbers, and can travel long distances (partial migrations). These factors probably will enhance spread of disease. Domestic birds with MG are regarded as infected for life, and MG may be transmitted via eggs to the next generation, therefore, this disease may become readily entrenched in nature.

The possibility that domestic or wild birds other than the house finch may be susceptible to infection is another concern. Experimental work is under way to determine the pathogenicity of the MG strains obtained from house finches. Preliminary characterization of the MG isolated from house finches indicated that the strains differ somewhat from the MG strains associated with disease in poultry. Domestic chickens and turkeys have been inoculated with house finch-derived MG and monitored for development of disease at the Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia. Experiments to reproduce the disease in unaffected house finches and to evaluate disease transmission between infected and uninfected birds are underway.

XI. Problems ensuring freedom of biologics from RETICULOENDOTHELIOSES VIRUS.

Dr. R. L. Witter, U.S. Department of Agriculture, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, Michigan, gave the following presentation:

Reticuloendotheliosis virus (REV) was isolated in 1994 by Dr. Aly Fadly from a commercial fowlpox vaccine that had been administered off label in conjunction with reovirus vaccine to two broiler breeder flocks in Alabama at 7 days of age. Both flocks developed a lymphoid leukemia-like syndrome.
The same vaccine had been given at about 10 weeks of age, as recommended by the manufacturer, to other broiler breeder flocks in this operation where seroconversion occurred but no tumors were noted. Also, the same subtype of REV was isolated from the vaccine and the chickens. Thus, there appeared to be a correlation between the early use of this contaminated vaccine and the RE lymphomas.

There were some difficulties associated with the demonstration of REV in the vaccine. Although PCR assays were consistently positive, cell cultures inoculated with the vaccine were negative by ELISA and, when tested by indirect immunofluorescence, only a small number of cells appeared to contain viral antigen. However, REV was consistently demonstrated in blood samples obtained from 2-week-old chicks that received the vaccine at one day of age. At least two other laboratories made unsuccessful efforts to demonstrate virus in the vaccine using complement fixation or other procedures, although one of these ultimately isolated REV from a production seed. This indicated that when mixed with fowlpox virus, REV was relatively difficult to demonstrate by conventional test systems.

This issue is not new. REV-contaminated Marek's disease vaccines caused major losses in Australia and Japan in the 1970s and have since been sporadically encountered in Brazil and other countries. APHIS considered requirements for REV testing of biologics in the late 1970s but no requirements were imposed, apparently because no contaminated vaccines were identified in a survey of production serials and there was no history of problems. This question may now need to be revisited.

Currently in the United States, SPF flocks used for vaccine production must be free of REV infection but there is no requirement that poultry biologics or master seeds be certified to be free of REV. Testing protocols are outlined in the European Pharmacopoeia and in requirements for some individual countries. Many biologics companies have recently started voluntary testing programs for REV. The National Broiler Council has appointed a committee to make recommendations on REV quality control. It is not yet clear, however, whether testing should be voluntary or required, and what specific tests should be used.

XII. Mycoplasmosis subcommittee
Dr. F. Hoerr, chair of the subcommittee on Mycoplasmosis presented the following report:

The contributions of the Southeastern Poultry and Egg Association to avian mycoplasma testing were acknowledged. The subcommittee recommends that the Transmissible Diseases of Poultry Committee send a written commendation to the SEPEA for providing funds to establish an Avian Mycoplasma Reagent Laboratory and to distribute a video, Avian Mycoplasma Serology Techniques, and the Proceedings of the 1994 Poultry Mycoplasma Workshop to diagnostic laboratories. The Western Poultry Disease Conference, the California Veterinary Diagnostic Laboratories, and
the American College of Poultry Veterinarians should be thanked for their role in sponsoring the wet lab and distributing the proceedings and the videotape.

Concerns were expressed about several current issues of mycoplasma reporting, regulation, and management of MG or MS-infected poultry. The subcommittee reached consensus agreement in offering the following recommendations:

a. that mycoplasmosis cases be reported responsibly to include the review of laboratory data to differentiate positive laboratory tests from actual positive flocks, and to identify the avian species or type of poultry infected;

b. that state veterinarians work in cooperation with poultry producers to develop a statewide notification network that identifies the location of MG and MS-infected flocks;

c. that there be cooperation among state veterinarians in sharing information about interstate movement of MG or MS-infected poultry and hatching eggs;

d. that young male broiler breeders raised to supplement the fertility of older breeder flocks (so-called spike males) be subjected to serological testing for MG and MS, and either culture or PCR prior to distribution to the breeder flocks;

e. that MG or MS-infected primary breeders be depopulated and the hatching eggs destroyed, and further, strongly encourages the same action for multiplier breeders.

XIII. Subcommittees

A. Avian Influenza: C.W. Beard; B.C. Easterday; D. Halvorson; R. Webster; J.E. Pearson; D. E. Swayne; R.J. Eckroade, Vice Chair; B.S. Pomeroy, Chair.

B. Food Safety: J.-W. Colby; D. Hill; G.T. Holder; G.E. Kolb; S. McCarter; A. Mutalib; P.E. Poss, Chair.

C. Infectious Bronchitis: C.W. Beard; R.J. Eckroade; H.N. Lasher; M. Opitz; H.L. Shivaprasad; P. Woolcock; S. Naqi, Chair.

D. Infectious Laryngotracheitis Eradication: W.C. Baisley; F.J. Hoerr; G.T. Holder; H.N. Lasher; E.M. Odor; H.M. Ghorri, Chair.

E. Mycoplasmosis. S.H. Kleven; E.T. Mallinson; H.M. Opitz; B.S. Pomeroy; H.W. Towers; R. Yamamoto; F.J. Hoerr, Chair.

F. Ratite Industry: H.L. Shivaprasad; S.A. Vezey; K. Coldwell; J.P. Sanders; F. Golan; R. Angel; H. Rubin; K.D. Hicks-Alldredge, Chair.

G. Broiler Industry: G.T. Holder, Chair.

H. Table Egg Industry: G.L. Waters, Chair.

I. Turkey Industry: G.Y. Ghazikhanian, Chair.

J. Program committee. R.E. McCapes; P.E. Poss; F.J. Hoerr, R. J. Eckroade; G.T. Holder, Chair.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

Chairman: Dr. David G. Thawley, St. Paul, MN
Vice Chairman: Dr. Beth Lautner, Des Moines, IA

Dr. Gary A. Anderson, KS; Dr. George W. Beran, IA; Dr. L. G. Biehl, IL; Mr. Neal Black, MN; Mr. Philip E. Bradshaw, IL; Dr. John R. Cole, GA; Dr. John M. Cunningham, NE; Dr. Paul B. Doby, IL; Dr. E. Gerald Duhamel, NE; Dr. Gene A. Erickson, NC; Dr. Wayne R. Freese, MN; Dr. Anthony M. Gallina, IL; Dr. Robert D. Glock, CO; Dr. Mark Hammer, NC; Dr. D. L. Harris, IA; Dr. Howard Hill, IA; Dr. Wade Kadel, KY; Dr. Charles L. Kanitz, IN; Dr. John P. Kluge, IA; Dr. Charles Massengill, MO; Dr. James McLean, IA; Dr. William L. Mengeling, IA; Dr. Rita D. Michaels, MO; Dr. F. J. Mulhem, MD; Dr. Phillip A. O’Berry, IA; Mr. Craig Olson, IA; Dr. R. E. Omohundro, TX; Dr. Linda Schlater, IA; Dr. Roy A. Schultz, IA; Dr. H. Wesley Towers, DE; Dr. Mahlon W. Vorhies, KS; Dr. Philip W. Widel, MO; Dr. Wallace B. Wren, IA; Dr. James C. Wright, AL.

Nine committee members and thirty-one guests were present for a program focused on the practice of feeding recycled commodities to swine.

The meeting began with a presentation by Dr. Kyong-Jn Yoon provided an update on the diagnosis of Porcine Reproductive and Respiratory Syndrome. Dr. Yoon presented a review of the molecular structure at the virus and the clinical signs of the disease. He reviewed the several diagnostic assays used as indirect tests (indirect florescent antibody test, immunoperoxidase, serum virus neutralizing test, and ELISA). He described the characteristics of these tests following the experimental infection of young pigs. He concluded that the immunoperoxidase test appears to be the best overall indirect test. Direct diagnostic tests reviewed by Dr. Yoon included virus isolation, the polymerase chain reaction (PCR) and histoimmunology. He noted that due to antigenic diversity among viral strains there is potential for misdiagnosis.

Dr. Barbara Corso, from the Center for Epidemiology and Animal Health of USDA/APHIS, presented a review of risk assessment of the practice of feeding recycled commodities to swine. She reviewed a survey study to quantify the risk of feeding uncooked waste materials by swine producers licensed under the Swine Health Protection Act. Foreign and Public Health Diseases were examined. The foreign diseases modeled included hog cholera, foot and mouth disease, African swine fever, and swine vesicular disease. The public health disease category included trichinellosis, toxoplasmosis, salmonellosis, and campylobacteriosis.

Dr. Corso noted that in most cases plate waste represents the vast majority of recycled commodities fed to swine by licensed operators. Most
TRANSMISSIBLE DISEASES OF SWINE

sources of plate waste are institutions (restaurants, jails, hospitals, etc.) as opposed to household waste. In Puerto Rico, however, household waste represents the majority of waste fed to swine.

Results form the study indicated that the hog cholera and foot and mouth disease introduction was significantly higher (approximately 4-6% per year nation wide) than the other foreign diseases. These risks were much higher in Puerto Rico. Of the public health diseases, a near 100% risk per producer per year was established for toxoplasma, salmonella, and campylobacter.

Dr. Linda Detwiler from USDA/APHIS in New Jersey discussed regulatory and philosophical issues related to recycled commodities feeding. She described some of her experiences as a regulatory official. In the absence of complete compliance with regulations, she noted that it is important for producers to be educated to report unusual disease occurrences. Dr. Detwiler stressed that there have been a recent trend toward more recycled commodities feeding to swine. This has raised the question as to the increased risk of disease introduction due to this practice.

Dr. Michael Westendorf from Rutgers University reviewed some of the scientific issues relating to the feeding of recycled commodities. Dr. Westendorf reviewed the nutrient content of waste commodities. He noted that most sources provide an adequate balance of nutrients. With efficiencies ranging between 2.2:1 for military waste to 5-6:1 for municipal waste. From his studies, Dr. Westendorf concluded that food waste has a current value of over $26/ton on an as-fed basis, or $76/ton on a dry-matter basis. He noted several issues in need of more research. These include a study of carcass quality characteristics and meat palatability from swine fed recycled commodities. Supplementation needs should also be investigated.

There is also a need to further study digestibility and feeding value of waste commodities. Other research needs include the investigation of management information for waste feeding and studies to further determine the assessment of cooking food waste from a nutritional point of view.

Dr. Joseph Annelli addressed the issue of international disease surveillance. At the 1993 meeting of the USAHA Transmissible Diseases of Swine Committee a resolution was passed to encourage USDA to encourage a means by which technical issues of disease surveillance can be standardized on an international basis. He described a proposal by which any country that wishes to impose an import embargo based on the risk of disease introduction, is required within a reasonable time frame to give proof it is free of the disease. Following this technical evaluation the embargo may be classified as truly due to disease or it may be classified as a true trade barrier. In the latter case the issue would be outside the prerogative of USDA. Dr. Annelli noted that to further address the USAHA resolution there is a need to internationally standardize testing procedures.

Dr. Frank Wilson, USDA/APHIS briefly described the HACCP (Hazard Analysis Critical Control Point) program as it relates to the pork industry.
He described HACCP as an holistic approach to disease prevention. HACCP draws on several management principles, good manufacturing practices, total quality management, and ISO 9000. The program has been designed to identify and control the critical points relating to foodborne disease. This would involve all aspects of the food production chain. At the industry level, a likely outcome would be to deliver HACCP guidelines for producers.

Two resolutions were passed by the committee. These were:

RESOLUTION:

The Transmissible Diseases of Swine Committee urges USDA to 1) continue to examine the risks and benefits of recycled commodity feeding with regard to foreign animal diseases and public health diseases including organizing a national symposium to explore the future of expanded recycled commodity feeding and its impact on swine health; 2) increase funding available for Swine Health Protection regulatory activities; 3) increase funding for research for alternative methods of recycling food waste.

RESOLUTION:

Safety of foods of animal origin is an all encompassing endeavor of concern to the consumer and the U.S. poultry and livestock industries, but which lacks a national forum for discussion, policy and program.

The U.S.A.H.A. throughout its nearly one hundred year history has shown that it is an organization through which all stakeholders in the food safety issue could develop market oriented, industry compatible, economically non-disruptive programs to control designated pathogens to live poultry and animals, and ultimately, the finished raw product, and

A past precedent has been set by F.D.A. requesting U.S.A.H.A. to be the national forum in developing recommendations to control the presence of salmonella in feeds and which has resulted in the formation of the Feed Safety Committee with representation from all industries involved in this issue, now

Therefore the Committee on Transmissible Diseases of Swine strongly resolves that the U.S.A.H.A. use its committee system to establish a working group to address the issue of pre and post-harvest food safety and other related programs involving animals, poultry, and their products. This group would then be available to participate with government in formulating policy and programs to achieve the food safety goals of this country in an expeditious and economically feasible manner.

