PROCEEDINGS

EIGHTY-THIRD
ANNUAL MEETING

of the
UNITED STATES
ANIMAL HEALTH
ASSOCIATION

THE TOWN AND COUNTRY HOTEL
October 28-31, November 1-2, 1979
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Committee on Tuberculosis and Johne’s Disease—1980

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Carl E. Boyd, Elgin, SC
A. M. Carey, Wheaton, MD
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G. H. Frye, Mitchellville, MD
G. F. Hoffsis, Ashville, OH
D. R. Hughes, Phoenix, AZ
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Norman Lichtman, Westville, NJ

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M. S. Silberman, Reynolds, GA
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G. R. Snyder, Reston, VA
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K. M. Weinland, W. Lafayette, IN

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Vice-Chairman: James S. Smith, Hyattsville, MD

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Delmar R. Cassidy, Ames, IA
Joe B. Finley, Jr., Encinal, TX
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Erskine V. Morse, West Lafayette, IN
Mitchell J. Rogers, Little Rock, AR
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Milton Friend, Madison, WI
D. E. Herrick, Bowie, MD
C. J. Mikel, Oklahoma City, OK

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Second Vice-President

J.C. SHOOK
Third Vice-President

R.J. STADLER
First Vice-President

JOHN R. RAGAN
Secretary-Treasurer
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Oct. 11-12, 1898</td>
<td>Omaha, Neb.</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Taylor Riddle, Kan</td>
</tr>
<tr>
<td>3. Oct. 11-12, 1899†</td>
<td>Chicago, Ill.</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Mortimer Levering, Lafayette, Ind</td>
</tr>
<tr>
<td>5. Oct 8-9, 1901</td>
<td>Buffalo, N.Y.</td>
<td>*Dr. E. P. Niles, Va</td>
<td>*Mr. W. H. Dunn, Tenn</td>
</tr>
<tr>
<td>8. Aug. 23-24, 1904</td>
<td>St. Louis, Mo.</td>
<td>*Mr. M. P. Smith, Monticello, Ill</td>
<td>*Dr. S. H. Ward, St. Paul, Minn</td>
</tr>
<tr>
<td>11. Sept 16-17, 1907</td>
<td>Richmond, Va.</td>
<td>*Dr. Charles G. Lamb, Col</td>
<td>*Dr. C. E. Cotton, St. Paul, Minn</td>
</tr>
<tr>
<td>12. Sept 14-16, 1908</td>
<td>Wash., D.C.</td>
<td>*Dr. Charles G. Lamb, Col</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill</td>
</tr>
<tr>
<td>15 Dec 5-6, 1911</td>
<td>Chicago, Ill.</td>
<td>*Dr. John F. Devine, Goshen, N.Y.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill</td>
</tr>
<tr>
<td>16. Dec 3-5, 1912</td>
<td>Chicago, Ill.</td>
<td>*Dr. Macoy P. Ravenel, Madison, Wis</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill</td>
</tr>
<tr>
<td>17. Dec 24, 1913</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter F. Bahnsen, Atlanta, Ga.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill</td>
</tr>
<tr>
<td>18. Feb 16-18, 1914</td>
<td>Chicago, Ill.</td>
<td>*Dr. S. H. Ward, St. Paul, Minn</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill</td>
</tr>
<tr>
<td>22. Dec 2-4, 1918</td>
<td>Chicago, Ill.</td>
<td>*Dr. M. Jacob, Knoxville, Tenn</td>
<td>*Dr. S. H. Ward, St. Paul, Minn</td>
</tr>
<tr>
<td>23. Dec 1-3, 1919</td>
<td>Chicago, Ill.</td>
<td>*Dr. G. W. Dumphy, Knoxville, Tenn</td>
<td>*Dr. S. H. Ward, St. Paul, Minn</td>
</tr>
<tr>
<td>25. Nov 28-30, 1921</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. F. Crewe, Bismarck, N.D.</td>
<td>*Dr. Theo A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>26. Dec 6-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>27. Dec 5-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Mont</td>
<td>*Dr. O. E. Dyson, Kansas City, Mo.</td>
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<td>Date</td>
<td>City, State</td>
<td>Name and Location</td>
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<td>Dec 24, 1925</td>
<td>Chicago, Ill</td>
<td>*Dr. J. H. McNeil, Trenton, N.J.</td>
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<tr>
<td>Dec 13, 1926</td>
<td>Chicago, Ill</td>
<td>*Dr. John R. Mohler, Wash., D.C.</td>
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<tr>
<td>Nov 30-Dec 1-2, 1927</td>
<td>Chicago, Ill</td>
<td>*Dr. L. Van Es, Lincoln, Neb.</td>
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<td>Dec 7, 1928</td>
<td>Chicago, Ill</td>
<td>*Dr. C. A. Cary, Auburn, Ala.</td>
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<td>Dec 4-6, 1929</td>
<td>Chicago, Ill</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
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<td>Dec 3-5, 1930</td>
<td>Chicago, Ill</td>
<td>*Dr. A. E. Wight, Wash., D.C.</td>
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<tr>
<td>Dec 24, 1931</td>
<td>Chicago, Ill</td>
<td>*Dr. J. W. Connaway, Columbia, Md.</td>
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<tr>
<td>Nov 30-Dec 1-2, 1932</td>
<td>Chicago, Ill</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
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<td>Dec 6-8, 1933</td>
<td>Chicago, Ill</td>
<td>*Dr. E. T. Faulder, Albany, N.Y.</td>
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<tr>
<td>Dec 5-7, 1934</td>
<td>Chicago, Ill</td>
<td>*Dr. T. E. Robinson, Providence, R.I.</td>
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<td>Dec 4-6, 1935</td>
<td>Chicago, Ill</td>
<td>*Dr. Edward Records, Reno, Nev.</td>
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<td>Dec 24, 1936</td>
<td>Chicago, Ill</td>
<td>*Dr. Walter Wisnicky, Madson, Wis.</td>
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<td>Dec 1-3, 1937</td>
<td>Chicago, Ill</td>
<td>*Dr. R. W. Smith, Concord, N.H.</td>
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<tr>
<td>Nov 30-Dec 1-2, 1938</td>
<td>Chicago, Ill</td>
<td>*Dr. D. E. Westmoreland, Frankfort, Ky.</td>
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<tr>
<td>Dec 6-8, 1939</td>
<td>Chicago, Ill</td>
<td>*Dr. J. L. Axy, Indianapolis, Ind</td>
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<td>Dec 4-6, 1940</td>
<td>Chicago, Ill</td>
<td>*Dr. H. D. Port, Cheyenne, Wyo</td>
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<tr>
<td>Dec 3-5, 1941</td>
<td>Chicago, Ill</td>
<td>*Dr. E. A. Crossman, Boston, Mass</td>
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<td>Dec 24, 1942</td>
<td>Chicago, Ill</td>
<td>*Dr. I. S. McAdory, Auburn, Ala.</td>
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<tr>
<td>Dec 1-3, 1943</td>
<td>Chicago, Ill</td>
<td>*Dr. W. H. Hendricks, Salt Lake City, Utah</td>
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<td>Dec 6-8, 1944</td>
<td>Chicago, Ill</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
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<td>Dec 5-7, 1945</td>
<td>Chicago, Ill</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif</td>
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<td>Dec 46, 1946</td>
<td>Chicago, Ill</td>
<td>*Dr. William Moore, Raleigh, N.C.</td>
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<td>Dec 3-5, 1947</td>
<td>Chicago, Ill</td>
<td>Mr. Will J. Miller, Topeka, Kan</td>
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<tr>
<td>Oct 13-15, 1948</td>
<td>Denver, Colo</td>
<td>*Dr. Jean V. Knapp, Tallahassee, Fla</td>
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<tr>
<td>Oct 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. O. Brandenburg, Bismarck, N.D.</td>
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<tr>
<td>Nov 14-16, 1951</td>
<td>Kansas City, Kan</td>
<td>*Mr. T. E. Mollin, Denver, Colo.</td>
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<tr>
<td>Oct 29-31, 1952</td>
<td>Louisville, Ky</td>
<td>Dr. Ralph L. West, St. Paul, Minn</td>
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<tr>
<td>Sept 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
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<td>Nov 10-12, 1954</td>
<td>Omaha, Neb</td>
<td>*Dr. T. C. Green, Charleston, W.Va.</td>
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<tr>
<td>Nov 16-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wulkins, Helena, Mont</td>
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<td>Nov 28-30, 1956</td>
<td>Chicago, Ill</td>
<td>Dr. A. L. Brueckner, Baltimore, Md</td>
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<td>Nov 13-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyo</td>
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<td>*Dr. O. E. Dyson, Wichita, Kan.</td>
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<td>*Dr. L. Enos Day, Chicago, Ill.</td>
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<td>*Dr. L. Enos Day, Chicago, Ill.</td>
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<td>Dr. Mark Welsh, College Park, Md.</td>
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<td>Dr. Mark Walsh, College Park, Md.</td>
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<td>Dr. Mark Welsh, College Park, Md.</td>
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<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<td></td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
</tr>
<tr>
<td>--------------</td>
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<td>----------------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, Fla.</td>
<td>Dr. John G. Milligan, Montgomery, Ala.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>65. Oct. 3-Nov. 1-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, N.M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R.I.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>80. Nov. 7-12, 1976</td>
<td>Miami Beach, Fla.</td>
<td>H. E. Goldstein, Columbus, Ohio</td>
<td>Dr. W. L. Bendix, Richmond, Va.</td>
</tr>
</tbody>
</table>

**Resigned Dec. 12, 1977

*Deceased  †Reprinted in 54th Annual Report ††Reprinted in the 66th Annual Report
+ This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION AND MEMORIAL SERVICE

F. James Schoenfeld, DVM
Salt Lake City, Utah

Our Father who art in Heaven, Hallowed be Thy Name.

We have assembled here this night as members of the 22nd Annual Conference of the American Association of Veterinary Laboratory Diagnosticians and the 83rd Annual Meeting of the United States Animal Health Association.

We are grateful for this privilege and opportunity to learn and to exchange knowledge concerning the animal kingdom and those conditions which may afflict them. May we accept this responsibility and do all we can to preserve their lives in health that we as mankind throughout the world may enjoy the blessings of this responsibility. May thou grant us the knowledge and means that we may continue in our work.

We know that we are living in a time of many diversities in the world and we are grateful to live in this choice land of America—a choice land. May we so live our lives to continue to make this the great country that it is. As thou hast promised that it will be, if we but serve the God of this land—Who is Jesus Christ.

We pray for the leaders of these organizations, our presidents, who devote so much time to the success of these organizations. For the committee chairman, and members who gather together the knowledge that is essential to the health of our animal industries. We pray for the office staff and especially “our Ella” who keep us up-to-date and informed.

We pray for our families who are at home that they may be protected and watched over, and for those family members who are with us may they enjoy the proceedings of these meetings.

We pray for the leaders of this nation, the President and the Congress, that they may seek Thee in guidance. For the leaders of the Nations of the World, that the people of all the Nations may feel Thy influence in their lives.

We pray for those who are ill amongst us, or who are caused to mourn, may their burdens be lightened. We especially pray for Mrs. Norman Powers, who is seriously ill, grant unto her strength and peace of mind at this time that she may have the blessings that she needs, also for her husband who stands at her side.
We are thankful for the privilege of meeting in this great city of San Diego, in this great state of California. We are thankful for this hotel, and its facilities to make this convention a success.

As we begin these great meetings this week, may we accomplish the purposes for which we are gathered, and take with us the knowledge to our various states and communities that we may have a better and safer world to live in. We are thankful for the privilege of meeting together this night and we offer this prayer unto Thee in the name of Jesus Christ.

Amen.
MEMORIAL SERVICE

October 29, 1979

Mr. President, members of these associations, ladies and gentlemen.

At this time, as is our custom, we pause for a moment of silent prayer and reverence to pay tribute to those friends and colleagues who have now completed their mortal existence and have passed on to paradise.

1978-79 Deceased

Dr. Gordon L. McNeilly — Mt. Pleasant, Michigan — November 11, 1978
Dr. Edgar P. Morphet — Elko, Nevada — October 10, 1978
Dr. S. S. Newcomb — Albany, New York — January 8, 1979
Dr. E. R. Goode — College Park, Maryland — February, 1979
Dr. M. D. Mitchell — Pierre, South Dakota — May 20, 1979
Dr. M. L. Weldy — Goshen, Indiana — May, 1979
Dr. J. B. Taylor — Montgomery, Alabama — July 22, 1979
"WELCOME TO CALIFORNIA"

to
United States Animal Health Association and
American Association of Veterinary Laboratory Diagnosticians
Joint General Session
Monday, October 29, 1979

By
R. E. Rominger
Director
Department of Food and Agriculture

Welcome to California, the number one agricultural state in the United States with 1978 production of $10.4 billion and 1979 looking like it should be even better. Of that production, we export almost 25 percent outside the U.S. (I understand this traditional welcoming address from the host state is kind of a statistical one-upmanship.) Over 200 commercial crops are grown in California, and we lead the nation in the production of 47 of those crop and livestock commodities. No one of them dominates our diverse production, but you will recognize your importance when I tell you that number one in dollar value are cattle and calves at $1.3 billion; and number two, milk and cream at $1.2 billion. The livestock and poultry sector totals $3.3 billion, almost one-third of our production, and that doesn’t include horses and goats!