The U.S.A.H.A. should inform the appropriate federal food safety authority of the formation and the availability of this working group of experts.

The meeting was adjourned at 4:10 p.m.
The United States Animal Health Association (USAHA) during their November 1992 meeting recommended that a comprehensive Mexico/United States tuberculosis eradication and control program be established as well as a joint Mexico/United States tuberculosis committee to provide oversight.

The first meeting of the Mexico/United States Committee for Cooperation on Bovine Tuberculosis was held in Denver, Colorado on August 5, 1993, during the meeting of the National Cattlemen's Association meeting. Five other meetings were held as follows:

- Second Meeting Las Vegas, NE October 23, 1993
- Third Meeting Reno, NE January 26, 1994
- Fourth Meeting Guadalajara, Mex. May 17, 1994
- Fifth Meeting Denver, CO July 21, 1994
- Sixth Meeting Grand Rapids, MI October 30, 1994

The joint committee is made up as follows:
- Two regulatory veterinarians from each country.
- Three industry representatives from each country.
- One research representative from each country.
- One representative of veterinarians of border states from each country.

It was recommended that the industry representatives include one beef producer, one dairy producer, and one general farm representative.

The co-chairmen of the committee are Dr. Donald Luchsinger, Acting Deputy Administrator of APHIS for Veterinary Services, and Dr. Hector Campos, Director General of Animal Health in Mexico.

The primary focus for the meetings has been to work through issues that can assist Mexico in the development and implementation of its national tuberculosis eradication program. A progress report of the Mexican program has been presented in each meeting.

At this time, a national commission has been established in Mexico for eradication of bovine tuberculosis and brucellosis. US $92 million of federal funds will be spent in this commission for the next five years, in addition to the funds provided by state governments and the cattlemen.

A total of 241 federal veterinarians were hired during 1994 for supervision of program activities throughout the country. A total of 1,492 veterinarians are accredited for tuberculosis and brucellosis.
The state of Sonora is in the eradication phase and five other states have active testing programs underway. All other states in Mexico are preparing to begin state wide testing.

Examples of issues considered by the joint committee are testing procedures, slaughter surveillance, and laboratory establishment and support. Training has been held on various program components, including post-mortem procedures, comparative cervical testing, and epidemiology and traceback. There has also been an effort to make the Mexican TB regulations equivalent to the Uniform Methods and Rules of the bovine tuberculosis program in the United States and to establish the criteria by which the Mexican TB states would be considered equivalent to the United States TB modified-accredited states.

Research activities have also been discussed and carried out between United States and Mexican scientists. A recent project was completed to compare the sensitivities of the United States and Mexican tuberculins.

A key issue for discussion has been the import regulations of the United States for cattle from Mexico. Also, an important issue has been the monitoring of some state programs in Mexico by United States officials.

The joint committee provides a forum for the United States and Mexico to actively interact and address important issues that appear to be hampering TB eradication progress in Mexico.

The interest and leadership shown by industry representatives from both countries are very much appreciated and are considered key to the success of this effort.
During the 1994 fiscal year (FY), there were many accomplishments toward the final eradication of bovine tuberculosis. In response to a cooperative State-Federal initiative, an area test of Dona Ana county in New Mexico evaluated 35,300 animals in 23 dairy herds during April, 1994. This area test identified one tuberculosis-infected herd. The Bovine Tuberculosis Eradication Uniform Methods and Rules (UM&R) were amended to include Cervidae species. Federal regulations are being promulgated to regulate the interstate movement of Cervidae. The United States is working, in cooperation with the Mexican government and the United States-Mexico Bi-National Tuberculosis Committee, to identify all cases of tuberculosis attributed to Mexican-origin cattle in the United States. Herd plans, to eradicate tuberculosis infection, have been developed for two of the five tuberculosis-infected herds in the El Paso milkshed.

The National Academy of Science, National Research Council, completed an evaluation of the Cooperative State-Federal Bovine Tuberculosis Eradication Program. Their evaluation concluded that the eradication of bovinetuberculosis is both economically and biologically feasible. The committee recommended that tuberculosis eradication also be vigorously pursued in deer, elk, and other farmed exotic hoofed species and that producers of all affected species financially support the eradication program.

During FY 1994, the national program added Louisiana and New York to the list of States that are Accredited-Free and suspended Virginia's Accredited-Free status. There were 11 herds infected with bovine tuberculosis this fiscal year. Of these, 5 were carried over from the previous fiscal year and 6 were detected during fiscal year 1994. The newly detected herds consisted of 2 beef herds in Virginia, one beef and one dairy herd in Texas, one dairy herd in Puerto Rico, and one dairy herd in the New Mexico portion of the El Paso milkshed. The Animal and Plant Health Inspection Service (APHIS) finalized a rule that bans the importation of Holstein steers and spayed heifers from Mexico. This rule, as well as the initiation of the Mexican national tubercu-
In accordance with resolutions by the United States Animal Health Association (USAHA) in 1993, APHIS is considering regulations that limit exposure of domestic cattle to infected Mexican imports and impose movement requirements on Mexican steers after entry into the United States. APHIS has finalized a rule recognizing the Mexican blue metal ear tag as the official permanent identification for imported Mexican cattle.

In support of Mexico's bovine tuberculosis eradication efforts, APHIS has participated in cooperative training programs in the areas of epidemiologic case development, post-mortem inspection, and field testing. Additionally, APHIS has provided the Mexican Government and Mexican industry groups with technical seminars and site visits concerning the eradication of bovine tuberculosis. These technical seminars and site visits have been conducted in the Mexican States of Sonora, Chihuahua, and Coahuila. Animal health officials from the States of Arizona, California, and Texas have participated in these efforts.

APHIS, the Montana Board of Livestock, and the Montana Game, Fish, and Parks Department carried out a wildlife survey around a tuberculosis-infected cervid herd in Montana. This survey identified one case of tuberculosis in a free-ranging mule deer doe, as well as 3 tuberculosis-like lesions in the survey area. APHIS also assisted the Wyoming Fish and Game Department with a hunter's survey of elk killed around the Wyoming National Elk Refuge. To date, no evidence of bovine tuberculosis has been found with this survey.

The submission of thoracic granulomas from slaughter animals, by USDA, Food Safety and Inspection Service (FSIS) and State meat inspection personnel, is the principle method of tuberculosis surveillance in the United States. USDA, APHIS, Veterinary Services (VS) has determined that the optimal submission rate for adequate surveillance is one sample per 2,500 animals slaughtered or one sample per 2,000 adult animals slaughtered. During fiscal year 1993, the national average for slaughter submissions was approximately 33 percent of the optimal submission rate.

This granuloma submission data reflects complex problems in the existing surveillance program for tuberculosis. VS and meat inspection personnel must continue to work together toward increasing the submission of granulomas for surveillance purposes. A national surveillance data base, planned for the beginning of fiscal year 1995, will allow Regional and Area APHIS personnel to more closely monitor slaughter submissions and to target problem areas during the fiscal year. As a result of this monitoring, granuloma submission rates should increase.

The inclusion of individual animal identification devices with slaughter samples is essential for the traceback of tuberculosis infected animals to their herds of origin. Of the 182 tuberculosis investigations conducted on...
feedlot animals of Mexican origin, 78 (43 percent) had Mexican eartags submitted with the slaughter samples. This submission rate represents a 12 percent increase over last year's rate. Proper identification substantially increases the likelihood of a successful tuberculosis investigation. The USDA, Food Safety and Inspection Service (FSIS) is working with VS to increase the submission of identification devices with surveillance samples collected by its inspectors.

Bovine tuberculosis continues to persist at a very low level in some large dairy operations in the El Paso milkshed. Total depopulation of such large herds is the most effective method for eliminating the disease, but is rarely an economically acceptable option for either the Government or the herd owner. The Regional Tuberculosis Epidemiologist stationed in El Paso, Texas has developed clean-up plans for two of the four herds in the milkshed. These plans employ new testing methods and schedules meant to enhance the eradication of tuberculosis in these large dairies. The re-appearance of tuberculosis in herds previously released from quarantine by testing has long been recognized as a deterrent to eradication. The rate of reinfection in such herds is estimated at 32 percent and appears to increase as herd size increases.

The Bovine Tuberculosis UM&R were amended to include Cervidae species on May 15, 1994. These rules provide for accredited cervid herds, official tuberculosis tests, and requirements for interstate movement. The UM&R represents a cooperative effort between USDA, State regulatory agencies, the cervid industry, and the USAHA. These rules are a significant step toward accelerating the eradication of the disease in Cervidae.

There has been a rekindling of interest in tuberculosis research in recent years that will help the program to continue its progress. Colorado State University, Cornell University, Iowa State University, and Texas A&M University have ongoing research programs in bovine tuberculosis. APHIS continues to support these efforts by providing diagnostic specimens and reagents to these institutions. The Scientific Advisory Subcommittee of the Tuberculosis Committee (TBSAS), established by USAHA during fiscal year 1993, provides a means for unbiased evaluations of scientific issues relevant to the national tuberculosis program. In cooperation with the International Llama Association, APHIS also sponsored tuberculosis in llama research projects in both the Republic of Argentina and in the United States.

Figure 1—Fiscal year 1994 began with 42 States plus the U. S. Virgin Islands having Accredited-Free State status for tuberculosis. During the year, Louisiana and New York achieved Accredited-Free status by meeting all standards of the UM&R. Seven States plus Puerto Rico have Modified-Accredited status. The Accredited-Free status of Virginia was suspended during FY 1994.

There were 11 tuberculosis infected herds on record in FY 1994. Five of these were carried over from previous fiscal years and six were newly
detected in FY 1994.

Figure 2—The six newly detected herds during FY 1994 included one dairy herd and one beef herd in the State of Texas, two beef herds in the State of Virginia, one dairy herd in the Commonwealth of Puerto Rico and one New Mexico dairy herd. The New Mexico dairy herd is located in the New Mexico portion of the El Paso milkshed and was detected as a result of the Dona Ana county area test.

Figure 3—This figure depicts the numbers of imported Mexican steers since 1985. In 1994, 1,181,828 feedlot type animals were imported from Mexico. This represents an increase of 191,604 animals from the 1993 level, and the second highest number of importations on record.

Figure 4—This figure depicts slaughter investigations of feedlot origin since 1985. In 1994, there were 249 feedlot investigations, a decrease of 139 cases over 1993. The proportion of feedlot investigations that traced to Mexico has remained at a relatively constant 72 percent over a 10 year period. Even though the percentage has remained uniform, the 1994 data shows an overall decrease in the absolute number of cases traced to Mexico.

Figure 5—This graph represents the cases of tuberculosis per 10,000 animals imported, based on the average number of feedlot type animals imported from Mexico during the previous two fiscal years. During FY 1994, there were 3.44 cases of tuberculosis per 10,000 Mexican imports, a 22 percent decrease over the previous fiscal year. This rate was chosen to depict an average time spent prior to slaughter for imported Mexican cattle.

Figure 6—Two of the 11 herds on record in FY 1994 were depopulated. One was a small Texas beef herd, and the other consisted of a small Virginia beef herd.

Figure 7—During the period 1985-1994 there were 109 tuberculous herds detected of which 81 (74 percent) were depopulated. Eighteen of the herds during this period have been released from quarantine following testing and the slaughter of reactors, or are still being tested. Nine herds remain under quarantine for tuberculosis.

Figure 8—During the period 1985-1994, 28 herds were released from quarantine or are still under test. During this same period 9 herds in which Mycobacterium bovis was confirmed had a previous history of bovine tuberculosis (32 percent).

Figure 9—Suspicious tuberculous lesions or thoracic granulomas were submitted by Meat Inspection personnel from 3,961 slaughter cattle in FY 1994. Of these, 318 (8 percent) were positive for tuberculosis on laboratory examination. Only 12 (3.8 percent) of the positive cases were adult cattle with the balance of 306 cases being immature feedlot animals.

Figure 10—This figure depicts the granuloma submission rates for the 1989-1993 time period by VS region. Slaughter statistics for 1994 were not available at the time of this report. A goal of one granulomatous lesion submitted per 2,500 cattle inspected at regular slaughter was adopted by
VS in 1970 as optimal for efficient tuberculosis surveillance. During FY 1993, the Western Region of VS submitted 85 percent of the optimal rate, the Central Region of VS submitted 23 percent of the optimal rate, the Southeastern Region of VS submitted 19 percent of the optimal rate, and the Northern Region of VS submitted 18 percent of the optimal rate. The total U.S. submission rate only achieved 33 percent of the optimal rate.

Figure 11—This map shows the distribution of 12 captive cervid herds on record during FY 1994, which have had bovine tuberculosis confirmed in one or more animals. Five of these herds were newly detected during FY 1994 and 7 were carried over from the previous fiscal year. These herds are located in 10 States, 6 of which are Accredited Free of bovine tuberculosis.

Figure 12—This figure shows the species distribution of infected Cervidae herds during FY 1994. This includes 4 deer herds (33 percent), 5 elk herds (42 percent) and 3 mixed deer and elk herds or exotic exhibits (25 percent).

Distinct progress was made during this fiscal year toward the eradication of bovine tuberculosis. The national challenge that we face is to overcome all remaining obstacles and to achieve the tuberculosis eradication goal. This will require that all factions of industry and the State and Federal Governments remain firmly committed to the eradication of tuberculosis from this country.

The authors wish to acknowledge the assistance of Dr. Granville H. Frye, Chief Staff Veterinarian, Ms. Fran Shields, Program Assistant, and Ms. Julie Munsey, Office Automation Assistant, Cattle Diseases and Surveillance Staff, USDA, APHIS, VS, Hyattsville, Maryland.
Bovine Tuberculosis State Status and Location of 11 Tuberculosis-Infected Herds – FY 1994

Accredited free States (42) plus Virgin Islands

Modified accredited States (7) plus Puerto Rico

Accredited-Free Suspended (1)
Location of 6 Tuberculosis-Infected Herds
Found in FY 1994

3 - Beef Herds
2 - Dairy Herds
1 - Dairy Herd from El Paso Milkshed

Tuberculosis Eradication

figure 2

VANTIEM, ESSEY
Imported Mexican Steers

figure 3
Closed Slaughter Investigations of Feedlot Origin

Tuberculosis Eradication

% Cases Attributed to Mexican-origin cattle

VANTHEM, ESSAY

figure 4
Cases of Tuberculosis as a Function of Imported Mexican Cattle

Cases of Tuberculosis per 10,000 imports.*

*Based on the average number of Mexican imports during the previous 2 years.

figure 5
Proportion of Tuberculosis-Infected Herds
Depopulated – FY 1994

2 herds depopulated
11 Tuberculosis-Infected Herds

figure 6
Tuberculous* Herds Newly Detected vs. Herds Depopulated

*Infected and exposed

Figure 7

Total Herds - 109

Herds depopulated - 81 (74%)

27 (26%) Herds not depopulated
Reappearance of Tuberculosis in Herds Previously Released from Quarantine

**Figure 8**

- **Herds previously released from quarantine found infected** – 9 (32%)
- **Herds not depopulated** – 28
Granuloma Submission Rates by VS Region: 1989–1993

Percent achievement of optimal goal of 1 submission per 2,500 inspections.

Figure 10
Location of 12 Tuberculosis-Infected Cervidae Herds During FY 1994
Tuberculosis Distribution of Infected Cervidae Herds

- Mixed/Exhibit (25%)
- Deer (33%)
- Elk (42%)

5 - Elk Herds
4 - Deer Herds
3 - Exhibits or Mixed Herds

- 5 newly infected herds
- 7 herds carried over from last fiscal year

Figure 12
REPORT OF THE COMMITTEE ON TUBERCULOSIS

Chairman: Dr. Bob R. Hillman, Boise, ID
Vice Chairman: Dr. Dennis L. Thompson, Sacramento, CA

Dr. L. Garry Adams, TX; Dr. Robert D. Angus, ID; Dr. Daniel L. Baca, TX; Dr. Lowell R. Barnes, IN; Dr. Carole A. Bolin, IA; Dr. Richard E. Breitmeyer, CA; Dr. Raleigh D. Buckmaster, IA; Mr. Jess Burner, Jr., TX; Dr. Thomas F. Conner, IN; Dr. Robert A. Cook, NY; Dr. Donald S. Davis, TX; Ms. Kim Dowling, SD; Dr. Steven R. England, NM; Dr. M. A. Essey, MD; Mr. Joe B. Finley, TX; Dr. Murray E. Fowler, CA; Dr. J. Clay Freeny, OK; Mr. Robert Frost, CA; Dr. G. H. Frye, MD; Dr. Belinda Goff, IA; Dr. Francisco Gurria, MEX; Dr. Thomas J. Hagerty, MN; Dr. William L. Hartmann, MN; Dr. Burke Healey, OK; Mr. Del E. Hensel, CO; Dr. E. Ray Hinshaw, AZ; Dr. John W. Hunt, MO; Dr. Sarah B. S. Hurley, WI; Dr. Samuel Hutchins, 3rd, VT; Mr. Ralph D. Jones, SD; Mr. Denis Joyce, ND; Dr. Victor P. LaBranche, MA; Mr. Peter Lies, ND; Dr. Herbert Lloyd, FL; Dr. Charles Massengill, MO; Dr. Clifford W. McGinnis, NH; Dr. A. R. McLaughlin, WI; Dr. Robert M. Meyer, CO; Dr. Rita D. Michaels, MO; Dr. Michael W. Miller, CO; Dr. Janet B. Payeur, IA; Mr. J. O. Pearce, Jr., FL; Mr. Scott Petty, Jr., TX; Dr. J. T. Prichard, NM; Dr. James P. Quigley, GA; Mr. James E. Rich, WA; Dr. William A. Rotenberger, ND; Mrs. Sherry Seubert, WI; Dr. Charles R. Sherron, TX; Dr. Clarence J. Siroky, MT; Dr. Donald Sackett, WI; Dr. C. D. Stumpff, KS; Mr. George Teagarden, KS; Dr. Joe W. Templeton, TX; Dr. Robert L. Tharp, MO; Dr. C. O. Thoen, IA; Dr. E. Tom Thome, WY; Dr. Daryl K. Thorpe, SD; Dr. Gerald F. Toms, NY; Dr. Paul O. Ugstad, NE; Mr. Alejandro Varela, AZ; Mr. Jay Whetten, NM; Ms. Diana L. Whipple, IA; Mr. Dave Whittlesey, CO; Dr. Richard D. Willer, AZ; Mr. David Winters, TX; Mr. Sam Withiam, OK; Mr. Steve Wolcott, CO; Dr. Jerry M. Woodall, OK; Dr. Glen L. Zebarth, MN.

The Committee on Tuberculosis met on Wednesday, November 2, 1994 from 1:30 to 5:30 P.M. and Thursday, November 3, 1994 from 1:30 to 4:45 P.M. Sixteen papers and reports were presented to over 100 committee members and guests.

Dr. Barry Stemshorn, Agriculture Canada, presented the status report on the Canadian Tuberculosis Program. The Canadian bovine tuberculosis eradication program for cattle is modeled closely after the program in the United States. The program is based on abattoir surveillance and the submission of tuberculosis-like lesions to federal laboratories for histopathology and culture. Histopathology compatible animals are traced and the herd of origin quarantined. If Mycobacterium bovis is isolated, all susceptible animal species on the quarantined premises are ordered destroyed, and compensation is paid.

All provinces with the exception of Ontario are classified as tuberculo-
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sis-free. Ontario's status was suspended as a result of the discovery of an infected herd this year. The premises found infected in 1994 had been depopulated because of tuberculosis in 1992. The infection in 1992 was the result of contact of cattle with an infected cervid herd. Following depopulation, cleaning and disinfection was carried out and the premises was restocked. The initial tuberculosis test of the restocked herd was negative. The one year retest was done and several cattle were classified as reactors. The herd was then ordered destroyed.

The recent results of testing cervid herds under the Captive Ungulate Program suggest that bovine tuberculosis in very nearly eliminated from ranched cervid herds in Canada. As with the cattle tuberculosis eradication program, the finding of an occasional tuberculosis infected herd can be anticipated. The changing emphasis of the ranched cervid industry from the sale of breeding stock to the marketing of venison will result in changes to the Captive Ungulate Program. As abattoir surveillance increases the ability to monitor the health status of ranched cervids, on the farm testing will be phased out.

The Canadian policy on tuberculosis in zoos is being reviewed and modified to limit federal involvement to actions essential to protect Canada's livestock industries and human health.

Dr. Francisco Gurria, Executive Director of the National Commission for the Eradication of Bovine Tuberculosis and Brucellosis, presented a status report on the Mexican Tuberculosis Eradication Program. Dr. Gurria reported that during the past six months of 1994 the organization of the tuberculosis program in Mexico has been completed. Since the creation of the National Commission for the Eradication of Bovine Tuberculosis in January of 1994 and the allocation of a federal budget of 92.5 million dollars for the first five years in June of 1994, technical, administrative and logistic aspects of the program have been outlined and are already in place and working.

The publication of the Mexican Official Norm (NOM) in March, 1994, provided the necessary legal framework to operate and to enforce the mandatory components of the campaign.

A total of 244 veterinarians have been appointed as federal officials to perform activities relative to the bovine tuberculosis and brucellosis programs. These veterinarians will function as state coordinators, area supervisors or laboratory staff.

The Commission has the responsibility to establish eight regional laboratories with the capability to process tissues for histopathology and bacteriology. These laboratories are expected to be fully operational by the spring of 1995. Presently diagnostic work is being performed by university laboratories and the central SARH Laboratory in Tecamac, Mexico. In an effort to encourage diagnostic work at the laboratory level, the Commission has an agreement with Pronavibe (Federal Biologics Laboratory) to produce the
necessary media to isolate *Mycobacterium bovis* and *Brucella spp.*. These media will be distributed without cost to all regional laboratories. Pronabive also produces all the reagents and tuberculin necessary for the campaign. In 1994 the laboratory will produce approximately 6.5 million doses of PPD (both bovis and avium).

During the last four years Mexico has implemented an accreditation program through which it has accredited 1706 veterinarians. 448 of these veterinarians have also attended a week long epidemiology course.

One of the most significant indicators of the progress of the program is the reduction in the number of lesioned Mexican steers found at U.S. slaughter establishments. The number has dropped from 436 in FY 92 to only 182 in FY 94. In spite of this reduction, lesioned animals of Mexican origin still represent 73% of all U.S. tuberculous slaughter cases. We expect that in FY 95 the number will drop after all holstein cattle of Mexican origin that are in U.S. feedlots are slaughtered. Holstein cattle represented 40% of the lesioned cases discovered in FY 94.

From January to October 1994 1.8 million skin tests were performed by accredited veterinarians. By the end of the calendar year this number is expected to exceed 2.2 million tests.

Due to fact that the program has been in place for just a few months, we recognize that some aspects have to be reviewed and improved. Slaughter inspection and surveillance, data gathering and management are some of these elements that are now being revised. Mexico is aware that even one single case represents an unnecessary risk to the U.S. livestock industry, therefore, federal, state and cattle industry representatives have made this problem a priority that needs to be solved as quickly as possible in order to keep the export markets open for further cattle trading.

Dr. Mitchell Essey, Cattle Diseases and Surveillance Staff, presented the U.S. Status report. Dr. Essey reported that forty-two states and the Virgin Islands are Tuberculosis Accredited-Free. Virginia's Free status was suspended following the detection of an infected herd. There were 11 infected cattle herds during FY94, of which five were carried over from previous years. The six newly detected herds were: two beef herds in Virginia, one dairy herd in Puerto Rico, one dairy herd in New Mexico (El Paso milkshed) and two herds in Texas (one dairy and one beef).

Two infected herds totaling 900 cattle were depopulated, leaving 20,000 dairy cattle under quarantine and testing. For the decade, 81 (74%) herds were depopulated of 109 total infected herds found. During this same period in which 28 herds were released from quarantine, 9 (32%) previously released herds were found again infected.

There were 261 slaughter surveillance tuberculosis investigations completed of which 12 were adult cattle and 249 were feedlot cattle. Of the latter, 182 (73%) were traced to Mexico. The total number of submissions on slaughter surveillance continues at about 4,000 annually, but the num-
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ber of positives diminished to 318. This is a 50% decrease from the 1992 high of 613 cases. The decrease can be attributed to prohibiting holstein steers from Mexico (first self imposed by Mexico) and advances in Mexico's tuberculosis eradication program. U.S. regulations prohibiting importation of holstein steers and spayed heifers became effective May 13, 1994.

There were 12 M. bovis infected captive cervid herds under quarantine during the year, of which seven were carried over from previous years. The 5 newly detected cervid herds were located as follows: 2 deer herds in Vermont, one deer herd each in New York and Pennsylvania, and one elk herd in Colorado. One wild mule deer was confirmed with M. bovis. The animal was part of about 160 deer collected in a wildlife survey surrounding an infected captive elk herd in Montana. M. bovis infection was confirmed in one of 16 coyotes examined in this survey. The Uniform Methods and Rules for Tuberculosis in Cervidae became effective May 15, 1994, which act brought cervidae officially into the National program.

The National Academy of Science, Special Committee on Tuberculosis completed its evaluation of the National Bovine Tuberculosis Eradication Program. They concluded that bovine tuberculosis eradication was economically and biologically feasible and the program be vigorously pursued in cervidae and other non-traditional hoofed species. They recommended further that producers of all affected species financially support the eradication goal.

Dr. Scott Hurd, Centers for Epidemiology and Animal Health presented a Benefit/Cost and Performance Analysis of the Gamma Interferon Test for Bovine Tuberculosis. The Objectives of this analysis were to provide an estimate of the sensitivity and specificity of the gamma interferon test and to evaluate the benefit/cost considerations of using this test as an alternative to the intradermal caudal fold skin test.