Many people don’t realize that, because we may be more famous for the fact that we produce 40 percent of the nation’s fruits and nuts (and I don’t want any wisecracks) and one-third of the vegetables.

Of our major commodities, we rank first in the nation, listed in order of dollar value, in grapes, lettuce, nursery products, processing tomatoes, eggs, flowers and foliage, almonds, walnuts, strawberries, peaches, sugar beets, celery, broccoli, prunes, lemons and cantaloupes. We rank second in the U.S. in milk and cream, cotton, hay, oranges, turkeys and fresh-market tomatoes. We’re third in rice, potatoes and barley.

We’re producing almost 10 percent of the U.S. agricultural production with 3 percent of the farms and although our state has become more urbanized (with 22 million people, we lead the nation), agriculture is our leading industry. We hope we are as interested in quality as we are in quantity—we are told that our inspection and weights and measures people are among the most highly trained in
their profession. With the help of you all and our natural barriers—dessert, mountains and ocean—we try to keep pests and diseases out.

In the area of your concerns, we are all very interdependent. Each state is affected by the animal health programs of other states because there is so much movement of poultry and livestock throughout the nation. A quick review of official health certificates just since July 1 of this year shows we received shipment of livestock and poultry from 47 states and sent shipments to 49 states. This underscores the interest California has in seeing that the U.S. Animal Health Association is successful in helping to bring about a higher level of health standards for livestock and poultry throughout the nation.

You are among the unsung heroes. Some of the most important things that are done seem to be our best-kept secrets, while those who “blow it” and have scandals make the 6 o’clock news. As my wife said, “It’s kind of like managing a household—nobody notices until something is not done.”

We, in government agriculture, are continually being challenged. Can you encourage agriculture and at the same time regulate it? I think it’s a reflection of the good guys/bad guys mentality—unless you’re throwing people in jail, you’re not enforcing the law. We do a little of that; but in any good enforcement program, the best compliance is achieved through education, working together, and sharing some responsibility. When we have to hire lawyers and go to court, it’s expensive in time and dollars—the system has failed if we have to do that very often. Most of all, we have going for us something I call Economic Behavior Modification. In regular police situations, the offender destroys other people’s property; in our situation, a violator will usually be ruining his own crops or animals. If you spray the wrong pesticide on your crop, it will be quarantined; or if you mismanage your herd, it’s money out of your own pocket.

There’s a new ERA debate in the California Legislature as the result of a resolution introduced this past season—Equal Rights for Animals. Immediately, visions of India, with animals roaming the streets, popped up in people’s minds, and livestock people became alarmed at thoughts of outlawing common practices—tagging ears, branding, etc. It seems to me it would be to the advantage of livestock people not to be so predictable, not in such a hurry to act out the role of “bad guy” or “the enemy” which is so necessary for anyone to have to rally the troops against. Rather, we might honestly say, “We are fond of animals, too; that is why we are ranchers or veterinarians—we do many things to keep them well and comfortable and are open to ideas which might improve animal life.” I think our credibility might
be more intact, then, when we offer criticism of specific proposals which might be economically unreasonable, or not result in any less harm to animals. We allow ourselves to be "used" as the bad guy too often.

You can help us solve a particular animal health problem that is serious, not only in California, but in several other states; and that is the eradication of cattle scabies. The problem is severe because of the growing concern over the use of pesticides in agriculture. We are concerned in our State with the use and disposal of those materials used successfully over many years to treat scabies. Our range operators have a significant problem in trying to comply with the USDA requirement for withholding treated cattle from streams and ponds for seven days after treatment. We have asked for further consideration of this requirement where a cattleman's pond contains no fish and is under his complete control. I challenge this conference and the Parasitic Diseases and Parasiticides Committee to address this and other issues related to that program. Dr. Bill Utterback, a USDA veterinarian from California, is a member of that committee and is fully conversant with our problems here in California.

My expectations for this conference are high, because of your unique talents—the nation's greatest minds in the field of animal health.
RESPONSE TO WELCOME

T. S. Maddox, D.V.M.
Frankfort, Kentucky

Director Rominger, President Zweigart, President Elect Hawkins, ladies and gentlemen.

Although I was hoping I might see it some day, I did not realize I would arrive in Heaven so soon and that the purpose of my visit would be to participate in this, the 83rd annual meeting of the U.S.A.H.A. No one would even envision a group of veterinarians meeting in Heaven.

Those of you who are from the northern parts of our great nation will appreciate my sentiments best; for you, too, will languish in this temperate climate, enjoying a brief respite from worry about the atmospheric conditions we anticipate as another winter approaches. But, it is not only the temperature which makes this city so appealing. It is also the beautiful geography which allows us to be in the mountains, desert, river valleys and shoreline—all at once.

I can imagine that the Spanish explorers who first sailed into San Diego Bay in 1594 must have been certain they had found a place of uncommon beauty, although they did not return to settle this area until 200 years later. Even then, it was California’s first Spanish mission and the first European settlement. The Spanish influence is still felt today in San Diego where a graceful, leisurely pace of life is reflected in its people and this is evident to us as we enjoy the warm hospitality of this convention site.

Even the industry located in this thriving populous city of San Diego mirrors the lifestyle of its inhabitants. Known as the “oceanographic capital of the world,” more than 100 businesses engage in ocean studies or their support. The intellectual climate, not just sea and sun, attracts these organizations and others such as electronic firms, research groups and higher education institutions. The resources of this community make it understandable why contemporary business wish to locate here. San Diego today is nothing like the image those of us who are pre-World War II had imagined it to be when it was dominated by U.S. Navy Forces. The magnificent 14-mile-long bay that served our nation well in time of war now serves this city as a tourist attraction as well. All we need to do is gaze over the Bay and catch a glimpse of the yachts and boats at anchor or at sail and we have the feeling of the wonderful blend of leisure and business this community has been able to capture in its development.

Inasmuch as I hate to mention something could possibly be wrong with America’s nearest approach to Shangri-La, but I was told that nuts
are one of the leading agricultural exports of California, and I can see why—you have regularly scheduled tremors and quakes to shake the trees for easy harvest. Seriously, all the nation recognizes California’s agricultural industry and its great contribution to our nation’s food supply and it is fitting that we convene here to make decisions that will contribute to the health of the nation’s livestock and agricultural economy.

I know I speak for all the conferees when I thank you, Director Rominger, for your welcome to San Diego and the Golden State, and say it is our pleasure to convene here in the Plymouth Rock of California.

We shall look forward to the challenge of matching your hospitality and enthusiasm of welcome in Kentucky next year at Louisville.
Commissioner Rominger, Members of AAVLD, USAHA and guests:

Welcome to Sunny California! When we selected this site for the 1979 meeting, we felt it would be a little different than the downtown hotels we have become accustomed to, but were excited about the facilities and the climate. It was February 1 and when I left Pennsylvania it was 12°, in Chicago it was 8° and when I arrived here it was overcast and 60°. They immediately began making apologies for the cool weather. Here I was with a heavy lined top coat, hat, gloves and the whole bit. They assured me it would be sunny and warmer the next day and it sure was. What a pleasant relief from winter winds and snow.

My year as executive secretary has been a busy one and hopefully for the association a successful one. The assistance of our office staff—Ella Blanton, Linda Ragland and Betty Miller, my secretary at Frederick, have made my job very pleasant. They should all be congratulated on their attitude and dedication to the affairs of U.S.A.H.A.

My president, Tom Zweigart, and the board of directors have done an excellent job of looking after the affairs of the association and seeking ways for improvement.

The membership of the association keeps growing and there is increasing interest from a variety of segments of the livestock industry and veterinary profession in our meetings and publications. Approximately 80 individual members and 2 allied groups have applied for membership in our Association.

For the Minneapolis and Buffalo meetings, the U.S.D.A. information office loaned us the services of Miss Kathy Ellis to handle our public relations activities prior, during and following the meetings. This work received wide acceptance from the Agricultural Press and I am sure was at least partially responsible for the increased interest in our association activities that I mentioned earlier. U.S.D.A. was no longer able to provide this service on the previous basis so representatives from the Public Relations and Information Committee and Board of Directors interviewed several applicants and contracted with Mr. James Madison of P J Associates in Falls Church, Virginia, to handle this work for the 1979 meeting. We trust the officers, committee chairmen and members will give Mr. Madison their full cooperation in
his first year as Public Relations coordinator. Our work force is limited this year, so if the Committee Chairmen can't get their papers typed before their report is due, give your presentation and then have your report typed and turn it in at the registration desk.

During the year, we represented the association at a number of meetings with U.S.D.A. and regional animal health organizations. We also consulted with the animal health committee of the National Academy of Science regarding disease problems that need to have additional emphasis and support to maintain a viable livestock economy.

The proceedings of last year's meeting were published the earliest ever, thanks to the cooperation of the speakers, the printer and a lot of diligent work by our staff. For the first time, we have made preregistration available to avoid some of the long waiting lines experienced in the past. We were pleased that so many of you took advantage of this service.

I am sure that you have all noted the absence of Norm and Jay Powers at the registration desk. This was due to a personal emergency in the Powers family and I am sure the membership unanimously wishes them well and looks forward to seeing them at future meetings.

We hope your stay in San Diego is pleasant and rewarding. If there is anything the secretary's office can do to assist you during the week or any time during the year, please don't hesitate to contact us.
STATEMENT OF CASH RECEIPTS and DISBURSEMENTS
FOR PERIOD OCTOBER 1, 1978 through SEPTEMBER 30, 1979

CASH BALANCE — OCTOBER 1, 1978:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Cash on Hand — October 1, 1978</td>
<td>$214.50</td>
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<tr>
<td>Southern Bank and Trust Company</td>
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<tr>
<td>Checking Account</td>
<td>$33.46</td>
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<td>Savings Account</td>
<td>$21,767.64</td>
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<td>$22,015.60</td>
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INCREASED BY CASH RECEIPTS:

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<tr>
<th>Description</th>
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<tbody>
<tr>
<td>Individual Dues</td>
<td>$16,570.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>10,200.00</td>
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<tr>
<td>Proceedings</td>
<td>5,830.01</td>
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<tr>
<td>Reprints</td>
<td>3,371.43</td>
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<td>Foreign Animal Books</td>
<td>890.10</td>
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<tr>
<td>Registration Fees</td>
<td>31,560.00</td>
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<tr>
<td>Tours</td>
<td>3,246.25</td>
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<tr>
<td>Interest Income</td>
<td>2,157.40</td>
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<tr>
<td>Rosters</td>
<td>15.00</td>
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<td>73,840.19</td>
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TOTAL BEGINNING BALANCE and RECEIPTS $95,855.79

ADD — RECEIPTS RECEIVED FOR YEAR
1979-1980 prior to September 30, 1979:

<table>
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<th>Description</th>
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<tbody>
<tr>
<td>Individual Dues</td>
<td>$2,160.00</td>
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<tr>
<td>Registrations</td>
<td>7,150.00</td>
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<tr>
<td>Tours</td>
<td>5,683.45</td>
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<tr>
<td>Interest</td>
<td>13.36</td>
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<tr>
<td></td>
<td>15,006.83</td>
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<td>$110,862.60</td>
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UNITED STATES ANIMAL HEALTH ASSOCIATION  
SUITE 205, 6924 LAKESIDE AVENUE  
RICHMOND, VIRGINIA 23228  

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS  
FOR PERIOD OCTOBER 1, 1978 through SEPTEMBER 30, 1979  

DECREASED BY EXPENDITURES:  