The annual government costs of shifting to the gamma interferon test are estimated to be $2.6 million. Beef and dairy producers would experience a net savings of $425,000 per year. For the gamma interferon test sensitivity and specificity were set 82% and 91%, respectively. They were set at 82% and 97.5% for the caudal fold test. Data from the Tuberculosis Information Management system indicated that 11% of tests are a result of traceback and infected herd testing. These costs are born by the state and federal government. Costs increase largely due to decreases in specificity of the gamma interferon test compared to the caudal fold test. Lower specificity results in more costs of confirmatory testing, indemnification, post-mortem examination, and histopathology and cultures of culled reactors.

Dr. Don Davis, Texas A&M University, presented a report on the use of the Blood Tuberculosis Test (BTB) in deer, zoo animals and cattle. He reported that during the first four months of operation of the BTB laboratory at Texas A&M, the test was conducted on over 500 animals including 100 deer, 400 cattle and 130 zoo animals. The zoo animals included over 30
species. The specificity and sensitivity of the BTB in 153 cattle of known Mycobacterium bovis culture status was 96.6% and 91.6%, respectively. BTB results are reported as positive for M. bovis, positive for M. avium, equivocal, negative or no data. The equivocal and no data results are usually the result of poor sample collection and/or improper handling or shipping of the samples.

Very few problems have been experienced with the BTB testing during the implementation phase. The BTB has correctly diagnosed tuberculosis infected animals and has been useful in exonerating non-infected animals incorrectly characterized as reactors by the Single Cervical Test. As more data are collected in North America, minor adjustments to the BTB criteria will be implemented to account for the occurrence of cross reactions of non-pathogenic Mycobacteriums, particularly M. intracellulare.

Dr. Clarence Siroky, Montana State Veterinarian, presented the results of a wildlife tuberculosis survey in Montana. Bovine tuberculosis was discovered in a wild mule deer in association with an infected captive elk herd. This is the fifth case of spread of tuberculosis beyond a known focus of infected domestic animals to resident wildlife reported in North America.

There is concern that if this disease spills over to wildlife, a reservoir may become established that will be difficult to control and will represent a threat to wildlife and livestock health. Confirmation of the infected mule deer prompted the Montana Department of Fish, Wildlife and Parks and the Montana Department of Livestock to initiate a plan of tuberculosis control that would determine the prevalence of the disease and simultaneously reduce the possibility of intra- and inter-species spread. The plan was to establish a 4- mile perimeter surrounding the game farm as the control area and collect wildlife for testing. Approximately 120 wild deer were to be collected for testing by gross examination, histology and culture. Also, a small mammal inventory and sampling would be conducted within or very near the game farm fence. A second, and possibly third, sampling may be required based on the results of the first effort. Aerial survey revealed approximately 5.4-7.7 wild mule deer per square mile.

A total of 128 wild deer, 16 coyotes, 1 pronghorn, 1 elk, 3 porcupines, 1 rabbit, and 15 captive deer were collected in August 1994. Gross lesions consistent with tuberculosis were seen in only 1 coyote. One wild mule deer and 1 coyote had histologic lesions compatible with tuberculosis and 1 mule deer had suggestive lesions. Most culture results are pending, however, 1 coyote with no gross lesions was culture positive for Mycobacterium bovis. Arrangements have been made to depopulate the infected elk herd. Depopulation is expected to be complete in November 1994.

Dr. Dennis Thompson presented the following report of the TB Scientific Advisory Subcommittee's evaluation of the IDEXX Gamma Interferon Test:
Last year the TBSAS was asked to make recommendations about the IDEXX Gamma Interferon Test (IFN) using a bovine to avian ratio of 1.8 as the cutoff point between positive and negative. This year we've been asked to evaluate an IDEXX Gamma Interferon Test protocol that uses new screening calculations prior to computing the ratio, and then uses a 1.25 bovine to avian ratio as the cutoff point. All of the questions and answers which refer to the IFN test in this report pertain only to the 1994 version using the new calculations and cutoff of 1.25.

1. Given the results of the re-evaluation of the Gamma Interferon (INF) test in the Hillcrest herd as well as over 20,000 animals from free and low prevalence herds, does the INF test provide sensitivity and specificity equal to the caudal fold (CF) test?

**ANSWER** - The TBSAS has concluded that the IDEXX Gamma Interferon Test (IFN) is not equivalent to the caudal fold test (CFT). Specificity of the IFN test is lower than the caudal fold test. Data sets and information used to determine specificity were far ranging. They included: 1430 cattle in 7 herds in the continental United States - IFN= 86.0% specificity, CFT= 98.7% specificity; 1452 cattle in 2 herds in Puerto Rico - IFN= 87.3%, CFT= 83.7%; pooled data of above U.S. and Puerto Rico data sets- IFN= 86.7%, CFT= 91.2%; 2005 cattle in Maine tested by IDEXX - IFN = 95.21%, CFT not done; 10 herds with low prevalence in Texas- IFN= 86.2%, CFT= 97.6%. Table 3 from the report prepared by the Center For Epidemiology and Animal Health shows estimated specificities in ten Texas herds. Table 7 is from a report by IDEXX submitted to us in October 1994. Table 7 shows the number of positive cattle actually classified in those same ten herds by the caudal fold test and the IFN test. The number of positives actually classified by each test in those ten herds was 346 by the CFT; and 2654 by the IFN, indicating performance of the IFN in low prevalence herds as compared to the caudal fold test.

IDEXX claims that the IFN test has specificity of 91.35%. Information that supports specificity of the caudal fold test being higher than the IFN test includes:

**A.** A report from the National Research Council entitled
"Evaluation of the Cooperative State-Federal Bovine Tuberculosis Eradication Program" states that "the specificity of the caudal fold test can be estimated to be greater than 98 percent".

B. Annual reports by USDA summarize all tuberculin tests in the United States and repeatedly demonstrate specificity of 98% or higher.

C. A recent test of 35,300 dairy cattle in New Mexico demonstrated a specificity of 96.0%.

Sensitivity of the Gamma Interferon test (IFN) may be equal to the caudal fold test. However, this is supported by data from one herd and a small number of other infected cattle. Available data are not sufficient to verify that similar results would be obtained in other infected cattle populations.

2. Does the INF test provide equivalent performance to the CF test?

ANSWER - No, the IFN test does not provide equivalent performance to the caudal fold test. The specificity is lower and sensitivity has been determined in only one herd and a few other infected cattle.

3. Should the INF test be approved as a presumptive diagnostic test, equivalent to the CF test, for testing: a. Herds of unknown status?, b. High risk herds?, c. Infected herds where the prevalence of disease is: (1). High? (2). Low?

ANSWER - a. No, the IFN test should not be used as a presumptive (stand alone) diagnostic test for reasons described in answers to questions number 1 and 2 above. b. No c. No

4. Should the INF test be approved as a supplemental diagnostic test, equivalent to the comparative cervical (CC) test?

ANSWER - Limited data are available indicating that the INF test may be used as a test that is equivalent to the comparative cervical test (CCT). More data on test performance are required to more adequately respond to this question.

5. Should the INF test be approved as a supplemental diagnostic test, to be used in concert (in parallel) with other diagnostic tests?

ANSWER - Yes, the TBSAS recommends approval of the INF test as an ancillary test for use in parallel at the 1.8 cutoff with the caudal fold test or the single cervical test. In herds of unknown status, approval is also recommended for use with the new screening calculations and 1.25 cutoff in parallel with the comparative cervical test. Such use will be especially appropriate when increased sensitivity is desired and a lower specificity can be tolerated.

6. Are there more appropriate methods of calculation and bovis/avium ratios for the test, depending on the category of cattle being tested? Should there be different cut-off points for any or all of the following categories of cattle: a. cattle of unknown status? b. high risk cattle?
c. cattle in infected herds where the disease prevalence is (1) low; (2) high?
ANSWER - There have not been sufficient data presented to enable the Scientific Advisory Subcommittee to address this question.

7. Provide recommendations as to the appropriate uses of the INF test in the national tuberculosis eradication program.
ANSWER - It is clear that more data need to be developed to justify approval of the IFN test as a stand alone test. It is also clear that industry has a desire for a convenient, scientifically sound test. Therefore the TBSAS expresses the following recommendations, concerns and comments.

Specificity should be determined by using the test in series with the comparative cervical test in herds of unknown and known negative status. Sensitivity should be determined by use in parallel with the single cervical or caudal fold test in several infected herds having varying prevalence.

The test kit for the IFN has no provision for a positive control for each sample in order to assure that lymphocytes are capable of producing gamma interferon when stimulated. Without the inclusion of appropriate positive controls, false-negative results are possible. The TBSAS is concerned that there is no current mechanism to distinguish between negative samples and those samples unsuitable for analysis.

The TBSAS is concerned about the influence of recent, prior tuberculin injections on performance of the IFN. Until data validate the use of IFN test in such cattle, the TBSAS can not recommend its use.

The TBSAS has concerns about the effects of stress on IFN test performance. Little data exist regarding the effects of stress on IFN test sensitivity, but available information and statements by IDEXX officials indicate that a significant negative impact occurs. Therefore, the IFN test should be evaluated using samples from a large number of cattle subjected to various types of stress found in normal market conditions and report the results.

The TBSAS has requested additional data regarding performance of the test in stressed cattle, especially its sensitivity. A resolution in 1992 and the TBSAS reply to question number 2 last year both expressed such a need. Yet, only one study of 34 cattle containing only one confirmed infected dairy animal has been reported.

Requests for additional data regarding sensitivity in more infected cattle were made by the TBSAS last year. Very little new data have been submitted to the TBSAS.
Mr. Robert Frost, Research Committee, International Llama Association, presented an update on llama research projects. The International llama association has been pro-active in its cooperative efforts with governments, states, and provinces to find a diagnostic test for bovine tuberculosis in llamas and alpacas. These domestic livestock species belong to the camelid family and have blood characteristics, immune system responses, and behavioral patterns different from other North American livestock species. These differences in blood characteristics and immune responses have mandated that species-specific research be carried out to insure that diagnostic tests are sensitive and specific to llamas and alpacas. Skin test studies since 1988 have led to a major study going on now in a cooperative agreement effort between USDA and Argentina's INTA (National Institute of Agricultural Technology) in Argentina. This study's primary objective is to evaluate different intradermal tuberculin testing procedures for the diagnosis of tuberculosis infection in llamas. In addition to exhausting the skin sites, the evaluation of hematological and serological tests for the diagnosis of bovine tuberculosis in llamas and other camelids is being carried out. The Pan American Health Organization and the World Health Organization under Dr. Isabel Kantor will do the first ELISA work, and then USDA, Ag Canada, and United States institutions will continue with various serologic testing procedures. Results of these studies will be forthcoming in 1995. Currently the axillary site for PPD tuberculin skin testing is the USDA recommended site. To date, New York and many other states have used the axillary site with no reactors. There is no evidence that any herds of llamas or alpacas in North America have bovine tuberculosis. The "Assessment of Risk Factors for M. bovis in the U.S.", published in 1992 by USDA-APHIS-VS states, the "current evidence indicates that camelids have not been a factor in the spread of M. bovis."

The International Llama Association wished to express its appreciation of all the above parties. We especially appreciate APHIS and NVSL for its latest efforts at Ames to utilize llamas used in the recent brucellosis study for an additional study concerning diagnostic test possibilities in bovine tuberculosis. This effort, now underway, will certainly compliment the major cooperative effort being undertaken in Argentina.

Mr. Frost also reported on a model regulation developed by the llama industry. This model was endorsed by the Infectious Diseases of Cattle, Bison and Llamas Committee. Currently, 29 states require tuberculin testing for interstate transportation of llamas. The draft model regulation recognizes that new information is at hand for the antemortem detection of M. bovis and indicates that states should use USDA recommended protocols.

Dr. Joe Templeton, Texas A&M University, discussed natural disease resistance of cattle, bison and deer to tuberculosis. He reported that natural disease resistance is found in all these species and work being done at Texas A&M is identifying mechanisms to determine disease resistance.
Dr. Dan Baca, Texas Animal Health Commission, presented an update and progress report on the tuberculosis situation in Texas. He reported that FY94 began with 4 dairies remaining under quarantine in the El Paso milkshed. Eradication efforts in affected herds continued through 1994. One herd is scheduled for quarantine release test in December. Surveillance tests on two herds released from quarantine in FY93 and two area herds have not discovered any evidence of infection.

Bovine tuberculosis was confirmed in a large dairy located in Comanche county in early 1994, following a successful slaughter traceback. Although initial tuberculin test results indicated a high prevalence of infection (118 reactors out of 759 cattle tested), only three animals were confirmed with disease on postmortem. Five follow-up herd tests have not detected additional cases of infection.

A beef herd in Uvalde county was detected with tuberculosis in December 1993 following a trace from slaughter. The initial herd test identified 52 reactors among 193 head tested. Disease was confirmed in 67% of reactors at slaughter. Depopulation was completed in January 1994.

Extensive epidemiological investigations associated with both newly detected herds has not resulted in defining a source of infection nor evidence of spread.

An elk herd in northeast Texas was found to be infected with tuberculosis in FY93, as a result of an epidemiological trace from Oklahoma, still remains under quarantine.