<table>
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<tr>
<th>Item</th>
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<tr>
<td>Annual Meeting</td>
<td>$11,882.94</td>
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<td>Printing</td>
<td>16,114.11</td>
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<td>Office Supplies</td>
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<td>Salaries</td>
<td>14,332.82</td>
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<td>Wages</td>
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<td>Social Security Tax</td>
<td>778.33</td>
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<td>Communication</td>
<td>4,956.97</td>
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<td>Travel:</td>
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<td>Dr. J.C. Shook</td>
<td>349.75</td>
</tr>
<tr>
<td>Dr. W. L. Bendix</td>
<td>215.75</td>
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<tr>
<td>Dr. T. F. Zweigart</td>
<td>339.96</td>
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<tr>
<td>Dr. L.W. Hinchman</td>
<td>355.03</td>
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<tr>
<td>Dr. Glenn Rea</td>
<td>694.09</td>
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<tr>
<td>Ella R. Blanton</td>
<td>576.00</td>
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<td>Rent — Office Space</td>
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<td>American Association of Veterinary</td>
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<tr>
<td>Livestock Diagnostics</td>
<td>6,250.00</td>
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<td>Virginia Unemployment Insurance</td>
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<td>Surety Bond — Treasurer</td>
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<td>Other Meetings</td>
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<td>Miscellaneous Expenses</td>
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<td>Bank Service Charge</td>
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<td>$67,558.92</td>
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CASH BALANCE — September 30, 1979:  

<table>
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<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash on Hand — September 30, 1979</td>
<td>$898.18</td>
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<tr>
<td>Bank of Virginia</td>
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<tr>
<td>Richmond, Virginia</td>
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<td>Checking Account</td>
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<tr>
<td>Savings Account</td>
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<td>Savings Certificate</td>
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<td>$43,303.68</td>
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</table>
UNITED STATES ANIMAL HEALTH ASSOCIATION
SUITE 205, 6924 LAKESIDE AVENUE
RICHMOND, VIRGINIA 23228

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1978 through SEPTEMBER 30, 1979

REVENUE:

Total Cash Receipts $ 73,840.19
Less — Expenditures 67,558.92
Excess of Receipts over Expenditures $ 6,281.27

NET WORTH — SEPTEMBER 30, 1979:

Cash on Hand — September 30, 1979 $ 898.18
Balance:
Bank of Virginia
Richmond, Virginia
Checking Account 923.99
Savings Account 16,474.70
Savings Certificate 10,000.00
Prepaid Dues, Tours and Registration 15,006.81
Accounts Receivable 5,175.00
Petty Cash Fund 100.00
Deposit — C & P Telephone Company
Richmond, Virginia 100.00
Inventory — Supplies and Proceedings 27,800.00
Furniture and Fixtures 4,772.16

NET WORTH — SEPTEMBER 30, 1979 $ 81,250.84
ANALYSIS OF CHANGE IN NET WORTH:

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<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net Worth — September 30, 1978</td>
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<tr>
<td>Increased by:</td>
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<tr>
<td>Cash on Hand</td>
<td>683.68</td>
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<tr>
<td>Checking Account</td>
<td>890.53</td>
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<tr>
<td>Savings Certificate</td>
<td>10,000.00</td>
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<tr>
<td>Prepaid Dues, Tours and Registration</td>
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<tr>
<td>Accounts Receivable</td>
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<td>$87,074.02</td>
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<td>Furniture and Fixtures</td>
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<td>NET WORTH — SEPTEMBER 30, 1979</td>
<td>$81,250.84</td>
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Henry H. Budd, Accountant.
ANALYSIS OF MISCELLANEOUS EXPENSE:

<table>
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<td>Ella R. Blanton — Hospital</td>
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<td>Dr. M.D. Mitchell</td>
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<td>Dr. J.B. Taylor</td>
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ANALYSIS OF BANK SERVICE CHARGES:

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<td>Safe Deposit Box Rent</td>
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<td>Bank Service Charges</td>
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<td><strong>$104.57</strong></td>
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ADDRESS OF THE PRESIDENT-ELECT

B. W. Hawkins
Ontario, Oregon

USAHA Members and Guests:

It is with a great deal of humility and pleasure that I address you as President-Elect of this great organization.

I feel pleasure because I have long looked forward to the time when Industry and Regulatory could truly work together for the attainment of a common goal of disease control or eradication, whichever was the most attainable with the tools at hand and the cost benefit ratio ever in mind. I feel that time is now and I will continue to do everything within my power to enhance the work that has preceded this point in time.

I feel humility because I am only too aware of the breadth and scope of this organization's influence in Animal Health matters. As a non-professional member of USAHA I will draw heavily on members who have expertise in the fields that professional or scientific decisions need to be made in.

I want to allay any fears that some of you may have in this area. Technical and professional decisions need to be made by professionals. but there are other decisions to be made in Animal Welfare and in these matters I intend to use the power of this office to promote to the best of my ability good Animal Health with reasonable regulations.

It is to the latter point that I would like to speak at this time. There are regulations and even laws in this great country of ours that are completely without rationalization and because of the breadth of USAHA's membership I intend to use it's influence to correct or at least attempt to correct these inequities.

The preservation of our minor species, I think is vital. Because of antiquated legislation our sheep industry is having unwarrented problems in many areas. The liver fluke problem to mention one, is in my estimation a problem that need not have reached the stage that it has. This has been promulgated by the Delany amendment.

We need to fight for sound human and animal health, but we need to fight equally hard against crippling legislation that is unsound, that prevents good health. By working in concert with organizations like the Animal Health Institute and national industry groups we can be effective in these areas.

The Secretary of Agriculture is in the Cabinet office to speak for agriculture, and we must make him more aware of his responsibilities
by taking our concerns to him and securing his help in working legislation through Congress.

The withdrawal of antibiotics until proven to be detrimental certainly is within our purview to try and help attain a sound decision. Again, antiquated legislation requires administrators to do things they might rather not do, rationalization needs to be amended into the Delaney amendment.

I wholeheartedly endorse past presidents who have said the committees are the backbone of this or any organization. Our current president did a formidable job of realigning people on committees and he is to be congratulated for his two years of dedicated service and I do at this time want to thank Dr. Tom Zweigart for a job well done.

For those of our members who want to serve on specific committees, I would appreciate hearing of their desires. Much of this decision will be given to committee chairmen, but we give our best performance in an area we have concern in or have particular knowledge of.

Our visibility has been enhanced by the work of our Public Relations Committee. We need to expand this in our work on Capitol Hill. Again, because of the recent changes in our constitution and by-laws our combined strength can be used to an advantage because we truly speak for the best interests of the livestock industry with the consuming public the ultimate benefactor.

Now for areas of particular concern. Number 1 — Brucellosis:

Enough time has been spent on a study, asked for the industry and with suggested changes and received by APHIS, and discussed for one year by our Brucellosis Committee and industry, that it is my firm hope when we leave this Convention we will all go home with the commitment to achieve the goals that have so laboriously been hammered out. Let us be flexible when possible and firm when demanded.

With the increased mobility of livestock and the need to export more for various reasons Anthropode-born diseases need to be given more attention. Control measures continue to elude our researchers. I will be considering forming a committee to give these added emphasis. Anaplasmosis, Blue Tongue, Lucosis. All are involved in the exporting of livestock.

By mentioning a few areas of particular concern, I by no means want to diminish from the importance of other committees or their area of concern, for it takes continued vigilance and work in all areas for a healthy livestock industry.

In a democracy such as ours it has given me a lot of satisfaction the last several years to be able to work, debate and yes, even to argue, with the professionals of this organization in committee activities.
Decisions haven't always been to my liking but if we all remember that right will prevail and if we believe in it and continue to work hard for it we will achieve right in the end and that is to achieve the healthiest livestock industry in the world, with the minimum of regulation and at the least possible cost.

I will be asking the Executive Committee for authority to change a long standing practice of giving a copy of the proceedings to each individual member. I believe that the dues paid for official or allied membership is sufficient to entitle them to proceedings, but with escalating printing and mailing costs it becomes a matter of either increasing the individual dues or asking them to pay for the cost of the proceedings, and we need the input of the members. At the time of paying dues, a box could be checked and the cost of printing and mailing added to the dues for the proceedings.

I am concerned that as we progress and become a more vocal force in legislative matters that a system could be developed to get a concensus of opinion from the Executive Committee in the interim between conventions and will propose a system to the committee at this Convention.

These are a few items of particular concern, and in closing I would like to use this little quotation that has been used so many times before—“God grant me the serenity to accept the things I cannot change, Courage to change the things I can, and the Wisdom to know the difference.”
B. W. Hawkins, President-Elect presents plaque to outgoing President, Dr. T. F. Zweigart, for his outstanding leadership for the years 1977-1979.
REMARKS OF THE PRESIDENT
T. F. Zweigart, D.V.M.
Raleigh, North Carolina

Members of the United States Animal Health Association, members of the American Association of Veterinary Laboratory Diagnosticians and honored guests. As your lame duck president, I shall confine my remarks mostly to expressions of appreciation. First, I would like to thank members of the USAHA for honoring me with the privilege of serving as their president, and for the plaque given in recognition of my service.

Presidents come and go so the real strength of the Association is in the membership—especially those who take active parts as committee members. I would like to thank the members who have served on Committees and especially those who worked as Chairmen.

It is easy to take the staff for granted since they always seem to be there when you need them, yet without their support, necessary things would not get done. The staff is small but they gave me good support and honored all of my requests for assistance. I would like to thank Dr. J. C. Shook, Mrs. Ella Blanton and Mrs. Linda Ragland for all of the cheerful, willing help they gave me.

The membership has chosen an outstanding slate of officers to follow me, so I know the near term future of our Association is in capable hands. I am sure you will give them the same good support you have given me.

From all indications this meeting promises to be one of our best, and with your help it will be. Please join me in making the 83rd Annual session of the USAHA one we will all remember as both productive and enjoyable.
Dr. F. J. Mulhern, Administrator, presents Animal and Plant Health Inspection Service's Animal Health Award to Dr. A. G. Boyd, former State Veterinarian of California.
REPORT OF THE COMMITTEE ON NOMINATIONS
AND RESOLUTIONS

The Committee on Nominations and Resolutions presents the following slate of candidates for election at this meeting:

President ........................................ B.W. Hawkins
Ontario, Oregon

President-Elect .................................. L. W. Hinchman
Indianapolis, Indiana

1st Vice President .............................. R. J. Stadler
Hartford, Connecticut

2nd Vice President .............................. G. B. Rea
Salem, Oregon

3rd Vice President .............................. J. R. Ragan
Nashville, Tennessee

Region Representatives

Northeast ......................................... F. G. Buzzell
E. S. Bryant

North Central ................................... J. R. Bishop
Bill Gallagher

South ................................................ J. O. Pearce
Joe Finley

West .............................................. O. H. Timm
Robert Nicholas
RESOLUTIONS PASSED AT THE SAN DIEGO MEETING

(Full background copies may be obtained from the USAHA office, Richmond, Virginia.)

Resolution No. 1

RESOLUTION

The United States Animal Health Association (USAHA) will continue urging the Secretary of Agriculture to consider fever tick eradication in Texas as a national problem for which there is no assurance that this devastating disease potential will remain within said state; and

Members of this body (USAHA) also support all sound means and measures that can be feasibly arranged and implemented between the United States and Mexico for creating a fever tick buffer zone between the two countries.

Resolution No. 2

RESOLUTION:

The United States Animal Health Association recommends that Veterinary Services, APHIS, USDA, seek immediate means for providing sufficient funding to establish and maintain an efficient program for monitoring and investigating diseases of wildlife that potentially relate to domestic livestock and poultry.

NOTE: This identical Resolution was unanimously adopted on October 2, 1979, by the USDA, APHIS, Advisory Committee on Foreign Animal and Poultry Diseases.
Resolution No. 3
USAHA Meeting
Held at San Diego, CA Dates October 30, 1979
Source Committee on Wildlife Diseases - Frank A. Hayes, Chairman
Subject Matter Wildlife Management

RESOLUTION

The United States Animal Health Association will join with IAPWA in opposing pending municipal, state and federal legislation directed toward abolishing legal annual harvest of surplus wildlife populations; and

USAHA recognizes sport hunting, humane trapping and fishing as legitimate tools for the scientific management of surplus wildlife populations, and will thus assist in maintaining the health of this resource.

Resolution No. 4
USAHA Meeting
Held at San Diego, CA Dates October 31, 1979
Source Swine Brucellosis Subcommittee
Subject Matter Swine Brucellosis Study

RESOLUTION

The Brucellosis Scientific Advisory Committee of ASAHA, in conjunction with the Swine Brucellosis Subcommittee of the Brucellosis Committee of USAHA, and in consultation with state and federal regulatory officials, pork producer leaders and marketing interests, shall conduct a study of the present Swine Brucellosis Eradication Program, as established in the Uniform Methods and Rules, with the aim of modifying the program to solve the problems outlined above; such study to be completed within the next year and a report made at the 1980 meeting of USAHA.
Resolution No. 5  U.S. Animal Health Association Meeting
Held at San Diego, CA  Date: October 30, 1979
Source: Submitted by The Committee on Sheep and Goats
Subject Matter: Resolution on Bluetongue

RESOLUTION:

Be it resolved that the current level of research by State and Federal Agencies be maintained and that serious consideration be given to increasing the level of support, and

Be it further resolved that an appropriate agency or institution be encouraged and financially supported to conduct a precise economic impact study on Bluetongue virus infection in our food and wildlife animals.