Bovine tuberculosis was confirmed at a zoo in north Texas in February 1994. Routine tests conducted on two animals for shipment to another zoo led to confirmation of disease in one animal. Additional surveillance on other animals in the index and adjacent exhibits has resulted in confirmed cases in several species, including pygmy goats, sitatunga, gerenuk, and nilelechwe. Epidemiological evidence indicates the current outbreak is related to an earlier episode in 1985. All affected exhibits were voluntarily depopulated. Additional surveillance testing is currently in progress in other hoofstock exhibits.

At the end of FY94, four dairies in the El Paso milkshed, one dairy in Comanche county and one elk herd in northeast Texas remain under quarantine.

Mr. Brad Bouma, a dairy producer from the El Paso milkshed gave an excellent presentation on a producers perspective of tuberculosis in the El Paso milkshed. Mr. Bouma has two tuberculosis quarantined herds that milk 3,000 cows, a high risk herd of 300 milking cows and an Accredited-Free herd of 2,800 milking cows. He employs approximately 100 people.

There are approximately 150,000 Mexican steers crossed into the U.S. in the local area each year. Additionally, dairies in the Juarez, Mexico area have approximately 20,000 dairy cattle and are only a few miles away across the river. He indicated that cattle can and do stray across the river.

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Two of his herds have been released from quarantine and then found to be still infected through several cycles.

He feels that the caudal fold test is not quantifiable and cross reacts with other Mycobacteria organisms. Purchased additions comprise 10% of his herds, but represent 25 - 40% of the reactors. Only 13 lesioned animals have been found out of 495 reactors and 3338 animals slaughtered.

The economic impact of the tuberculosis program on an infected dairy is great. Indemnity rates only represents 60% of the value of the animal. "S" branding of steers and culled cows also reduces the value of the animals at the market place. Depopulation of a large dairy herd would most likely result in bankruptcy of the owner.

Mr. Bouma estimates that his quarantined herds contribute 30 million dollars to the local economy and his four herds provide 150 primary jobs and at least 200 secondary jobs.

Mr. Bouma points out a number of problems with the tuberculosis program from his perspective. He feels that USDA enforces the parts of the programs that are convenient and ignores the parts that require effort or funds. The bureaucracy is inefficient. No one seems to know who is in charge or who can make decisions. Veterinarians and departments often blame each other and refuse to take responsibility. Regional epidemiologist has only visited once, yet he second guesses field decisions and makes recommendations without ever being on the site. USDA points to the 7 Rivers Feedlot in Carlsbad, New Mexico as a possible source of tuberculosis, yet refuses to follow up on 7 Rivers and trace back the other cattle that were fed there and refuse to do research on other possible vectors. There are major communications break-downs between region, Hyattsville and NVSL.

He also believes there are a number of possibilities for solving the disease problem. A national position should be created for a border epidemiologist who could operate in Texas, New Mexico and Mexico and who could work with the Bi-National Committee. Treat the problem as an area problem not a Texas or New Mexico or Mexico problem. Create a statistician position to compile data, analyze the data and search for trends. Research for local vectors and other means of transmission. Get involved in new research. Use funds for research instead of indemnity. Re-analyze the rules and funds available and use them in the most efficient manner. Become pro-active.

Dr. Hector Campos presented a report on the activities of the Bi-National Committee on Tuberculosis Eradication Efforts in Mexico. The USAHA during its November 1992 meeting recommended that a comprehensive Mexico/U.S. tuberculosis eradication and control program be established as well as a Joint Mexico-U.S. Tuberculosis Committee to provide oversight. The first meeting of the Mexico-U.S. Committee for Cooperation on Bovine Tuberculosis was held in Denver, Colorado on August 5th, 1993,
THE COMMITTEE ON TUBERCULOSIS during the meeting of the NCA.

Since that time five other meetings have been held, one in Mexico and four in the U.S.

The joint committee is made up as follows: two regulatory veterinarians from each country; three industry representatives from each country; one research representative from each country; one representative of veterinarians of border states from each country. It was recommended that the industry representatives include one beef producer, one dairy producer and one general farm representative. The Co-Chairmen of the Committee are Dr. Donald Luchsinger, Deputy Administrator of APHIS and myself as Director General of Animal Health in Mexico.

The primary focus for the meetings has been to work through issues that can assist Mexico in development and implementation of its national tuberculosis eradication program. A progress report of the Mexican program has been presented at each meeting.

At this time, a National Commission has been established in Mexico for the Eradication of Bovine Tuberculosis and Brucellosis. $92 million of federal funds will be spent in this Commission over the next five years, besides the funds provided by State Governments and cattlemen.

A total of 241 federal veterinarians were hired during 1994 for supervision of program activities throughout the country. A total of 1492 veterinarians are accredited for tuberculosis and brucellosis.

The state of Sonora is in the eradication phase and another five states have active testing programs underway. All other states in Mexico are preparing to begin state wide testing.

Examples of issues considered by the joint committee are testing procedures, slaughter surveillance, and laboratory establishment and support. Training has been held on various program components, including post-mortem procedures, comparative cervical testing, epidemiology and traceback. There has also been an effort to make the Mexican TB regulations equivalent to the Uniform Methods and Rules of the Bovine Tuberculosis Program in the U.S. and to establish the criteria by which the Mexican states would be considered equivalent to the U.S. Modified Accredited states.

Research activities have also been discussed and carried out between U.S. and Mexican scientists. A recent project was completed to compare the sensitivities of the U.S and Mexican tuberculins.

A key issue for discussion has been the import regulations of the U.S. for cattle from Mexico. Also, an important issue has been the monitoring of some state programs in Mexico by U.S. officials.

The joint committee provides a forum for the U.S. and Mexico to actively interact and address important issues that appear to be hampering TB eradication progress in Mexico.

The interest and leadership shown by industry representatives from both countries are very much appreciated and are considered key to the
success of this effort.

Dr. Terry Beals, Texas Animal Health Commission, presented an overview of the Texas Strategic Plan for Cattle and Cervidae Tuberculosis. The plan was developed as a result of increased levels of concern generated by TB cases in Mexican cattle and captive non-traditional livestock along with the continuing difficulty with endemic low grade infection in the El Paso milkshed.

Political, industry and public concerns created a level of anxiety throughout the state that drove seven resource agencies and entities in Texas to see the need for a total plan to manage tuberculosis in cattle and deer in Texas.

The plan included background, historical and other basic information. It develops the areas in need of better technology, of research and defines proposals to generate needed data along with creative and innovative new technology.

The plan was circulated widely to industry, regulatory, political and other groups for input and is envisioned as a dynamic plan.

The proposed budget request from the legislature is approximately $6 million. Over half of these funds would be dedicated to research needs with the majority of the balance for improved regulatory program delivery.

Dr. Mitchell Essey presented a report on the status of six proposed amendments to the CFR affecting the tuberculosis program: The proposed rule that cattle from Mexico moving interstate be accompanied by a health certificate listing official identification and other vital information was published November 1993, and the comment period extended to February 1994. Action on this rule is still pending; Proposed rule that would add spayed heifers and breeding cattle to the "M" brand requirement for cattle from Mexico was published November 1993 and withdrawn in August 1994. A new proposal was published August 1994 that would delete breeding cattle from the amendment and require a legible, distinct, permanent "mark" placed on the right hip instead of a hot iron brand placed on the right jaw. The comment period closed October 24, 1994 and the final rule is pending; Proposed rule to recognize only Mexico's blue metal eartag marked SARH CNG in cattle imported from Mexico became final December 1993; Interim rule prohibiting Holstein steers and spayed heifers from being imported into the U.S. from Mexico became effective May 13, 1994; Interim rule prohibiting Bush tail possum and hedgehogs from New Zealand became effective May 31, 1994; Proposed rule providing post-entry restrictions on steers and spayed heifers from Mexico and recognizing Mexican states achieving modified-accredited equivalency and providing for restricted cattle status was published May 1994, and the comment period closed September 1994. Final action is pending.

Dr. Richard Breitmeyer, State Veterinarian for California, presented an overview of the Consensus Document from the State Veterinarians of the
Border States of Arizona, California, New Mexico and Texas. This proposal, which was developed after the last USAHA meeting and which has been presented as comment on the proposed rule for the importation of Mexican cattle proposes to establish in the rules for the importation of Mexican cattle for three program phases. These phases are Control, Eradication and Free. Entry requirements would be decreased as Mexican states progress through these phases. This consensus document provides incentive for Mexican producers to eradicate tuberculosis.

Dr. Garry Adams, Texas A&M University, presented an overview of a proposed USDA TB Research Initiative. This initiative would require $4 million dollars in funding each year for five years for research on diagnostic tests, development and evaluation of improved vaccines, disease processes, natural resistance, epidemiologic and economic modeling.

The Committee extensively discussed a number of these topics, made six recommendations and approved three resolutions.

The following recommendations were made by the Committee to USDA:

I. Evaluate the Blood Tuberculosis test (BTB) for use as a confirmatory diagnostic test after caudal fold tuberculin tests. This test was developed for diagnosis of bovine tuberculosis in deer. One hundred fifty three cattle with known Mycobacteria bovis status have been tested as part of Phase I of a study for use in cattle.

II. Change the Uniform Rules and Methods for Cervids as follows:

Part III- Herd Status Plans (on Page 14 - 1st paragraph)

A. Accredited Herd Plan for Cervidae

Currently reads -

1. Animals To Be Tested - Testing of herds for accreditation or reaccreditation shall include all Cervidae and all other hoof stock over 6 months of age that are not natural additions.

Change to -

1. Animals To Be Tested - Testing of herds for accreditation or reaccreditation shall include all Cervidae over 12 months of age and all animals under 6 months of age that are not natural additions.

III. Adopt the following to facilitate tracebacks of Cervidae. These paragraphs are similar to paragraphs in the Bovine Tuberculosis Uniform Methods and Rules and are recommended for insertion into the Uniform Rules and Methods for Cervidae.
Definitions:

Dealer: Any person engaged in the business of buying or selling Cervidae in commerce either on his or her own account or as the employee or agent of the vendor and/or purchaser, or any person engaged in the business of buying or selling Cervidae in commerce on a commission basis. The term shall not include a person who (1) buys Cervidae as part of his or her own bona fide Cervidae production operation; (2) is not engaged in negotiating the transfer of Cervidae; or (3) receives Cervidae exclusively for immediate slaughter on his or her premises.

Dealer Registration and Record Keeping: Any dealer who purchases, deals in, or sells Cervidae; or who acts as a commission representative or broker; or who operates and conducts an auction where Cervidae are sold must be registered or licensed with the appropriate State agency and maintain required records that will facilitate traceback of affected, exposed, or reactor animals by State authorities to the herd of origin or other point of original infection.

1. Dealer Registration - The State agency shall have authority, after due notice and opportunity for hearing to the individual or firm involved, to deny an application for registration and to suspend or cancel the registration when the agency is satisfied that one or more of the following conditions prevail: a. there is adequate evidence to establish intent to violate or circumvent record keeping requirements of this section and/or other animal health regulations. b. there is a demonstrated history of repeated inability to trace to the point of origin those affected, exposed or reactor animals handled by the dealer.

2. Records Required - Each registered or licensed person, firm or corporation shall keep sufficient records, for a minimum of 2 years, of all animals purchased for resale to enable the State agency to trace such animals satisfactorily to their herd of origin.

3. Violation Remedies - Provision shall exist for State animal health officials to institute such action at law or in equity as may appear necessary to enforce compliance with any provision of the recommended procedures discussed here. This shall include the authority for the appropriate state officials to petition the local court having venue for an order to enforce such subpoenas.

A brand law or regulation that accomplishes the traceback purposes of this section will be considered an acceptable alternative. Acceptance of this alternative will be based on an overall review of the provisions and accomplishments of the State program in achieving the effective traceback of all animals to the herd of origin, or other point of original infection.

IV. Add the following paragraph to the Uniform Methods and Rules for Cervidae.

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A newly assembled herd shall assume the herd status of the herd from which the animals originated. If the herd is assembled from more than one herd it shall assume the status of the originating herd which is lesser. A newly assembled herd shall also assume the testing schedule of the herd which status it assumes. These animals must have no exposure to Cervidae from herds of lesser status than the herd of origin which is determining the status of the newly assembled herd.

V. Amend 9 CFR, Ch. 1, Part 50 to add Cervidae to the existing indemnity program for tuberculosis in cattle and bison.

VI. Change the Uniform Methods and Rules for Cervidae as described below.

A. Reference Part II, Section D - Allow veterinarians employed on a part time basis by State or Federal Governments to administer the single cervical test. Delete the current requirement for veterinarians to be employed by governments "in full-time capacity".

B. Reference Part II, Section K - Require reactors to be branded with a "T" brand on the left hip. Delete the requirement to brand reactors with a "T" on the jaw.

C. Reference Part II, Section L, paragraph 1 - Delete language which permits "S" brands to be on the left jaw. Require "S" brands to be on the left hip only, and require hot brands.

D. Reference Part III, Section A - Change the length of the intervals allowed to conduct the second and third herd tests for establishing Accredited Herd status. The interval should be changed from 10 to 14 month intervals to 9 to 15 month intervals. This is greatly needed to adjust to breeding seasons and take advantage of times when male Cervids lose their racks.