Resolution No. 6  U.S. Animal Health Association Meeting
Held at San Diego, CA  Date: October 30, 1979
Source: Submitted by the Committee on Sheep and Goats
Subject Matter: Resolution on Foot Rot

RESOLUTION

Foot Rot is a serious disease problem of the sheep industry. This Committee requests that U.S.D.A. give priority to this problem with funds for Foot Rot research.

Resolution No.: 7  U.S. Animal Health Ass'n. Meeting
Held at San Diego, CA  Date: October 30, 1979
Source: Submitted by the Committee on Sheep and Goats
Subject Matter: Ram Epididimitis

RESOLUTION

Be it resolved that the Veterinary Biologics Division of U.S.D.A. reconsider their standards for production upon the application for production of Ram Epididimitis vaccine previously produced by the former licensee or other licensee.
RESOLUTION:

The advent of serological testing for bovine leukemia virus infection has resulted in restrictions on the movement of cattle in international trade, this slowly spreading infection has become of increasing significance to producers engaged in the exportation of breeding stock.

Therefore be it resolved:

1. That the U.S.A.H.A. request that the USDA Animal and Plant Health Inspection Services undertake and encourage activities that will lead to the standardization of serological testing procedures used by official laboratories for the identification of bovine leukemia virus infected cattle.

2. That the USDA Animal and Plant Health Inspection Services conduct seroepidemiological surveys for bovine leukemia virus infection in an attempt to better evaluate the prevalence of the infection in the United States.

3. That the USDA Animal and Plant Health Inspection Services undertake a study of the feasibility of developing voluntary official programs, on a state or national basis, for the control and/or eradication of bovine leukemia virus infection within cattle herds. Certification should be provided for herds that have initiated specific control programs or herds that are bovine leukemia virus free.

4. That the special interest groups involved in exporting cattle be encouraged to fund and participate in additional research regarding bovine leukemia virus control and eradication programs.

5. That the USDA Animal and Plant Health Inspection Services recognize that current studies indicate bovine leukemia virus is neither transmitted by, nor present in, semen from infected bulls.

RESOLUTION:

Be it resolved that that the United States Animal Health Association advocates the development of regulations by the states prohibiting the sale of raw milk or raw milk products.
Resolution No. 10

Held at San Diego, Ca.

Source Food Animal Hygiene Committee

Subject Matter Condensation

RESOLUTION

Be it resolved that a national survey of meat and poultry plants be conducted to determine the possible pathogenicity of the microbiological content of condensate that forms in product holding and processing areas.

Resolution No. 11

Held at San Diego, CA

Source Northeast U. S. Animal Health Association

Subject Matter Foot and Mouth Disease

RESOLUTION:

The USAHA requests APHIS, in cooperation with the states, review the eradication program for FMD as soon as practicable.

Resolution No. 12

Held at SAN DIEGO, CALIFORNIA

Source PARASITIC DISEASES AND PARASITICIDES COMMITTEE

Subject Matter PSOROPTIC SCABIES CONTROL

RESOLUTION

Therefore, be it resolved that: The U.S. Animal Health Association recommends to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service that no relaxation from the present quarantine and compulsory dipping regulations be allowed and that funds at least equal to those appropriated during the past 2 years be made available each year for the purpose of continuing quarantine and dipping regimens until psoroptic scabies of cattle is finally eradicated from the United States.
Resolution No. 13

Held at SAN DIEGO, CALIFORNIA Dates 10/28/79 to 11/2/79

Source PARASITIC DISEASES AND PARASITICIDES COMMITTEE

Subject Matter PSOROPTIC SCABIES RESEARCH

RESOLUTION

Therefore, be it resolved that: The USAHA go on record as favoring increased financial support by USDA, SEA/AR and CR of ongoing research projects involved with Psoroptes at Kerrville, Texas, Las Cruces, New Mexico and Ft. Collins, Colorado.

Resolution No. 14

Held at San Diego, Calif. Dates October 31, 1979

Source Establishment of a National Leptospiral Reference Laboratory

Subject Matter to be Supported by the U. S. Department of Agriculture

RESOLUTION

Therefore be it resolved that the United States Department of Agriculture utilize the funds appropriated by the 95th Congress to develop and implement (1) expanded research efforts to develop improved techniques to detect and rapidly identify leptospires in animals, (2) to expand leptospirosis epidemiologic services activities for domestic livestock, and (3) an intensified centralized leptospira stereotyping services including strain identification.

Resolution No. 15

Held at San Diego, California Dates October 30, 1979

Source Animal Welfare Committee - Chairman, E. Mickey Stewart

Subject Matter Animal Welfare

RESOLUTION

The USAHA recommends to APHIS, USDA, that these holding periods should be waived by regulation for breeders or buyers if dogs and cats are less than 12 weeks of age and sold for use as pets and still remain within the intent of the Animal Welfare Act.
RESOLUTION

The USAHA urges USDA and USAID to develop an international symposium on Avian Influenza, to cover such topics as occurrence, epizootiology, virology and control.

A working committee, including international representatives, work out the details for such a symposium to be held in the U.S. and develop guidelines to the classification of highly virulent strains of the virus and their control.

RESOLUTION

Be it resolved that the USAHA strongly urges USDA, APHIS to grant contractual awards only to responsible manufacturers of metal ear tags who can demonstrate adequate performance of the products.
Resolution No. 18  USAHA Meeting

Held At: San Diego, CA Dates: 10/31/79

Source: Committee on Transmissible Swine Diseases

Subject Matter: Pseudorabies

RESOLUTION

Be it therefore resolved that:

1. Additional federal and state funds be made available for the support of the pseudorabies program and

2. A feasibility and cost effect study of an eradication program be conducted by APHIS through an ad hoc committee consisting of scientists and representatives of all aspects of the swine industry. The committee shall gather information relative to the current and future costs to the swine industry as well as design a program outline which will establish the time frame for an eradication program and recommend regulation structure for the program.

3. The USDA is to be encouraged to continue its support for pseudorabies research, and

4. State, federal, and extension veterinarians are encouraged to assemble and disseminate information describing the advantages and disadvantages in control and eradication programs.

UNITED STATES ANIMAL HEALTH ASSOCIATION:

Resolution No. 19  USAHA Meeting

Held at: San Diego, CA Dates: October 30, 1979

Source: Committee on Parasites and Parasiticides

Subject Matter: Integrated Pest Management of Livestock Pests

RESOLUTION

The U. S. Animal Health Association supports the Budget increase proposal for integrated pest management of insects affecting livestock and poultry and urge the USDA-SEA to fund this proposal as soon as possible.
Resolution No. 20

Held at: San Diego, CA

Source: Committee on Parasites and Parasiticides

Subject Matter: Research on Ticks in Puerto Rico

RESOLUTION

The U. S. Animal Health Association urges the USDA-SEA AR to establish a temporary research laboratory in the Commonwealth in order for scientists to study for biology, life history, and control of these species, in order to provide the necessary information to the action agencies, so that they can efficiently and effectively eradicate these agents.

Resolution No. 21

Held at: San Diego, CA

Source: Committee on Livestock Identification

Subject Matter: MCI Traceback and Brucellosis Surveillance

RESOLUTION

1. Records be made in each state that identifies the apparent reason for each unsuccessful traceback. These should be summarized quarterly and annually. APHIS should collect and summarize this information nationally. Records for traceback from first market of arrival tests should be summarized separately. This information should be utilized at county, state and national level as a basis of program improvement. Priority should be given to establishing these records in the southeastern and southcentral states. After three years, the need for continuing such records should be reviewed.

2. Methods of maintaining surveillance where multiple dealer and/or market movement should receive special study with a view of developing a system or systems which is practical for the Brucellosis Program, the dealer, and the market operator. In this study, priority should be given to the southeastern and southcentral states.
Resolution No. 22

USHA Meeting

Held at: San Diego, CA Dates: October 28 - November 1, 1979

Source: Epizootic Attack Committee and Transmissible Diseases of Poultry

Subject Matter: Importation of Exotic Birds

RESOLUTION

The U. S. Animal Health Association urges the Department of Agriculture to construct or modify existing facilities so that all importation of exotic birds will be through properly designed and operated government owned quarantine stations, as is done with all other animals.

The U. S. Animal Health Association urges that no additional privately owned stations be approved and the presently approved private stations be phased out of use as rapidly as is feasible.

Resolution No. 23

USHA Meeting

Held at: San Diego, CA Dates: October 28 - November 1, 1979

Source: Import-Export Committee

Subject Matter: Comptroller General of the U.S. Study GC-79-84

RESOLUTION

Now therefore be it resolved that the USAHA alert other agricultural organizations and their informational services to the dangers of animal disease introduction by passengers and/or their baggage. Reducing current inspectional procedures, as suggested in the GAO report, could increase the risk of exotic animal or plant diseases entering the U.S.
Resolution No. 24

USAHA Meeting

Held at: San Diego, CA

Source: Import Export Committee

Subject Matter: Control of Any Species as Potential Vectors of Exotic Diseases.

RESOLUTION:

Therefore, be it resolved that the committee on import export, USAHA recommends that USDA, Veterinary Services in co-operation with the U.S. Public Health Service or other regulatory agencies work to assure that the Department of Agriculture's interests are met as well as those of other agencies and that such importations are made only to APHIS approved facilities when livestock and poultry diseases could be involved.

Resolution No. 25

United States Animal Health Association Meeting

Held at San Diego, California

Source: Import-Export Committee

Subject Matter: IMPORTATION OF HORSES FROM CONTAGIOUS EQUINE METritis COUNTRIES

RESOLUTION

be it resolved that the United States Animal Health Association oppose the proposed rule-making which would allow entry into the United States of stallions from Contagious Equine Metritis countries, without the treatment and the ancillary control of breeding virgin mares successfully prior to entry.

Resolution No. 26

USAHA Meeting

Held at

Source MORBIDITY AND MORTALITY

Subject Matter REPORTING PROGRAM

RESOLUTION

be it resolved that the U.S. Animal Health Association request the Secretary of Agriculture to implement procedures through appropriate Federal and State agencies, an animal morbidity and mortality reporting and data gathering system.
How To Become a Member of USAHA

To become an individual member of USAHA, write to:

USAHA
Suite 205, 6924 Lakeside Ave.
Richmond, VA 23228

and request an application. The individual dues are $20.00 per year and this entitles the member to receive the Annual Proceedings and all mailing materials from the USAHA office.
A VETERINARIAN'S GLIMPSE AT ANIMAL HEALTH
IN THE PEOPLE'S REPUBLIC OF CHINA

John L. Hyde

U.S. Department of Agriculture,
Science and Education Administration,
Agricultural Research, National Program Staff,
Livestock and Veterinary Sciences,
Beltsville, Maryland 20705

The information given below is based on that given to us by Chinese scientists during a visit of a group of animal scientists, an anthropologist, an economist, and a veterinarian to the People's Republic of China for about 28 days in 1979. This tour of the People's Republic of China (PRC) was sponsored by the National Academy of Sciences of the United States and the Chinese Academy of Sciences under the Committee on Scholarly Communication with the PRC. This part of the report will deal primarily with diseases of food-producing animals and some of the methods for controlling or preventing such diseases.

There appears to be no question that China has germplasm, especially of swine, that might benefit the American producer. At the same time, the Chinese want to obtain American germplasm, not only from swine, but also from other animals. Before we can consider importation of germplasm, we must have definitive information on the animal disease situation in China. Although the Chinese tried to give us the full picture, it seems likely that they have animal diseases of which they are not aware. This assumption is based, in part, on the knowledge that the Chinese have imported animals from countries where diseases exist that are said not to be present in China. Also, the Chinese diagnostic work, especially virological diagnosis, needs much improvement. Finally, we did not visit diagnostic laboratories enough to obtain firsthand information on the number of samples or animals presented each year and how well integrated the laboratories are.

One of the most significant observations was the great number of people taking care of animals. As a result, animal care is excellent and animals are very tame. It seemed that wherever we went, if there were only three or four ducks or pigs or even one animal, there was always someone with them. This continual care and observation probably helps to decrease the amount of mortality and morbidity among livestock and poultry in China.

We had no opportunity to see any diseases in the field nor to see autopsies or sick animals at the institutes we visited. The only obvious clinical lesion observed was in a single pig; it was an opening in the skin of the jowl area made either by a lance or by a break to the outside, probably a jowl abscess. Occasionally, we saw what appeared to be the beginning of atrophic rhinitis.
Bacterial and viral respiratory diseases, a problem in most animals, are, of course, more serious in areas where there is more stress due to environmental changes, i.e., changes in temperature, humidity, exposure, etc. With the possible exception of two or three facilities, we saw no diagnostic virology being done as we know it in the U.S.A. While knowledge of bacterial diseases is fairly good, the diagnostic capability for viral diseases leaves much to be desired in the way of accuracy and completeness. The research facilities we visited varied from poor to fairly good. The equipment was the same way. Some of it was 12 to 15 years old and appeared to have been used very little. One of the institutes we visited, the Veterinary Institute in Harbin, appeared to be doing the most advanced virological research on animal diseases in China.