E. Reference Part III, Section A, paragraph 4- Change the interval allowed for testing to reaccredit herds. An interval of 21 to 27 months is needed for reasons stated in E. immediately above.

F. Reference Appendix 3 - Make changes necessary to adequately reflect the changes of the interval for testing i.e. 9 to 15 month intervals are substituted for 10 to 14 month intervals.

The Committee approved resolutions on the following topics:

1. Request for USDA to adopt the recommendations included in the Border States Consensus Document on the importation of Mexican cattle.

2. Use of the IDEXX Gamma Interferon test.

3. Define and prescribe duties of TB Designated Epidemiologist.
OVERVIEW AND PROGRESS REPORT ON TUBERCULOSIS RESEARCH

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Programs for control and eradication of bovine tuberculosis in the United States were initiated in 1917. These programs reduced the prevalence of bovine tuberculosis, which is caused by Mycobacterium bovis, from 5% in 1918 to less than 0.02% in 1994. Despite the success of tuberculosis eradication programs, prevalence of disease has increased in recent years for several reasons. Importation of cattle from Mexico, where prevalence of bovine tuberculosis is high, has led to an increase in the number of infected animals detected at slaughter in the United States. Bovine tuberculosis is also present in several large dairy herds in the El Paso Milkshed. Eradication of tuberculosis from these herds has proven difficult because of insufficient funds to depopulate these herds and the inability to identify all infected cattle. In addition, tuberculosis caused by M. bovis has been diagnosed in several farmed deer and elk herds, and transmission of M. bovis from deer to dairy cattle raised on the same farm has occurred. Tuberculosis in llama, bison, and captive wild animals has also caused concern within the livestock industry because of the potential for transmission of disease from these animals to cattle.

Although bovine tuberculosis has nearly been eradicated from the United States, finishing this task, with current fiscal constraints, will require better use of existing diagnostic tests and development of improved tests. Research on bovine tuberculosis was discontinued many years ago with the expectation that the disease would be eradicated and research would no longer be needed. However, research is needed to develop a better understanding of the pathogenesis of tuberculosis in animals so that improved tests and control strategies can be developed.

Bovine tuberculosis research programs were initiated by the United States Department of Agriculture and several universities in 1992. Scientists met in August, 1992, at the First Workshop on Diagnostic Techniques for Bovine Tuberculosis to discuss and identify research priorities. Thirty-three scientists representing 4 countries, and 12 universities and federal agencies participated in the Workshop, which was held at the National Veterinary Services Laboratories, Ames, Iowa.

In June, 1994, the Second Workshop on Diagnostic Techniques for Bovine Tuberculosis was held at the National Animal Disease Center, Ames, Iowa, for the purpose of reporting results of research conducted since 1992. There were 64 participants from 7 countries representing 22 universities and government agencies. Twenty research papers were presented during
the one-and-a-half day meeting. Most of the reports focused on evaluation of existing diagnostic tests and molecular epidemiology of \( M. bovis \) isolates.

Current research efforts on bovine tuberculosis are directed toward accomplishing the following generalized objectives:

1. Evaluate tests for diagnosis of tuberculosis in cattle, deer, elk, and llama.
2. Develop improved diagnostic tests.
3. Determine molecular mechanisms of \( M. bovis \) infection.
4. Develop methods for differentiation of \( M. bovis \) strains.
5. Develop vaccines to protect against \( M. bovis \) infection.

Specific objectives for research being conducted at each of four universities and two federal agencies in the United States are as follows:

**USDA, ARS, National Animal Disease Center, Ames, IA**

1. Comparison of the sensitivity of the caudal fold skin test and a commercial gamma-interferon assay for diagnosis of bovine tuberculosis.
2. Analysis of tuberculins used in the United States, Canada, and Mexico.
4. Diagnosis and pathogenesis of \( M. bovis \) infection in elk.
5. Pathogenesis of \( M. bovis \) infection in cattle.
6. Immunohistochemical detection of \( M. bovis \) in formalin-fixed paraffin-embedded tissues.
7. Restriction fragment length polymorphism analysis of diverse \( M. bovis \) isolates.

**USDA, APHIS, National Veterinary Services Laboratories, Ames, IA**

1. Comparison of the NVSL (US) and Pronabive (Mexico) methods for production of PPD tuberculins.
2. Use of Microbial Identification (MIDI) system to identify \( M. bovis \) and determine subspecies differences.
3. Diagnosis of \( M. bovis \) infection in llamas.

**Texas A&M University, College Station, TX**

1. Genetics of macrophage function and natural disease resistance against tuberculosis in cattle.
2. The role of macrophage function in natural disease resistance against bovine tuberculosis.
4. Use of T-cell clones to identify protective immunogens of \( M. bovis \).
5. DNA species-specific probes for the diagnosis of brucellosis and tuberculosis.

**Cornell University, Ithaca, NY**
1. Develop DNA probes for use in diagnosis and molecular epidemiology.
2. Develop specific monoclonal antibodies for use in diagnostic tests.
3. Evaluate production of tumor necrosis factor as an indicator of cell-mediated immune response.
4. Develop improved methods for isolation of *M. bovis*.

**Iowa State University, Ames, IA**
1. Use of ELISA for diagnosis of tuberculosis in anergic animals.

**Colorado State University, Ft. Collins, CO**
1. Field evaluation of a five antigen panel ELISA system for the serological diagnosis of *M. bovis* in animal species.
2. Analysis of culture filtrate proteins from *M. bovis* field isolates.
3. Fingerprinting of *M. bovis* by ERIC-PCR.
4. Detection of *M. bovis* in tissue samples by use of PCR and DNA probes.

Bovine tuberculosis research is also being conducted in Mexico and Canada as well as many other countries around the world. Scientists are working in collaboration with each other to maximize use of limited resources and avoid unnecessary duplication of effort.

An International Symposium on Bovine Tuberculosis in Animals and Human Beings will be held May 8-11, 1995, at the University of Maryland, College Park, MD. The Symposium is sponsored by the U.S. Department of Agriculture, Centers for Disease Control and Prevention, National Institutes of Health, and the Agricultural Ministries of Australia, Canada, Mexico, and New Zealand. The purpose of the Symposium is to bring together scientists from around the world to communicate the most recent findings of bovine tuberculosis research.
REPORT OF THE COMMITTEE ON WILDLIFE DISEASES

Chairman: Dr. Victor F. Nettles, Athens, GA
Vice Chairman: Mr. Scott Petty, Jr., San Antonio, TX

W.L. Adams, GA; W.B. Amand, PA;*R.D. Angus, IA;*J.W. Armstrong, NV;*D.L. Baca, TX; C.S. Becvar, IA; W.M. Boyce, CA; W.W. Buisch, NY;*C.S. Card, PA;*D.R. Cassidy, IA;*L.M. Coffman, OR;*D.S. Davis, TX; B.J. Edmundson, WA;*M.A. Essey, MD; J.B. Finley, TX;*J.R. Fischer, GA;*B.R. Fox, MD;*R. Frost, CA; M.J. Gilsdorf, MD; C.A. Gipson, FL;*H. Hilderbran, TX; B.R. Hillman, ID; P.D. Hoctor, IN;*S.D. Holland, SD;*D.L. Hunter, ID;*S.B.S. Hurley, WI; D.A. Jessup, CA; D.C. Johnson, GA; D. Joyce, ND; W.E. Ketter, MD; P. Lies, ND;*C.W.S. Lum, HI; C.J. Mare, AZ; C. Massengill, MO; H.A. McDaniel, MD;*R.W. Mead, WA;*R.R Meyer, CO;*M.W. Miller, CO; J. Nehay, CA;*J.J. Rais, ID;*W.A. Rotenberger, ND;*M.S. Silberman, GA;*C.J. Siroky, MT; J.S. Smith, AZ;*D.E. Stallknecht, GA; C.D. Stumpff, KS;*A.B. Thiermann, MD;*C.O. Thoen, IA;*E.T. Thorne, WY;*J.K. Veatch, KS; R.D. Whiting, MD;*D. Whittlesey, CO; E.S. Williams, WY;*S. Wolcott, CO; J.M. Woodall, OK.(*denotes present)

The Committee on Wildlife Diseases met at 1:30 PM on Monday, October 31, 1994. A wide variety of wildlife health issues were discussed, some of which were Old Business along with several items of New Business. Summaries of reports to the Committee and the Committee’s recommended actions are as follows:

1. Report on the Greater Yellowstone Interagency Brucellosis Committee and National Symposium in the Greater Yellowstone Area

In 1993, Dr. Bob Hillman, Idaho Bureau of Animal Health, and Dr. Dave Hunter, Idaho Department of Fish and Game and Bureau of Animal Health, reported on efforts led by Wyoming Governor’s Task Force on Brucellosis to establish a 3-state, multiagency task force to address the important issue of brucellosis in elk and bison of the Greater Yellowstone Area (GYA). Considerable progress in this effort has been made, and Dr. Tom Thorne, Wyoming Game and Fish Department, reported on formation and the inaugural meeting of the Greater Yellowstone Interagency Brucellosis Committee.

in January 1994. At this meeting, all agencies agreed on the need for a cooperative approach to solving the brucellosis problem in the GYA and called for establishing the Greater Yellowstone Interagency Brucellosis Committee (GYIBC) along with Technical and Information and Education Subcommittees. Participants in this meeting accomplished the following: (1) they endorsed preparation of a memorandum of understanding creating the GYIBC, which has been signed by all three Governors and is now being considered by the Secretaries of Interior and Agriculture; (2) they appointed members to the executive committee and subcommittees; (3) they endorsed holding a National Symposium on Brucellosis in the GYA in September 1994 and formulated a draft agenda for the symposium; and (4) they adopted Goal, Mission, and Objectives statements for the GYIBC as follows:

I. It is the GOAL of the Greater Yellowstone Interagency Brucellosis Committee to protect and sustain the existing free-ranging elk and bison populations in the Greater Yellowstone Area (GYA) and protect the public interests and economic viability of the livestock industry in the three states.

II. Toward this end it is the MISSION of this Committee to facilitate the development and implementation of brucellosis management plans for elk and bison in the GYA.

III. This will be accomplished by subscribing to the following management OBJECTIVES which will, in turn, guide the Committee:

1. Recognize and maintain existing State and Federal jurisdictional authority for elk, bison, and livestock in the GYA;
2. Maintain numerically, biologically, and genetically viable elk and/or bison populations in the respective States, National Parks, and Wildlife Refuges;
3. Maintain the brucellosis free status of Wyoming, Montana, and Idaho, and protect the ability of producers in the respective States to freely market livestock;
4. Eliminate brucellosis-related risks to public health; and
5. Eliminate the potential transmission of *Brucella abortus* among elk, bison, and livestock;
6. Coordinate brucellosis-related management activities among all affected agencies;
7. Base brucellosis-related management recommendations on defensible and factual information while encouraging and integrating new advances and technology;
8. Aggressively seek public involvement in the decision making process;
9. Communicate to the public factual information about the need to prevent the transmission of brucellosis, the need for its eradication,
and the rationale for related agency management actions; and
10. Plan for elimination of *Brucella abortus* from the GYA by the year 2010.

The National Symposium on Brucellosis in the GYA was sponsored by the GYIBC, partially funded by the Wyoming Game and Fish Department and National Park Service, and co-hosted by Governors Sullivan, Andrus, and Racicot and Secretaries Babbit and Espy. Approximately 350 people attended the symposium, including all three Governors, representatives of the Secretaries of Interior and Agriculture and all the member agencies of the GYIBC, numerous news media representatives, and interested members of the public. Thirty-four invited papers made up sessions on Human Dimensions of Brucellosis in the GYA; Brucellosis From State and National Perspectives; Cattle, Elk, Bison, and Brucellosis of the Greater Yellowstone Area; Brucellosis-the Disease; Brucellosis in the Greater Yellowstone Area; Brucellosis From Federal Agency Perspectives; and Brucellosis Eradication and Future Needs.

The day after the symposium, the GYIBC held its inaugural meeting. The status of the Memorandum of Understanding was discussed, and charges for the Technical and Information and Education Subcommittees were adopted. It was agreed that Agricultural Research Service and National Biological Survey would be invited to participate on the GYIBC as nonvoting members. A position statement discouraging wildlife feedgrounds in the GYA was adopted, and several work assignments were given to the Technical and Information and Education Subcommittees.

Recommended Action: None required. The Committee was pleased with the progress of the multi-agency approach to this difficult problem.

2. Surveillance for TB in Montana Wildlife

Dr. Clarence Siroky, Montana State Veterinarian, gave a presentation on the discovery of bovine tuberculosis (TB) in a wild mule deer in association with an infected captive elk herd. The spread of TB beyond a known focus of infected domestic animals to resident wildlife has been reported on five different occasions in North America, with the latest being the finding of an infected wild mule deer adjacent to an infected captive elk herd near Hardin, Montana. Concern has been expressed that if this disease spills over into wildlife, a reservoir may become established that will be difficult to control and will represent a threat to wildlife and livestock health.