MISCELLANEOUS VIRAL DISEASES

Foot-and-mouth disease was said to be eradicated in some places and controlled in other places by vaccinating along the borders, especially the southern borders. The Chinese are vaccinating against types A and O; there is no vaccine for the Asia serotype of the disease. The vaccine used is a mouse-attenuated live vaccine.

Japanese B encephalitis is seen in virtually all parts of China. A vaccine with virus attenuated in cultured hamster kidney cells is used for horses and, in some areas, for pigs. There is also a vaccine for human use. Many species of animals susceptible to Japanese B encephalitis virus are not protected by vaccination.

OTHER IMPORTANT DISEASES

Swine influenza, colibacillosis, actinomycosis, coccidiosis (mostly in chickens and rabbits, but sometimes in other animals), indigestion in ruminants, pigs, and horses, and internal parasites in all species, including chickens, are problems in raising animals. Neoplasms much as we see in this country are found. There are plans to begin work next year at Harbin on colibacillosis and pseudorabies.

Anthrax as well as blackleg is seen in all ruminants, and malignant edema occurs in ruminants as well as in horses and swine. Necrobacillosis is seen in sheep and cattle. Salmonellosis occurs in ruminants, horses, chickens, and pigs. Tetanus can be a problem in ruminants as well as in horses and swine. Pasteurellosis is a problem in most animals, especially sheep, pigs, cattle, and goats. Colibacillosis is seen in all species. Although vaccines are used against it, brucellosis occurs, especially in cattle, sheep, pigs, and goats. It is questionable whether the mastitis-metritis-agalactia syndrome is present in China; however, mastitis from \textit{E. coli} does occur in sows.

Leptospirosis occurs in pigs, sheep, horses, dogs, cattle, and man. Although man is vaccinated against \textit{Leptospira pomona} 396 and \textit{L. grippotyphosa}, no \textit{Leptospira} vaccines are available for animals.
ANIMAL HEALTH IN THE PEOPLE'S REPUBLIC OF CHINA

PARASITES

Parasites are a problem in many areas of China. Roundworms are a significant problem in swine. In some areas, coccidiosis is not very severe in swine, and it is seldom seen by 1 year of age; in others, coccidiosis is severe, especially in poultry. The swine kidney worm is seen, but how much damage it causes is not known. *Sarcosporidia* is a problem in swine as well as cattle. *Echinococcus granulosus* is seen in pigs, cattle, and sheep. The tapeworms *Taenia saginata* and *T. solium* are known. *Trichinella spiralis* is reported in pigs.

NUTRITIONAL DEFICIENCIES

Selenium deficiency is found in certain parts of China. Essentially all species of animals, including man, are affected. The principal lesions in animals are seen in the skeletal muscles, much like those we see in lambs and calves in this country in so-called "white muscle disease." Deficiencies in other minerals, including iron and sulfur, also cause disease. There is avitaminosis A, B₁, and B₂, especially in poultry.

DISEASES OF SWINE

We asked about the presence of vesicular diseases in China every place we traveled. We were especially interested in finding out whether anything resembling swine vesicular disease had ever been present. The Chinese said they had never seen it. This is curious since the Italians concluded from their traceback after the first outbreak of the disease in Italy that swine imported from China were the source of the infection.

The major disease problems in swine are digestive diseases, transmissible gastroenteritis, swine edema, erysipelas, atrophic rhinitis, enzootic pneumonia (mycoplasmosis pneumonia), and swine abscesses.

Swine edema is caused by a hemolytic *Escherichia coli*. It appears at 3 to 4 months of age or shortly after weaning. The edema is seen along the mesenteric and gastric mucosa and is accompanied by an abundance of ascitic fluid, sometimes with fibrin.

Erysipelas, pasteurellosis, and hog cholera are controlled by use of a triple vaccine, really a virus-bacterin mixture. The attenuated bacterins for erysipelas and *Pasteurella* are not as effective as the Chinese would like. They are administered orally or parenterally, but more organisms are needed for the oral route than for the parenteral route. Hog cholera vaccine is a virus attenuated in rabbits (lapinized). It is lyophilized and administered twice a year. Vaccination is compulsory and apparently is very effective, since hog cholera or classical swine fever is not much of a problem.

In the Harbin area, research is in progress on transmissible gastroenteritis and atrophic rhinitis. Work on prevention of atrophic rhinitis is concentrated on the *Bordetella bronchiseptica* organism.
Enzootic pneumonia, caused by *Mycoplasma hyopneumoniae*, is a problem especially in breeding herds. Work on enzootic pneumonia is being done at Peking, Shanghai, Nanking, and Canton.

Swine abscesses are a problem, mostly in the south. The disease is referred to as streptococcosis of swine.

Brucellosis is a problem in swine, especially in the southeastern part of China. Sows are vaccinated. Any animals found positive are slaughtered. Strain 19 brucella organisms used to be used for vaccination against brucellosis, but this vaccine is no longer being produced. Instead, the Chinese use two strains referred to as Strains 2 and 5. Strain 2 is developed from *Brucella suis*. It is given orally in drinking water to pigs and sheep. If it is injected parenterally, it produces abscesses. The Strain 5 brucellosis vaccine is made from *Brucella melitensis*. It is administered by inoculation or aerosol mainly in sheep and cattle, and apparently is satisfactory.

Beta hemolytic streptococci produce an acute, very high fever with hemorrhage. This disease is differentiated from that caused by *E. coli* in that it is more acute and involves a higher fever. The disease can occur at any age and is most prevalent in the south of China. It can resemble erysipelas. Once it is detected, antibiotics are very effective in treating and controlling this disease.

Some other diseases that occur in swine, as well as in other animals, are anthrax, salmonellosis (serotypes unknown), tetanus, and toxoplasmosis. In some parts of China, the incidence of toxoplasmosis in pigs is as high as 30 percent. There is concern about rotaviruses, and there is some evidence that rotaviruses are a problem in China. Pseudorabies is present but apparently sporadic. Trichinosis is present. Mycobacteriosis is also present; however, most of the tuberculosis in swine originates as bovine tuberculosis. The Chinese have not seen the avian type yet; if it is present, it has not been reported.

**DISEASES OF CHICKENS**

*Viral*

Newcastle disease is controlled by vaccination with chick embryo vaccine containing virus of Strains 1, 2, F, and LaSota attenuated. Strain 1 is the Indian strain Mukteswar; Strain 2 is the Hitchner strain. Newcastle vaccine is administered by aerosol, which is calculated to insure 1 ml of virus-containing allantoic fluid for 500 chickens at 1 month of age. The vaccine is also administered in drinking water and intranasally at 1 day; then, at 1 month, the chickens are exposed to the aerosol. If chickens are exposed by aerosol at an early age, i.e., 1 day, they often develop mycoplasmosis. Fowl plague or pathogenic influenza in birds has not been observed. Infectious bronchitis is a problem, especially in state-owned confined-rearing operations; research is underway in hopes of developing a vaccine.
Leukosis is widespread. The Chinese are doing what everybody else is, i.e., living with it. Leukosis is the greatest obstacle to the establishment of specific-pathogen-free birds.

Infectious laryngotracheitis is quite common in the southern part of China. There are no protective measures, but work is underway to develop a vaccine.

Adenovirus infections are being looked for, and indications are that they may be present in some flocks.

Chicken pox was a problem, but it is now controlled by use of a pigeon pox virus vaccine. Marek’s disease is controlled by vaccination. Viral encephalitis has been suspected in birds, but no virus has been isolated as yet.

Other

*Mycoplasma synoviae* is present in broilers in China but apparently is not a major problem; there is no vaccine and the development of one has been given low priority. Chlamydial infections are thought to be present but so far have not been identified; if present, they must not be a serious problem. Chronic respiratory disease is seen mostly in confined operations.

Aflatoxin problems are seen in pigs and poultry fed “poor grain.” Aflatoxin in chickens is seen mostly in the southern part of China and is associated with moldy peanut meal. In the north, it is due to moldy corn.

Immunization against fowl cholera is by aerosol so that each bird is exposed to 100 to 200 million attenuated *Pasteurella multocida* organisms. The organisms are cultivated at 20°C to 22°C and passaged several times, until they are determined to be safe for use.

Salmonellosis in poultry is widespread.

Infectious coryza is not seen much; no protection is yet available. The use of antibiotics in feed is said to be effective.

Internal and external parasites are a problem, especially in birds raised on ground. Histomoniasis (blackhead) is seen in chickens, but it is not important. The Chinese have nothing to prevent it.

**DISEASES OF DUCKS AND GEESE**

Fowl cholera, salmonellosis, and parasites (especially coccidia) are the most serious diseases of ducks. Piperazine is used to control roundworms.

Pesticide residues were a big problem when DDT was used. More organophosphates are used today, and these can have serious effects including paralysis and deaths.

In addition to diseases mentioned above, some major diseases of ducks in the U.S.A. are also seen in China. They are duck virus hepatitis, *Pasteurella anatipestifer* infections, colibacillosis, salmonellosis, necrotic...
enteritis, and aflatoxin poisoning. Duck plague (duck virus enteritis) has not been reported in China.

According to the Chinese, the most serious disease of geese is a viral disease, “gosling plague.” The characteristic lesions are granular swellings on the intestine. Apparently, every few years, there is an epidemic of this disease. It is seen only in young geese. For control, older geese are vaccinated, which results in passive antibody transfer to the newborn, protecting them until they pass the age of susceptibility or become immune from exposure while carrying protective antibody. Fowl cholera is not a serious problem in geese. According to the Chinese, coccidiosis is not seen in geese.

DISEASES OF CATTLE

The shipping fever syndrome in cattle that we see in this country either does not exist or is not recognized in China. When animals are shipped by rail, they are offloaded, fed, and watered along the way. The estimated time for shipping cattle from the railroad depot, 400 km from Huhehaote in Inner Mongolia, down to Shanghai, Canton, or Hong Kong is about a week.

The Chinese stated that they do not see bovine virus diarrhea or mucosal disease and parainfluenza-3; if these diseases are present, they have not been recognized. Bluetongue is not seen in China in either cattle or sheep.

The malignant catarrhal fever that occurs in China is much like that seen occasionally in the United States. It is always associated with the presence of sheep, and usually one or two animals in a herd are affected. The 20 to 30 percent infection rate that we have seen in the United States in recent years has not been seen in China.

Parasitic

Trypanosoma evansi is seen mostly in water buffalo. There are no clinical signs of infection in cattle, but the parasite can be found in cattle. Babesiosis is seen occasionally throughout the country, mostly in the low ranges around the Yellow River. The Chinese state that it is seen in imported breeds. The yellow indigenous cattle are resistant but can be carriers of the parasite, which is transmitted by ticks. Schistosomiasis (caused by Schistosoma japonicum) is seen in cattle, water buffalo, and humans. Fasciola hepatica (and, in southern China, Fasciola gigantica) is seen in sheep, cattle, and water buffalo. Lungworms are present in sheep, pigs, and cattle.

Other

Listeriosis is recognized in swine, chickens, and sheep, but indications are that it is not found in cattle. Pasteurella hemolytica evidently is a significant problem in cattle.

Paratuberculosis is seldom found and, when found, it is in imported
ANIMAL HEALTH IN THE PEOPLE'S REPUBLIC OF CHINA

cattle. Here again, it is questionable just how hard the Chinese are looking for this disease. As in the United States, paratuberculosis is probably far more prevalent than is recognized.

Foot rot is rarely a problem in cattle.

Bovine leukemia is seen clinically and histopathologically; frank lymphatic leukemia also occurs, mostly in the northwestern part of China in cattle imported from Germany. This is another instance of the hazards of importing animals from foreign countries apparently without any prior testing or regulations requiring testing for diseases that do not exist in China.

Mycotoxicosis in cattle, from eating moldy sweet potatoes, used to be seen quite frequently. Lesions are primarily in the lungs with severe pulmonary emphysema. In 1955, there was a severe outbreak in the central part of the northwestern part of China. However, mycotoxicosis today is no problem since the Chinese know the cause and avoid it.

Ringworm occurs in cattle.

As in this country, poisonous plants can be a problem, especially in the pastural areas and under conditions of drought and over-grazing.

DISEASES OF SHEEP

Foot rot does occur in sheep but evidently it is not as big a problem as it is in the United States. Caseous lymphadenitis has not been seen in sheep.

Enterotoxemia is primarily a problem in sheep. *Clostridium perfringens* vaccine, types C and D, is used for prevention.

Rabies outbreaks occur along the border in mountainous areas where foxes are found. It is seen in cattle and horses, and other species may also become infected.

Sheep pox is occasionally seen in the pastural areas (Inner Mongolia); sheep are vaccinated to prevent the disease.

Internal parasites, including *Echinococcus granulosus*, are one of the more economically important problems in sheep, as well as in other species of animals.

“White muscle disease” (vitamin E deficiency) occurs in sheep much as it does in the United States.

DISEASES OF HORSES

The filaria, *Onchocerca cervicalis*, that gets into the spinal cord and brain and is sometimes seen in the eye of horses in this country also occurs in China.

Piroplasmosis is seen; the Chinese know that *Babesia caballi* is present, but they are not sure about *B. equi*.
Equine influenza occurs, and no effort is made to prevent it by vaccination.

Glanders and strangles are present. Glanders is diagnosed by the mallein test.

Aneurysms are occasionally found in horses.

Horses are vaccinated to prevent anthrax, tetanus, and Japanese B encephalitis, and a Pasteurella bacterin appears to be of some benefit against pasteurellosis.

In Inner Mongolia, we were told that horses contract "contagious pneumonia." Lesions consist of a heavy, thickened pleura.

ABORTION IN GOATS

There is a goat abortion problem in Inner Mongolia. The cause is not known, but the history indicates that abortion occurs only in the first or second pregnancy; that is, older animals do not have a problem with abortion like that seen in young does.

Also there is a problem of fly maggots in wounds sustained by animals in Inner Mongolia.

CONCLUSIONS

The trip to China was extremely informative, educational, and rewarding. Although the Chinese scientists are very up-to-date with the veterinary literature, general impressions from the scientific standpoint are two important areas of concern:

a. Do the Chinese know the animal disease situation in China today?
   The information we obtained from the Chinese researchers and a visit to one diagnostic laboratory suggests that they do not. After the expulsion of the so-called Gang of Four, Chinese scholars are just getting started again after many years, so there is a great shortage of the younger, well-trained people. However, the Chinese believe that things will be much better after a few years.

b. The importation of animals into China has been indiscriminate, probably because of a lack of uniform rules and regulations. At one point, we were told that the Minister of Trade issues import permits for semen. Unless he is following the advice of someone like a veterinarian, who is well informed about the world animal disease situation, the Chinese are certainly taking great risks in importing animals or germplasm from many countries. For example, although scrapie in sheep has not been reported in China, one wonders if it isn't there, since sheep have been imported from Europe. The Chinese are well aware, though, that some rapid and significant actions must be taken to prohibit the importation into China of animals with diseases that China doesn't have, as well as any domestic diseases. In other words, the Chinese must be sure to import only healthy animals.
After we returned to the United States, we met with the visiting Chinese Delegation while they were with the staff of the USDA Animal and Plant Health Inspection Service, who have responsibility for import and export. This was probably the most important meeting for the Chinese during their visit in the United States. When the Chinese left after the day and a half with this group, they must have known full well the job that lies ahead if China is to exchange germplasm in any form with countries such as the United States.
REPORT OF THE COMMITTEE ON RABIES

Chairman: W. G. Winkler, Atlanta Ga.

Co-Chairman: L. N. Butler, Phoenix, Ariz.


The Rabies Committee met on October 29 with a total of 16 members and guests present.

The Committee reviewed recommendations made at the 1978 meeting and noted the following action during the intervening year:

1. The Compendium of Animal Rabies Vaccines has continued to gain acceptance among states as the basis for rabies vaccination requirements. Progress is being made on acceptance of the standardized vaccination certificate. A definition of “high risk” areas has been promulgated which agrees closely with that proposed by this Committee.

2. The experimental human diploid cell strain vaccine is approaching licensure and is expected to become commercially available early in 1980.

3. No specific improvements in control of bat or cat rabies have been made in the past year.

The Committee then addressed those topics which it felt might call for specific action:

1. The problem of vaccine-induced animal rabies was discussed at length; 25-35 cases have been identified depending on the definition accepted for vaccine-induced cases. Most cases of vaccine-induced rabies have been associated with low egg passage FLURY strain vaccines which have now been withdrawn from the market; however, several cases have been associated with other modified live virus vaccine. The Committee recommended that the U.S.D.A., the Center for Disease Control, and the National Association of State Public Health Veterinarians collaborate to develop improved surveillance and diagnosis of vaccine-induced rabies.

2. The Committee reviewed the problem of accidental exposure of humans to modified live virus animal rabies vaccines. While recognizing the technical difficulties in establishing the risk of rabies following accidental exposure, the Committee felt a concise statement of risk was needed and requested the Center for Disease Control develop such a statement. The Committee also recommended that CDC and Veterinary Biologics, USDA, jointly review the criteria established for licensure of new biologics to determine if
human safety could be more heavily weighed in vaccine licensure evaluation.

3. Following a presentation on intravitam diagnosis of rabies using fluorescent microscopic examination of corneal impressions and skin biopsies by Mr. Dennis Howard, the committee discussed the desirability of recommending this technique for routine use in rabies diagnostic laboratories; it was decided to defer any recommendations pending additional data to support the sensitivity and practicality of this diagnostic procedure.

4. The Committee reviewed the 1980 Compendium of Animal Rabies Vaccines developed by the National Association of State Public Health Veterinarians and endorsed the document, commending the NASPHV for their work in this area.

5. The Committee reviewed the status of rabies in the United States and expressed concern over the general increase in rabies especially along the U.S.-Mexico border. They discussed possible means to increase awareness of the problem by the public and the veterinary and medical professions but made no specific recommendations.

6. The threat of rabies spread by pet wildlife, especially skunks, foxes, and raccoons was discussed. Major James Valey and Dr. Ashley Robinson presented data on the pet skunk industry in Minnesota noting that several thousand skunks are marketed to the pet trade each year from Minnesota and that some of these animals have developed rabies and exposed persons. The difficulty in differentiating between pen-raised animals might be incubating rabies when marketed. Preliminary rabies serologic testing of skunks in one major breeding facility has suggested that both wild caught and pen-raised animals had been exposed to rabies. The Committee felt that the only satisfactory way to control this threat to human health would be to severely restrict sales of such animals through the pet trade. Accordingly, the Committee proposes the following resolution: "Whereas the problem of wild animal pet associated rabies is recognized as an increasing hazard by public health authorities, and "Whereas no feasible means exists for assuring that wild animal pets, especially foxes, skunks, and raccoons, are immune to rabies, then "be it resolved that the Rabies Committee of the U.S. Animal Health Association requests that the USDA and Public Health Service together develop rules to prohibit the interstate traffic in skunks, foxes, and raccoons for sale in the pet trade."

"Further, the committee urges that the respective states promulgate legislation to prohibit interstate sale and possession of pet skunks, foxes, and raccoons."

7. There being no further business, the committee adjourned.

Respectfully submitted,
W. G. Winkler, Chairman
The Mastitis Committee addressed the issues affecting the use of antibiotics in the treatment of Mastitis. A summary of current research on antibiotic residues in milk was given by Dr. W. Menz. Six rapid antibiotic assay systems have presently been developed. Of these, two are currently commercially available. The remaining methods show promise of facilitating the rapidity and simplicity of detecting antibiotic residues in milk on the dairy farm.

The basic principle employed by all new test systems is the immunoassay method. As a result of these developments, it appears that adequate methodology will soon be available to the dairyman to determine antibiotic residues in milk that result from treatment of mastitis or other diseases.

Information on the current status of the STOP test, as developed by the U.S.D.A., was presented by Dr. C. Jordan. Under this program, official testing of cull dairy cows and calves at slaughter commenced in 25 states on October 1, 1979.

Preliminary survey results identified antibiotic residues in over 18% of the dairy cattle tested.

The Committee questioned the contribution of intramammary infusion products to these tissue residues in both cows and calves. Adequate information is currently unavailable on the effect lactating and dry cow intramammary preparations have on tissue residues of slaughtered calves. The Committee encourages additional data to be generated on the effect that intramammary antibiotic medicants have on the tissue residues of both the fetal calf and the newborn calf which is fed colostrum containing antibiotics.

This committee evaluated the progress of the Mastitis Survey initiated as a result of the 1978 Mastitis Committee meeting. It was decided to continue efforts to complete these national surveys sent to both dairymen and practicing veterinarians. It was the general consensus of this Committee that the information on mastitis treatment which results can be beneficial toward the development of intramammary preparations which can more effectively treat mastitis.
Animal disease surveillance is considered to be the aggregate of epidemiologic mechanisms needed to detect, evaluate and measure animal diseases (both qualitatively and quantitatively) with respect to their geographic and seasonal occurrence, behavior and economic importance.

Disease reporting is presenting an account of animal morbidity and mortality data on a regular, official, formal basis. Data must be collected, compiled, analyzed and interpreted and 'disseminated'; and incidence reported. Reporting should be based on surveillance of populations at risk.

In 1971, the Infectious Disease Committee (IDC) of the Minnesota Veterinary Medical Association (MVMA) was asked by its president to develop a meaningful reporting system. This occurred following a discontinuation of the "postcard system." The Minnesota Livestock Sanitary Board (MLSB) has the official responsibility by statute to report diseases.

The IDC, which was composed of private and public practitioners, developed the following purposes for reporting:

1. In order for the practicing veterinarian to recommend proper preventive measures to the livestock industry, surveillance of disease with a data base is essential.

2. To assist in eradication, control and prevention of disease, regulatory agencies need to have knowledge of when, where and under what conditions disease occurs. The MLSB has the official responsibility to compile, edit, publish and distribute this information.

3. Improved definition and control of the disease problems reduces economic loss to the livestock industry.

4. Disease reporting encourages the livestock producer to have a better understanding of the value of veterinary service and results in either taking measures to prevent disease or begin treatment early before serious losses occur.

5. Disease reporting identifies disease problems for veterinary
research and justifies research based on data indicating incidence and trends. It provides a data base which can be used to accurately estimate loss due to disease.

6. As a result of reporting, a meaningful data base gives:
   a. indicators of incidence and changing trends.
   b. geographic distribution of diseases.
   c. knowledge of emergence of new diseases.
   d. basis for certification of absence of diseases.

COLLECTION OF DATA

The Minnesota reporting system is based on collection of data at the farm level. Continual animal surveillance is conducted by the livestock owner and his veterinarian. The livestock owner plays an important role in daily observation and on-the-farm reporting on a special form. The practitioner is mailed or picks up the farm report each month and includes it in his monthly report (see Figures I and II) of morbidity and mortality occurring on that farm. Diagnosis is primarily clinical with an unknown amount of laboratory confirmation. The basic data unit is an animal month. The data reported is the occurrence or nonoccurrence of a new case in an animal during the present month of surveillance.

Minnesota veterinary districts form 13 strata and the private veterinary practitioners form primary sampling clusters in a district. Farmsteads (or herds) form secondary clusters of animals. All animals in a herd and the months in which the animals are actually under surveillance are tabulated.

The state or federal veterinarians (13) assigned to each of the districts constitute an important aspect. They contact the 40 practitioners, deliver and explain the guidelines and forms and either pick up or are mailed the forms each month. The data is then mailed to the MLSB office in St. Paul.

Each of the 13 districts defines the agricultural regions of the state. Each region is internally homogeneous with respect to type of farmsteads. Minnesota has about 400 food animal practitioners, and in any given 6 month period about 10% are drawn at random by the IDC and asked to report. They are exempt if they have participated during the 2 previous years.

Each chosen practitioner cooperator makes a list of as many farmsteads as possible from which he believes he can elicit the quality of surveillance required. A random sample of approximately 3-5 numbers is then designated from the list as the farmsteads that he will ask to cooperate. Recently the practitioner has been asked to report on the first 3 farms called on each month.

The procedure produces a probability sample which is stratified multi-stage cluster in design, and the universe is the set of all farmsteads which would be capable of an adequate quality of surveillance.
COMPILATION OF DATA

The data are centrally accumulated and compiled at the MLSB and processed at the Computer Center at the University of Minnesota into incidence rates of new cases based on the denominator of animal months kept under surveillance for the period being reported. In addition, incidence rates of herds are also identified. Cost of disease on farms is also tabulated. Reports by computer (IBM 360-30) output can be made monthly, quarterly or annually.

For most diseases with substantial prevalence, it is possible to expect reporting precision of rates to be within ± 30 cases per 1000 of the actual rate in the universe being sampled.

The system is able to pick up trends easily and it is possible to hold lag time down to several months.