Confirmation of the infected mule deer prompted the Montana Department of Fish, Wildlife and Parks and the Montana Department of Livestock to initiate a plan of TB control that would determine the prevalence of the disease and simultaneously reduce the possibility of intra- and inter-species spread. The plan was to establish a 4-mile perimeter surrounding the
game farm as the control area and collect wildlife for testing. Approximately 120 wild deer were to be collected for testing by gross examination, histology, and culture. Also, small mammal inventory and sampling would be conducted within or very near the game farm fence. A second, and possibly third, sampling may be required based on the results of the first effort. Aerial survey revealed approximately 5.4-7.7 wild mule deer per square mile.

A total of 128 wild deer, 16 coyotes, 1 pronghorn, 1 elk, 3 porcupines, 1 rabbit, and 15 captive deer were collected in August 1994. Gross lesions consistent with TB were seen in only 1 coyote. One wild mule deer and 1 coyote had histologic lesions compatible with TB and 1 mule deer had suggestive lesions. Most culture results are pending; however, Dr. Siroky announced that 1 coyote with no gross lesions was culture positive for *Mycobacterium bovis*.

Dr. Siroky also stated that arrangements have been made to depopulate the infected elk herd. Animals will be replaced by captive elk donated by the Game Breeders Association of Montana. This was applauded by several members of the Committee who were concerned by the fact that numerous wild animals were being killed for TB surveillance while a known infected herd had been kept alive.

Recommended Action: None required: The Committee commended the State of Montana and APHIS, USDA, for acting promptly to survey the wildlife affected by the infected game farm.

3. Adenovirus in California Black-tailed Deer

Dr. Leslie Woods reported on the discovery of a severe systemic vasculitis in black-tailed deer that has been associated with an adenovirus. Deer mortality was first noted in Northern California in mid-1993 and was characterized by bloody diarrhea, mucosal and cardiac hemorrhage, and pulmonary edema. Large numbers of deer were reported dead in the summer of 1993, and the syndrome was seen again on a smaller scale in 1994 in a rehabilitation facility. Attempts to isolate bluetongue virus (BTV) or epizootic hemorrhagic disease virus (EHDV) have been unsuccessful. Large intranuclear inclusion bodies have been seen in endothelial cells of damaged vessels of affected animals. Electron microscopy has demonstrated adenovirus particles in these inclusion bodies. An adenovirus has been isolated through use of a black-tailed deer pulmonary artery endothelial cell line, and fluorescent antibody testing has shown the virus to react with a bovine adenovirus type 5 conjugate. Tissues from affected deer have been negative for EHDV, but some have been positive for BTV on polymerase chain reaction tests. A retrospective search of deer cases revealed an adenovirus-affected deer that was examined in 1987. Dr. Woods indicated that inoculation trials are currently underway with penned deer.
Recommended Action: The Committee was concerned to hear about this new disease syndrome that is associated with an adenovirus. Our foremost recommendation is that all animal health regulatory agencies and fish and wildlife agencies should be apprised of this potential new deer disease and that they take appropriate actions to avoid the spread of this agent wherever possible.

4. *Mycoplasma gallisepticum* Infection in House Finches

Dr. John Fischer reported on a previously unrecognized conjunctivitis in house finches (*Carpodacus mexicanus*) that has occurred in Connecticut, Delaware, Maryland, Massachusetts, New Jersey, New York, North Carolina, Pennsylvania, Virginia and West Virginia. The first reports were received in February 1994 from Maryland and Virginia. Since that time, hundreds of sick birds, virtually all house finches, have been observed at feeders or submitted to wildlife rehabilitators. The most recent reports are from New York and Connecticut. The disease outbreak is simultaneously being investigated by the Southeastern Cooperative Wildlife Disease Study (SCWDS), the National Wildlife Health Center (NWHC), wildlife agencies in numerous states, and veterinary diagnostic laboratories in MD, VA, NC, and GA.

Clinical signs and gross lesions have ranged from mild to severe unilateral or bilateral conjunctival swelling with serous to mucopurulent drainage and nasal exudate. Microscopic lesions consisted of chronic lymphoplasmacytic conjunctivitis, rhinitis, and sinusitis. The cause of the conjunctivitis tentatively has been identified by culture and polymerase chain reaction as *Mycoplasma gallisepticum* (MG). Although MG is a well-known pathogen of domestic poultry, it previously has not been documented as a cause of disease in songbirds.

At this time, the disease seems important only for house finches, but the effects on house finch populations are unknown. House finches flock to bird feeders in large numbers and can travel long distances (partial migrations). These factors probably will enhance spread of disease. Domestic birds with MG are regarded as infected for life, and MG may be transmitted via eggs to the next generation, therefore, this disease may become readily entrenched in nature.

The possibility that domestic or wild birds other than the house finch may be susceptible to infection is another concern. Experimental work is under way to determine the pathogenicity of the MG strains obtained from house finches. Preliminary characterization of the MG isolated from house finches indicated that the strains differ somewhat from the MG strains associated with disease in poultry. Domestic chickens and turkeys have been inoculated with house finch-derived MG at the Poultry Disease Research Center, College of Veterinary Medicine, University of Georgia. Experiments to reproduce the disease in unaffected house finches and to evaluate dis-
ease transmission between infected and uninfected birds are underway.

Recommended Action: The discovery of *M. gallisepticum* in house finches could represent a new facet to the epidemiology of mycoplasmosis for poultry. Poultry producers and poultry health authorities should be aware that a potential exists for transmission of *M. gallisepticum* via house finches and possibly other passerine birds. Therefore, the standard practice of excluding wild birds from poultry facilities should be reinforced in the poultry industry.

5. EHD/BT in Deer: An Update

Dr. David Stallknecht reported on a recent increase in hemorrhagic disease in southeastern white-tailed deer populations. The Southeastern Cooperative Wildlife Disease Study, in cooperation with wildlife agencies in 14 states, has been serologically monitoring these populations since 1991. A decreasing trend in both EHDV and/or BTV antibody prevalence and serotype diversity during 1992 and 1993 suggested that herd immunity to these viruses was extremely low over most of the Southeast. It appears from preliminary 1994 data that this trend has changed dramatically. Virus isolations during 1994 were made from 18 deer from Delaware, North Carolina, South Carolina, Georgia, Tennessee, Mississippi, and Kansas. All isolates were serotyped as EHDV serotype 2 with the exception of a single BTV serotype 10 isolated from Georgia. Reports of HD, without confirmatory virus isolation, also were received from Virginia and Arkansas. Most of the virus isolations have been made from penned animals. Mortality associated with these infections in penned herds has been extreme in some cases, with losses exceeding 50%. To date, high losses have not been reported from wild populations.

Recommended Action: None required.

6. Search for Diagnostic Test Idiosyncracies in Wildlife

Dr. Victor Nettles reported that the Office of International Des Epizooties (OIE) has formed an ad hoc Group on Wildlife Diseases to assist in the collection of reports of wildlife diseases of concern to member countries. The Committee will work on surveillance, reporting, classification, and data banking wildlife health problems on a world-wide basis. One problematic area is diagnostic procedures for diseases in wildlife. The majority of standardized diagnostic tests and techniques listed in the OIE Manual of Standards are also appropriate for use in many wildlife species; however, this is not universally true. Notorious inaccuracies have been observed; bovine tuberculosis testing is a prime example. Additional problems have occurred when cattle tests for anaplasmosis have been applied to deer, and the interpretation of serological tests for African horse sickness in elephants and rhinos, bluetongue tests in wild ungulates, or Newcastle tests in wild birds
WILDLIFE DISEASES

are hard to assess. Furthermore, serums from some wild animals are anti-complementary on complement fixation testing.

The OIE Work Group on Wildlife Diseases wishes to obtain information on problems that exist with current diagnostic tests for wild species. This information will be provided to the OIE so that it can be incorporated in the OIE Manual of Standards.

Recommended Action: Persons with data to document problems that have occurred when standard diagnostic tests were applied to testing in wildlife are encouraged to communicate with the Wildlife Diseases Committee Chairman, Dr. Victor Nettles, so that this information can be made available to the OIE.

7. Spongiform Encephalopathy in Deer and Elk

Dr. Michael Miller, Colorado Division of Wildlife, reported on cases of chronic wasting disease (CWD) in free-ranging deer and elk in northcentral Colorado on behalf of Drs. T.R. Spraker, E.S. Williams, D.M. Getzy, W.J. Adrian, G.G. Schoonveld, and R.A. Spowart. Between March 1981 and October 1994, this group of investigators diagnosed 33 cases of CWD in 28 mule deer (Odocoileus hemionus), 4 Rocky Mountain elk (Cervus elaphus nelsoni), and a white-tailed deer (O. virginianus) submitted from northcentral Colorado. Emaciation accompanied by excessive salivation, behavioral changes, ataxia, or weakness were observed clinically. Severe emaciation was the only consistent gross finding; bronchopneumonia and/or other intercurrent diseases were also observed in over 50% of the cases. Spongiform encephalopathy was diagnosed microscopically in all cases. Although histologic lesions were indistinguishable from CWD in captive cervids, clinical and gross findings suggested the course of disease was more acute in free-ranging deer and elk. Mule deer appeared to be the primary species affected and accounted for 85% of all cases. Males were over-represented among mule deer cases: 16 (57%) of 28 affected mule deer were male. All but 1 of the 33 cases were from Larimer County; mule deer submissions were clustered near Estes Park, Fort Collins, and Loveland. Most affected animals were young adults. Some seasonality of case submissions was apparent: 27 of 33 cases were submitted during October through April period. Thirty of 33 cases were submitted since 1990; this pattern may be a product of intensified detection efforts, increasing prevalence, or both. Prevalence estimates, host range, distribution, origins, and management implications of CWD in wild deer and elk remain undetermined and warrant further investigation.

Recommended Action: As with the adenovirus situation in California, the Committee was concerned to hear about this new disease syndrome. Again, our foremost recommendation is that all animal health regulatory agencies
and fish and wildlife agencies are apprised of this potential new deer disease and that they take appropriate actions to avoid the spread of this agent.

8. Potential for Delivering Oral Medication to Deer

Dr. Nettles reported briefly on a pilot study that was conducted to estimate the potential for delivering medications/vaccines to a population of wild white-tailed deer. This study was done on a 4,600 acre hunting club in South Carolina during 1992 by researchers at the University of Georgia. The hunting club was feeding supplemental whole-kernel corn to wildlife on a large-scale basis. Through prior experimental trials, it was determined that the antibiotic tetracycline (TC) could be used as a biomarker for deer; deer that consumed as little as 300 mg of TC could be detected by examining their mandible for fluorescence under ultraviolet light. An extremely low level of TC was added to the corn that was being fed to wild deer for a 17-day period in the summer. Subsequent examination of hunter-killed deer revealed that 68% of the deer killed on the area were marked. Of the deer harvested off of the hunting club, approximately 30% were marked. It was concluded that it may be feasible to deliver oral vaccines to wild deer populations that are accustomed to supplemental feed sources.

Recommended Action: None required.

9. Delivery of Medications to Deer for Tick Control

Dr. John George reported on methods for the control of ticks on white-tailed deer that are being evaluated by the Knipling-Bushland U.S. Livestock Insects Research Laboratory in Kerrville, TX. White-tailed deer are the principal hosts for adults and, consequently, are the primary cause of increasing populations of both the deer tick (Ixodes scapularis) and the lone star tick (Amblyomma americanum). The white-tailed deer also is a suitable host for the southern cattle tick (Boophilus microplus) and the cattle fever tick (B. annulatus).

Practical technology to control or eradicate ticks on these wild animals is not available. One possible approach is to treat deer by providing bait medicated with a systemic parasiticide such as ivermectin. To determine if lone star ticks could be controlled by feeding white-tailed deer ivermectin-medicated corn, a 38.8 hectare deer-fenced pasture was cross-fenced to yield two similar-sized pastures. Both pastures were stocked with deer and ivermectin-treated corn was provided in one pasture. In both pastures, corn was dispensed at a rate of 0.45 kg/deer/day. For animals in the treatment pasture, the corn was treated with ivermectin at a rate of 10 mg/kg of corn to provide an approximate daily dose of 200 mcg/kg of deer body weight. Results from 2 years demonstrated that deer consumed sufficient corn to maintain blood ivermectin levels close to the target dose of 30 ppb
and well above the minimal efficacious dose of 8 ppb. The amount of ivermectin in deer blood dropped below the detectable level (2 ppb) within 28 days after removal of treated corn.

Efficacy of treatment was based on comparisons of indices of density of larvae, nymphs, and adult ticks obtained from random samples of clusters of larvae surveyed by a "flip-cloth" method and nymphs and adults collected with dry ice traps. The overall control of all life stages of *A. americanum* during 1992 was 54%, but in 1993 it increased to 92%. The absence of larval masses, suggestive of 100% control in treated pasture in 1993, indicated the remarkable impact that was made on the adult tick population. The systemic treatment of white-tailed deer with whole-kernel 'corn medicated with ivermectin appears to offer a new alternative for the control of ticks.

In addition to the medicated bait approach, Dr. George's group has developed a self-treatment device that allows appropriate quantities of tickicide to be applied directly to deer that are attracted to specially designed feeders. In the first year of testing, the prototype feeder was modified to better conform to antlered bucks, and methods for excluding feral swine and raccoons were developed. Initial indications on efficacy are encouraging.