ANALYSIS AND INTERPRETATION OF DATA

Initially, computer sheets with quarterly disease incidence rates were distributed by the MLSB without interpretation. These data were of little value to the practitioner or the livestock producer. Presently, monthly disease reports are analyzed and interpreted by veterinarians from the MLSB, College of Veterinary Medicine, IDC and a statistician from the Department of Applied Statistics, University of Minnesota.

DISSEMINATION

Disease trends and discussions are published monthly in the MLSB newsletter under a category called Disease Alert. This information is useful to veterinary practitioners and livestock producers for preventive medicine and herd health.

The IDC has disseminated information to livestock groups by publishing reports and discussions in farm magazines, extension newsletters and bulletins. Selected disease data on a specific species each month with interpretation in the Farmer magazine reaches 90% of the Minnesota farms.

CURRENT STATUS

In 1978, a 3-year State-Federal cooperative project was implemented to determine validity of the Minnesota Food Animal Disease Reporting System. The validation study is being conducted on 90 farms in 13 counties in southern Minnesota.

Since 1971, the practicing veterinarians and livestock producers have been voluntary (non-paid) participants. The annual cost of the ongoing system to the MLSB, USDA, APHIS and Veterinary Services is presently approximately $55,000. It has become increasingly difficult to obtain the voluntary cooperation of practicing veterinarians and the livestock producers. A bill has been introduced in the Minnesota Legislature to provide annual funding of $72,000. This money will be used
to reimburse the 40 private practitioners and 120 farmers for their reporting services.

Advantages of the Minnesota System

1. The system provides estimates of trends relative to geographic districts from a population of farmsteads.
2. The continual surveillance by the livestock owner and the private practitioner provides early diagnosis.
3. It estimates population at risk for rate determination.
4. It provides on-the-farm diagnosis of diseases and conditions that may not be observed in a market, slaughterhouse or diagnostic laboratory.
5. Does not discriminate farm size or amount of infection present in herd.
6. Allows us to employ statistical theory in the construction of estimators.

Disadvantages

1. Participation and willingness to cooperate in the voluntary system has decreased in the past 8 years. The voluntary nature gives concern to the State-Federal district veterinarian to put the “pressure” on the private practitioner to obtain reports. The private practitioner may be reluctant to put the pressure on the client-farmer to obtain reports.
2. Sampling on 120 farms may result in low incidence diseases being undetected in the system data. Lack of detection in the data still allows statistical construction of an upper limit on the low incidence rate.
3. The Minnesota System will require additional financing to obtain data. Livestock markets, slaughter plants, and diagnostic laboratories data are available.
4. This system is difficult to implement in areas where there are sparse livestock or few veterinarians in practice. An experiment is being conducted in counties in northern Minnesota where this situation exists. Here, State-Federal veterinarians are conducting surveillance directly on the farm with success.
### DAIRY HERDS

Record total number on farm in each category:
- Calves (up to 1 year)
- Heifers (1 - 2 years)
- Adults (2 yrs. of age & over)

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<tr>
<th>Calves</th>
<th>Heifers</th>
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<th>DISEASE CONDITION</th>
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<td>403 IBR (up to 1 year)</td>
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<td>404 Leptospirosis (up to 1 year)</td>
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<td>408 Etiology undetermined (up to 1 year)</td>
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<td></td>
<td>410 ACTINOMYCOSIS (lumpy jaw) (up to 1 year)</td>
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<td>411 ARNIBITIS (up to 1 year)</td>
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<td>412 Blackleg (up to 1 year)</td>
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<td>413 Enterotoxemia (Type B, C or D) (up to 1 year)</td>
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<td>416 Vitamin A (up to 1 year)</td>
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<td>417 Vitamin D (up to 1 year)</td>
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<td>418 Vitamin E (White Muscle Disease) (up to 1 year)</td>
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<td>422 Cysticosis (up to 1 year)</td>
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<td>423 Hypoderma (cattle grub) (up to 1 year)</td>
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<td>424 Faciesiosis (up to 1 year)</td>
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<td>425 Pseudocowpox (up to 1 year)</td>
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<td>426 Ringworm (up to 1 year)</td>
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<td>427 Warts (parasitosis) (up to 1 year)</td>
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<td>431 Leptospirosis (up to 1 year)</td>
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<td>432 Haliehaut Lymphoma (Leukemia) (up to 1 year)</td>
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<td>433 Sheep (up to 1 year)</td>
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<td></td>
<td></td>
<td></td>
<td>434 Staphylococcal (up to 1 year)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>435 Str. melitensis (up to 1 year)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>436 Etiology undetermined (up to 1 year)</td>
<td></td>
</tr>
</tbody>
</table>

### FIGURE 1: Form utilized by veterinarians in disease reporting.
DISEASE REPORT FOR DAIRY HERDS, MONTH

<table>
<thead>
<tr>
<th>Calves</th>
<th>Heifers</th>
<th>Adults</th>
<th>No. Died</th>
<th>DISEASE CONDITION</th>
<th>Est. of Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>451</td>
<td></td>
<td></td>
<td></td>
<td>PODODERMATITIS (infectious foot rot)</td>
<td></td>
</tr>
<tr>
<td>462</td>
<td></td>
<td></td>
<td></td>
<td>POISONINGS</td>
<td></td>
</tr>
<tr>
<td>463</td>
<td></td>
<td></td>
<td></td>
<td>Herbicides, Insecticides, Pesticides</td>
<td></td>
</tr>
<tr>
<td>464</td>
<td></td>
<td></td>
<td></td>
<td>Metals (Arsenic, Copper, Lead, etc.)</td>
<td></td>
</tr>
<tr>
<td>465</td>
<td></td>
<td></td>
<td></td>
<td>Plants and Feeds</td>
<td></td>
</tr>
<tr>
<td>466</td>
<td></td>
<td></td>
<td></td>
<td>Urea</td>
<td></td>
</tr>
<tr>
<td>467</td>
<td></td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td></td>
</tr>
<tr>
<td>468</td>
<td></td>
<td></td>
<td></td>
<td>PYELONEPHRITIS</td>
<td></td>
</tr>
<tr>
<td>469</td>
<td></td>
<td></td>
<td></td>
<td>RESPIRATORY DISEASES</td>
<td></td>
</tr>
<tr>
<td>470</td>
<td></td>
<td></td>
<td></td>
<td>BVD</td>
<td></td>
</tr>
<tr>
<td>471</td>
<td></td>
<td></td>
<td></td>
<td>Barn pneumonia (adults)</td>
<td></td>
</tr>
<tr>
<td>472</td>
<td></td>
<td></td>
<td></td>
<td>Calf Diphtheria (Necr. Laryn.)</td>
<td></td>
</tr>
<tr>
<td>473</td>
<td></td>
<td></td>
<td></td>
<td>Enzootic pneumonia (calves)</td>
<td></td>
</tr>
<tr>
<td>474</td>
<td></td>
<td></td>
<td></td>
<td>IBR</td>
<td></td>
</tr>
<tr>
<td>475</td>
<td></td>
<td></td>
<td></td>
<td>Para-influenza (PI-3)</td>
<td></td>
</tr>
<tr>
<td>476</td>
<td></td>
<td></td>
<td></td>
<td>Pasteurellosis</td>
<td></td>
</tr>
<tr>
<td>477</td>
<td></td>
<td></td>
<td></td>
<td>Shipping fever complex</td>
<td></td>
</tr>
<tr>
<td>478</td>
<td></td>
<td></td>
<td></td>
<td>Sporadic pneumonia (adults)</td>
<td></td>
</tr>
<tr>
<td>479</td>
<td></td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td></td>
</tr>
<tr>
<td>480</td>
<td></td>
<td></td>
<td></td>
<td>RETAINED PLACENTA</td>
<td></td>
</tr>
<tr>
<td>481</td>
<td></td>
<td></td>
<td></td>
<td>UNEXPLAINED DEATHS</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1 con't.
### Farmstead Surveillance and Disease Reporting

**Disease Report**

Record total number on farm in each category:

- Calves (up to 1 yr. of age)
- Heifers (1 - 2 yrs. of age)
- Adults (over 2 yrs. of age)

<table>
<thead>
<tr>
<th>Calves</th>
<th>Heifers</th>
<th>Adults</th>
<th>Disease Condition</th>
<th>Estimate of Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>408</td>
<td></td>
<td></td>
<td>Abortions</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
<td>Abscesses</td>
<td></td>
</tr>
<tr>
<td>411</td>
<td></td>
<td></td>
<td>Arthritis</td>
<td></td>
</tr>
<tr>
<td>412</td>
<td></td>
<td></td>
<td>Blackleg</td>
<td></td>
</tr>
<tr>
<td>416</td>
<td></td>
<td></td>
<td>EVD (Vascular Disease)</td>
<td></td>
</tr>
<tr>
<td>470</td>
<td></td>
<td></td>
<td>Calving Problem (delivered by owner)</td>
<td></td>
</tr>
<tr>
<td>471</td>
<td></td>
<td></td>
<td>Calving Problem (delivered by veterinarian)</td>
<td></td>
</tr>
<tr>
<td>475</td>
<td></td>
<td></td>
<td>Deficiencies (cause not known)</td>
<td></td>
</tr>
<tr>
<td>477</td>
<td></td>
<td></td>
<td>Bloat</td>
<td></td>
</tr>
<tr>
<td>478</td>
<td></td>
<td></td>
<td>Displaced Abomasum</td>
<td></td>
</tr>
<tr>
<td>479</td>
<td></td>
<td></td>
<td>Constipation</td>
<td></td>
</tr>
<tr>
<td>480</td>
<td></td>
<td></td>
<td>Hardens</td>
<td></td>
</tr>
<tr>
<td>481</td>
<td></td>
<td></td>
<td>Digestive Disturbances (cause not known)</td>
<td></td>
</tr>
<tr>
<td>482</td>
<td></td>
<td></td>
<td>Diphtheria in calves</td>
<td></td>
</tr>
<tr>
<td>483</td>
<td></td>
<td></td>
<td>Foot Rot</td>
<td></td>
</tr>
<tr>
<td>484</td>
<td></td>
<td></td>
<td>Ketosis (Acetosena)</td>
<td></td>
</tr>
<tr>
<td>485</td>
<td></td>
<td></td>
<td>Lice</td>
<td></td>
</tr>
<tr>
<td>486</td>
<td></td>
<td></td>
<td>Lumpy jaw</td>
<td></td>
</tr>
<tr>
<td>487</td>
<td></td>
<td></td>
<td>Maggots</td>
<td></td>
</tr>
<tr>
<td>488</td>
<td></td>
<td></td>
<td>Mastitis</td>
<td></td>
</tr>
<tr>
<td>489</td>
<td></td>
<td></td>
<td>Metritis (Womb infection)</td>
<td></td>
</tr>
<tr>
<td>490</td>
<td></td>
<td></td>
<td>Milk Fever</td>
<td></td>
</tr>
<tr>
<td>491</td>
<td></td>
<td></td>
<td>Milk Quality Report was satisfactory</td>
<td></td>
</tr>
<tr>
<td>492</td>
<td></td>
<td></td>
<td>Milk Quality Report was not satisfactory</td>
<td></td>
</tr>
<tr>
<td>493</td>
<td></td>
<td></td>
<td>Pneumonia, Adults and heifers</td>
<td></td>
</tr>
<tr>
<td>494</td>
<td></td>
<td></td>
<td>Pneumonia, calves</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2:** Form utilized by farmers in disease reporting.
<table>
<thead>
<tr>
<th>Calves</th>
<th>Heifers</th>
<th>Adults</th>
<th>No. Died</th>
<th>DISEASE CONDITION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>490 Poisoning (cause not known)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Please Record</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>486 Herbicides, Insecticides, Pesticides</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>487 Metals (arsenic, copper, lead, etc.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>488 Plants or Feed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>489 Urea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>495 Retained Placenta (Cow failed to clean)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>427 Ringworm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>421 Scours, Individual Adults and Heifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>420 Scours (Winter dysentery, in many adults)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>417 Scours - calves (White diarrhea)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>491 Scours - calves (Watery diarrhea)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>458 Unexplained deaths</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>428 Warts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>409 Wooden Tongue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>450 Worms</td>
</tr>
</tbody>
</table>

FIGURE 2 con’t.
REPORT OF THE COMMITTEE ON MORBIDITY-MORTALITY

Chairman: Harry Goldstein, Columbus, Ohio.

Co-Chairman: Gary P. Combs, Hyattsville, Md.


This committee was reinstituted this year after 23 years of non-existence. It is interesting to note that the 1920 proceedings of the former United States Livestock Sanitary Association notes a resolution adopted at that meeting. The resolution reads, “Be it resolved by the United States Livestock Sanitary Association that we recommend that the livestock sanitary authorities of each state take steps to gather reliable information concerning the health of livestock in the state with definite information as to any and all existing outbreaks of communicable diseases and that the information thus gathered be forwarded to the Chief of the Bureau of Animal Industry, United States Department of Agriculture, with the request that the statistics and information thus gathered be edited and published by the department and distributed to the various state sanitary boards in sufficient quantity so that distribution may be made among those interested in animal health and livestock sanitation in the various states.”