Recommended Action: None required.

10. Challenge to Duck Plague Resolution

Dr. David Hunter reported on a challenge by a private veterinarian to the USAHA's resolution that was adopted last year on duck plague. This resolution supports efforts by fish and wildlife agencies in controlling the spread of duck plague during outbreaks. An identical resolution also was adopted by the American Association of Wildlife Veterinarians (AAWV) and the Wildlife Disease Association (WDA). In response to the challenge to the resolution, both sides of the issue were addressed at a special session on duck plague at the WDA Meeting in California. After the topic was discussed, both the AAWV and the WDA upheld the resolution. AAWV will appoint a committee to develop two additional positions on duck plague. The first position would be a resolution calling for research to determine key issues on the epidemiology of duck plague. The second position will be recommendations on how to apply preventive measures to avoid duck plague outbreaks in the "typical scenario" involving non-migratory waterfowl.

Recommended Action: The Committee on Wildlife Diseases chose not to change the original USAHA resolution concerning duck plague.

11. Studies on *Elaphostrongylus cervi* in Native Deer

Dr. Alvin Gajadhar reported on results of experimental transmission
studies of *Elaphostrongylus cervi* in native mule and white-tailed deer. The red deer is the definitive host of *E. cervi*, a nematode parasite which invades the musculature and central nervous system of animals. Although it causes little or no clinical illness in red deer, it is capable of causing severe neurological disease in some species of cervids and perhaps other ungulates. The parasite does not occur in North America, and its effect on native ungulates is unknown. Attempts to quarantine animals and screen for *E. cervi* have been thwarted because of the lack of a reliable diagnostic assay and basic information about the parasite. In order to protect North American wildlife, several regulatory agencies in North America do not allow various imported cervid species within their regions. There is an urgent need to determine the susceptibility of native wildlife to this parasite.

Larvae of *E. cervi* were obtained from red deer in New Zealand and grown to the infective stage in North American snails. Young white-tailed deer and mule deer, bottle-raised in isolation, were orally inoculated with the parasite. An inoculated red deer and an uninoculated white-tailed deer served as controls for the experiment. The two infected mule deer developed progressive ataxia in both hind legs beginning 104 days after inoculation. Within 3 or 6 weeks, spinal cord injury caused by the parasite severely compromised the animals' ability to move, and they were euthanized when they were no longer able to stand or retain sternal recumbency. Larvae of *E. cervi* were found in the feces of both animals, indicating that mule deer could serve to disseminate the parasite in North America. Some of the seven white-tailed deer infected with *E. cervi* became lethargic for several days after inoculation but did not develop clinical illness or pass *E. cervi* in their feces. Dead parasites, surrounded by inflammation, were found in the wall of the gastrointestinal tract and other organs of the white-tailed deer.

Experimental evaluation of *E. cervi* in red deer showed that the prepatent period can extend to at least 206 days, much longer than the previously reported 86-125 days. This has significant implication for quarantine protocols aimed at keeping *E. cervi* out of North America. Furthermore, our experiments demonstrate that the pattern of excretion of *E. cervi* larvae by infected red deer and mule deer is sporadic and unpredictable, and the Baermann test used to detect the infection is not reliable.

Recommended Action: The Committee suggests that the national animal health authorities in the United States and Canada utilize these data in the development of policies on importation of animals that could be infected with *E. cervi*.

12. Michigan Captive Cervidae Survey

Dr. Karen Shank gave a brief presentation on the 1993 Michigan captive cervidae project. Michigan is one of the top three states in the num-
bers of captive cervidae (mostly white-tailed deer). A survey was completed in 1993 to gather baseline data on this industry. The captive cervidae population had a 7.9% increase during 1993. Nearly 85% of the purchases were made through private sales, and 60.9% of purchases were from within the State. Six percent of the survey respondents reported borrowing bucks for breeding purposes. Thirty-two percent of deer farmers quarantine new additions for periods ranging from 3 to 270 days. Nearly 40% of cervidae owners reported keeping other species on their premise.

Recommended Action: None required, although these data may be useful to future risk assessments.

13. Disease Issues Important to the Llama Industry

Mr. Bob Frost of the Research Committee of the International Llama Association (ILA) gave a brief report. He indicated that llamas and alpacas are not classified as wildlife or captive wildlife but that llamas have been domesticated for over 6,000 years. Nevertheless, the International Llama Association has recognized that with continued and expanding use of llamas in the wildlands of North America, there is a need for reliable diagnostic tests for various diseases of concern.

The United States Department of Agriculture has entered into a cooperative agreement with Argentina's National Institute of Agricultural Technology (INTA) to further develop a diagnostic test for bovine tuberculosis in llamas and alpacas. This research project, which used 70 llamas and bovids, was started last spring and will conclude this December at INTA's Research Center for Veterinary Sciences near Buenos Aires. The project has focused on a search for possible skin sites that can be best utilized for tuberculin injection in field testing. ELISA tests also will be done by Dr. Elizabeth Kantor at the Pan American Health Laboratory in Buenos Aires. Additional serum samples will be sent to Agriculture Canada and a few U.S. institutions for work with various ELISA and DNA tests. The ILA is looking forward to the INTA’s results in the upcoming year. At present, there are no natural herds of llamas or alpacas in the Americas that are known to have bovine tuberculosis.

A brucellosis study was recently completed by the National Veterinary Services Laboratories, and preliminary reports indicated the majority of the bovine diagnostic tests will work for llamas. As with tuberculosis, there have not been documented cases of naturally occurring brucellosis in llamas in North or South America. Mr. Frost presented the position of the ILA that llamas do not pose a threat to domestic livestock or wildlife in the wildlands in regards to infectious diseases.

Recommended Action: None required.
14. Use of Blood Tuberculosis Test in Deer, Zoo Animals, and Cattle

The use of the blood tuberculosis test (BTB) in the United States was reported by Dr. Don Davis. This test became fully operational at Texas A & M University as an official test for bovine tuberculosis under the USDA/APHIS/VS Uniform Methods and Rules for the Eradication of Tuberculosis in Cervidae on May 15, 1994. During the first 4 months, the BTB was conducted on over 500 animals, including 100 deer, 400 cattle, and 130 samples from over 30 species of zoo animals. The specificity and sensitivity of the BTB in 153 cattle of known *Mycobacterium bovis* culture status was 96.9% and 91.6%, respectively. The results of the BTB are reported to the client, the state veterinarian, and the federal area-veterinarian-in-charge as positive for *M. bovis*, positive for *M. avium*, equivocal, negative, or no data. The equivocal and no data results are usually the result of poor sample collection and/or improper handling or shipping.

Very few problems have been experienced with the BTB testing during the implementation phase. The BTB has correctly diagnosed TB-infected animals and has been useful in exonerating non-infected animals that were positive on the single cervical test. As more data are collected in North America, minor adjustments to the BTB criteria will be implemented to account for the occurrence of cross-reactions of non-pathogenic *Mycobacteria*, particularly *M. intracellularare*.

Recommended Action: None required.

15. Resolution on Brucella Vaccine Development

Dr. Dan Baca introduced a resolution that would urge the USAHA to encourage VS/APHIS/USDA to expedite the evaluation of alternative vaccines for brucellosis, viz., RB 51, a subunit vaccine, or a live mutant vaccine. The Committee agreed that vaccine development was an important issue in regard to addressing brucellosis in free-ranging wildlife, and we were supportive of this aspect of the resolution. However, the question of the use of vaccines in the long-term brucellosis eradication strategy for livestock was another matter. A motion was passed that indicated that the Committee on Wildlife Diseases would be willing to co-sponsor the resolution with the Brucellosis Committee if the resolution was acceptable to the Brucellosis Committee.
REPORT OF THE COMMITTEE ON
ZOLOGICAL ANIMALS

Chairman: Dr. Calvin W.S. Lum, Honolulu, HI
Vice Chairman: Dr. Robert Temple, Bristolville, OH

Dr. Wilbur B. Amand, PA; Dr. Jack N. Armstrong, NV; Dr. Robert A. Cook, NY; Dr. Richard L. Crawford, MD; Dr. P. M. Eppele, SD; Dr. Werner P. Heuschele, CA; Mr. Harvey Hilderbran, TX; Dr. Patrick D. HECTOR, IN; Dr. David L. Hunter, ID; Dr. David A. Jessup, CA; Dr. Chester J. Mikel, OK; Dr. John H. Olsen, FL; Dr. Lisa H. Rothe, CO; Dr. Morton S. Silberman, GA; Dr. C. O. Thoen, IA; Dr. E. Tom Thorne, WY; Mr. Dave Whittlesey, CO; Dr. Peregrine Wolff, MN.

The meeting of the committee on Zoological Animals was called to order by Chairman Calvin Lum at 1:30 P.M., November 2, 1994. The meeting was attended by 10 committee members and 18 guests.

The chairman reviewed the purpose and goals of the committee as stated in the by-laws of USAHA, and indicated that one of the chair's goals for the committee was to improve and facilitate the communications between state veterinarians and the captive wildlife and alternative livestock industry members. In addition, it is his intention to encourage more interaction between regulators and these industries. One of the more important outcomes of this committee will be the distribution to all state veterinarians of a directory/information manual and the designation of this committee as the clearinghouse of information and resources for all involved.

First on the agenda was a panel discussion among three state veterinarians: Dr. Jack Armstrong, Nevada State Veterinarian, Dr. Mike Marshall, Utah State Veterinarian, and Dr. Richard Hull, Illinois State Veterinarian. Their concerns were both with zoonotic diseases and diseases that affect captive wildlife and alternative livestock. They spoke of a lack of valid testing for individual animals as well as herd surveillance of captive wildlife species. They also wanted guidance on how regulators can deal with dangerous animal legislation in various states. They expressed the desire to meet their regulatory mandates with the least regulatory oppression on the industry.

The next speaker was Dr. Dale Schwindaman, Deputy Administrator for USDA, APHIS, REAC. Dr. Schwindaman gave an update on agency activities and reviewed the budget situation with REAC. He also reviewed the 1994 amendments to the Marine Mammal Protection Act. Also reviewed for the committee were issues pertinent to REAC, which included concerns with human deaths by elephants, roadside zoos, environmental enhancement and perimeter fencing. He identified future issues: 1) Morbillivirus (seals and dolphins); 2) negotiated rulemaking as it pertains to marine mammals; 3) zoo intern program.
REPORT OF THE COMMITTEE

Dr. Eric Miller, Director of Animal Health, St. Louis Zoo, followed with a review of the AZA accreditation process which included standards for health delivery and quarantine, and discussed various diseases and disease testing. He also stressed the importance of organizations involved in captive wildlife working with state and federal regulatory officials.

Next on the agenda was Dr. David Ligda, Chairman of Exotic Animals and Wildlife section of the AVMA Network of Animal Health System (NOAH). Dr. Ligda shared with the committee information on this interactive electronic network and how it pertains to the sharing of information regarding disease testing, husbandry and etc. He suggested that this system may be useful in answering many of the questions posed by members of the audience.

The next speaker was Dr. John Kopec, USDA, APHIS, VS, Senior Staff Veterinarian, Brucellosis Eradication Program. Dr. Kopec presented an update on the Llama Brucellosis Project. This project involved cooperation between industry and government, whereby two phases of the project are now complete. Phase I was development of valid brucellosis tests in vaccinated animals. Phase II was live challenge, utilizing testing methods developed in Phase I. Phase III - to be done in the future.

Dr. Robert Temple, Vice Chairman outlined the committee project of development of a central clearinghouse for information and resource. He reported that he met recently with the AAZV Board to discuss their participation. As a result of his meeting with AAZV, he was happy to report that this committee in cooperation with AAZV will be incorporating the manual of the AAZV Infectious Disease Committee into this committee's directory/information manual, which will be distributed first to the fifty state veterinarians.

The directory/information manual will contain the names and telephone numbers of regulatory and participating individuals, knowledgeable in their particular fields of expertise. It will contain the latest facts on certain diseases and husbandry practices. In addition to individuals, this committee will be working with various organizations such as AAZV and AZA. This committee will act as a clearinghouse to continually update and revise this manual so that it will always reflect the latest information.

The goal of the committee in implementing this project is to promote a more effective line of communication between regulatory agencies and individuals in the captive wildlife and alternative livestock industries.

The last speaker was Dr. Nancy Frank, National Association of Public Health Veterinarians. Dr. Frank presented a position statement from the organization and the Council of State and Territorial Epidemiologists regarding translocation of wildlife. The concern of these organizations was the interstate movement of wildlife species from endemic areas of zoonotic diseases. The committee decided to establish communications with these organizations to more clearly define the parameters of their concerns.

No resolutions were presented to the committee.

The meeting was adjourned at 5:00 p.m.

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99TH ANNUAL MEETING
October 28 - November 3, 1995
JOHN ASCUAGA'S NUGGET HOTEL
Reno, Nevada

100th ANNUAL MEETING
October 12-18, 1996
EXCELSIOR HOTEL
Little Rock, Arkansas

101st ANNUAL MEETING
October 17-24, 1997
GALT HOUSE HOTEL
Louisville, Kentucky