The Morbidity-Mortality Committee continued its efforts through 1955 in preparing a manual of reportable diseases stimulating national animal disease reporting. In 1956 the chairman of this committee recommended that this committee be discontinued.

This year’s president, Dr. Zweigart, is commended for re-establishing this committee. There is and always will be a need for a viable animal disease reporting program. A reporting program can be of great importance to those persons interested in disease control and eradication efforts, or the statistics can be just like a pile of dead fish, unless properly prepared and properly used.

Disease control programs depend upon several factors:

1. Where does the disease occur (geographical areas)?
2. When does the disease occur (seasonal occurrences)?
3. How does the disease occur (modes of transmission)?
4. What is the economic impact?
5. What are the tools available to control or eradicate?

In essence we are describing all the facets of an epidemiological approach to control or eradicate disease.

Accurate diagnosis is paramount to effective use of the forementioned factors. This year’s committee was apprised of the potential reporting program initiated by the American Association of Veterinary Diagno-
ticians. It was most refreshing to hear the comments and thinking of this group. Diagnosis from a competent laboratory is primary in disease evaluation and must be utilized in morbidity-mortality reporting.

The committee was presented a resolution from AAVLD to this committee for consideration. This resolution was presented to be a matter of record and appears in the committee report.

RESOLUTION:

WHEREAS, the need for a national animal disease incidence reporting system has long been recognized, and

WHEREAS, the National Academy of Sciences issued a bulletin on this subject emphasizing the need for such a report in 1974 and a similar subcommittee report calling for the same objective in 1966, and

WHEREAS, the United States Animal Health Association has also recognized the need for such a report, and

WHEREAS, several state veterinary diagnostic laboratories are currently collecting animal disease incidence data in their states, and

WHEREAS, the clinics in several colleges of veterinary medicine are currently collecting data from their admissions, and

WHEREAS, other governmental and private agencies are currently collecting or planning to collect animal disease morbidity data, and

WHEREAS, the American Association of Veterinary Laboratory Diagnosticians has a committee on this subject which at the 1979 meeting submitted a report recommending the activation of facilities currently available to prepare such a report from cooperating diagnostic laboratories and others, and

WHEREAS, the problem of a standard nomenclature has been resolved by the ready translation of one standard nomenclature to another, and

WHEREAS, the compilation of such data has met with enthusiastic endorsement on all sides, and all parties contacted to date have indicated a willingness to contribute their data to a national report, and

WHEREAS, the basic program for compiling this data is available in several diagnostic laboratories, and

WHEREAS, facilities are now available to prepare such a report in the Veterinary Services Laboratory in Ames, Iowa, and probably elsewhere in the country,

NOW, THEREFORE, BE IT RESOLVED that the American Association of Veterinary Laboratory Diagnosticians endorse the recommendations of their Committee on Animal Disease Reporting and recommend compilation of such a report to the United States Animal Health Association for their consideration and hopefully implementation by the United States Department of Agriculture.
Dr. Erich Hemphill of the Food Safety and Quality Service, (FSQS) informed this committee that a task force has been established in FSQS to study their needs in animal disease data collection and reporting. He identified that FSQS saw the need to cooperate with numerous other agencies and institutions in establishing an animal disease reporting system identification of slaughter animals was mentioned as a requirement for traceback to farm of origin. FSQS has already developed a computerized databank of conditions found in animals at slaughter plants by region throughout the United States.

Dr. Gary Combs of Veterinary Services, APHIS, USDA presented to the committee a short history of disease reporting and presented illustrations of a possible way to publish a quarterly disease report on livestock and poultry diseases containing highlights from Veterinary Services, Meat and Poultry Inspection Program, Veterinary Research, Veterinary Entomology, Military Veterinarians, and basic animal population statistics.

This committee recommends that the United States Department of Agriculture provide the leadership to work with the respective state regulatory officials, the respective state laboratories, the respective state and federal meat inspection officials to implement a program that will utilize all sources of animal disease data, to provide a method of reporting, and to disseminate both data and information involved with occurrence of animal disease conditions.
The U.S. Animal Health Association Committee on Professional Oversight met at 1:30 P.M. Thursday, November 1, 1979.

The following items were considered by the committee:

1. Review of 1978 report items—All items considered in last year's committee report were reviewed with further action taken on some items.

   The committee has been assured the AVMA Council on Public Health and Regulatory Veterinary Medicine will act on our request to review accreditation standards on November 8 and report to this committee in regard to their findings and recommendations.

2. The committee was requested to consider the possible implied warranties by statements on certain health certificates that could place the issuing veterinarian in unwarranted jeopardy. Following discussion the Committee requests the USAHA to enlist the cooperation of the AVMA Council on Public Health and Regulatory Veterinary Medicine to join in a cooperative effort in devising the necessary statement to assure the animals in movement are healthy, but to relieve the issuing veterinarian of responsibility for certifying the absence of inapparent disease conditions.

3. A resolution was presented to the Committee in regard to assured quality and performance standards for metal eartags furnished by USDA, APHIS for use in our regulatory programs. Following discussion with a review of the faulty eartags furnished in the past, the Committee adopted the resolution to be forwarded to the Resolutions Committee for action.
REPORT OF THE COMMITTEE ON ANIMAL WELFARE

Chairman: E. Mickey Stewart, Lincoln Nebr.

Co-Chairman: Ed Wilson, Crofton, Md.

Oscar Clabaugh, Kans.; A.E. Decoteau, Mass.; Bruce H. Ewald, Va.;
Michael W. Fox, D.C.; Thomas M. Gustafson, Nebr.; Wallace T. Nagao,
Hawaii; Donald H. Person, N.C.; R.H. Rumler, Vt.; Robert J. Schroeder,
Calif.; D.F. Schwindaman, Md.; Christine Stevens, D.C.; Walter Wehr,
Nebr.; Paul Zillman, Ill.

The animal welfare committee met Tuesday 10/30 at 1:30 PM. with
routine procedures and introductions. The chairman read a mission
statement which had been supplied by Dr. Zweigart.

Two similar presentations were made expressing concern over the
welfare of domestic animals in certain confinement conditions.

The committee directed a subcommittee be formed to search for data
relative to questionable practices. The subcommittee will also visit with
representative producers, researchers and feed systems designers
regarding current animal welfare conditions. The full committee will be
kept advised.

There was the annual discussion on pet animal shipping crates.

A resolution has been sent to the executive committee regarding a
holding period for pet animals.

We then had a very brief review of diesel fumes research in stock
trucks.

Meeting adjourned at 3:05 PM.

MINUTES

Meeting called to order at 1:30 PM. by E.M. Stewart, Chairman.

After introductions the chairman read a report of last year's meeting.
He then read a "Mission Statement" for the committee which had been
prepared by USAHA President, Dr. Tom Zweigart. Dr. Michael Fox
made a presentation about his concern for animal welfare under certain
confinement conditions. He specifically mentioned veal calf production,
chickens in batteries, swine crates, transportation over long distances.

Diane Halverson, representing Christine Stevens made a presentation
which was of a similar nature.

After discussion the chairman declared a consensus of the committee
to appoint a special sub-committee to accomplish the following:

1. to investigate the allegation made by Dr. Fox and Ms. Halverson
   a. by attempting to identify specific questionable practices
   b. by securing input from producers, manufacturers of feed sys-
      tems, research sources.
2. to report to the full committee in 3 months as to procedures and a progress report in 9 months. Said report to be forwarded to the committee by the chairman.

Next item of discussion was animal shipment crates. Dr. Schwindaman gave a status report which indicated research and analysis will be completed so that the regulations will be on board by warm weather—next year. Next item had to do with a waiver of the holding period for pet animals.

It was moved by Oscar Claubaugh and seconded by Dr. Fox that a resolution be drafted to waive the holding period. Motion carried. (Resolution attached)

Chairman reported on diesel fume research in stock trailers. His report indicated favorable progress.

Meeting Adjourned: 3:05 PM.

ATTENDANCE SHEET

<table>
<thead>
<tr>
<th>NAME</th>
<th>ADDRESS</th>
<th>ORGANIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dale F. Schwindaman</td>
<td>705 Fordham St. Rockville, MD</td>
<td>USDA</td>
</tr>
<tr>
<td>Bruce H. Ewald</td>
<td>203 Smithfield Blacksburg, VA</td>
<td>Virginia Tech</td>
</tr>
<tr>
<td>A.F. Clabaugh</td>
<td>Rt. 2, Box 71 McLouth, KS.</td>
<td>USDA VS</td>
</tr>
<tr>
<td>Melvin R. Crane</td>
<td>3312 Patricia Dr. Urbandale, IA</td>
<td>USDA, VS</td>
</tr>
<tr>
<td>A.E. Decoteau</td>
<td>424 Trapelo Rd. Waltham, MA</td>
<td>USDA, VS</td>
</tr>
<tr>
<td>D.H. Person</td>
<td>Rt. 3, Box 16C Zebulon, N.C.</td>
<td>USDA VS</td>
</tr>
<tr>
<td>J.R. Wheaton</td>
<td>P.O. Box 1268 Pinehurst, N.C.</td>
<td>USDA VS</td>
</tr>
<tr>
<td>Michael W. Fox</td>
<td>I.S.A.P. 2100 L St. NW Washington, D.C.</td>
<td>HSUS</td>
</tr>
<tr>
<td>Michael J. Polino</td>
<td>115 Charlotte Way Unit 101 Enola, PA</td>
<td>USDA, VS</td>
</tr>
<tr>
<td>D.J. Gilhooly</td>
<td>46-133 Punalei PC Kaneohe, H.</td>
<td>USDA, VS</td>
</tr>
<tr>
<td>Milo L. Johnson</td>
<td>2231 Devonshire Lincoln, NE</td>
<td>USDA, VS</td>
</tr>
<tr>
<td>J.R. Stauffer</td>
<td>13721 Carriage La Pickerington, O.</td>
<td>USDA, VS</td>
</tr>
<tr>
<td>Keith Farrell</td>
<td>Bx 2250 C.S. Pullman, WA</td>
<td>WSV</td>
</tr>
<tr>
<td>Paul Zillman</td>
<td>1100 Jorie Blvd. Oakbrook, IL</td>
<td>L.C.I.</td>
</tr>
<tr>
<td>Name</td>
<td>Address</td>
<td>Organization</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-----------------------------------</td>
</tr>
<tr>
<td>R. Van Gelder</td>
<td>8029 Larwin Dr.</td>
<td>USDA, VS.</td>
</tr>
<tr>
<td></td>
<td>Citrus Hts, CA</td>
<td>No. Livestck. Fdrs.</td>
</tr>
<tr>
<td>Thomas M. Gustafson</td>
<td>RR #1</td>
<td>Calif. Dept.</td>
</tr>
<tr>
<td></td>
<td>Wakefield, Ne.</td>
<td>Food/Agric. Bur.</td>
</tr>
<tr>
<td></td>
<td>Turlock, CA</td>
<td>Md. Dept. of Agric.</td>
</tr>
<tr>
<td>Mickey Stewart</td>
<td>2701 Beech St.</td>
<td>Animal Health</td>
</tr>
<tr>
<td>Leon F. Ackermann</td>
<td>Bakersfield, CA</td>
<td></td>
</tr>
</tbody>
</table>
Chairman: B.W. Hawkins, Ontario, OR.


CHAIRMAN'S PREROGATIVE

The State-Federal Relations Committee agrees that the present administration should provide a balanced budget in an overall effort to afford efficient operating costs and assist in reducing today’s inflationary trend. However, this Committee feels the need to point out that the efforts of the U.S.D.A., APHIS, SEA, and FSQS provide programs that in reality are returnable dividends; to the overall health and welfare for the consuming public; stimulate a more healthful economic climate for agribusiness; and provides billions of dollars that provide needs for many additional services not directly considered as being a part of agriculture.

A healthy agriculture provides the country with not only a bountiful food supply, but provides commodities for balance of trade, and contributes markedly to the overall national economy.

F.D.A

The Committee is pleased with the proposed programs of F.D.A. in regards to the implementation of a new, more meaningful medicated animal feed surveillance. The emphasis on the human risks associated with the use of such medicated feeds in animals is to be commended. It is hoped that this new program will continue to seek out the original sources of animal feed medication with certain drugs and seek full compliance with regulations.

The need for the training program for F.D.A. and state personnel in enforcement of regulations on the feed manufacturing industry is past due and the Committee hopes that in the new approach enforcement will be much more uniform.

It is hoped that F.D.A. will continue the focus attention on the need of the livestock industry and practicing veterinarian’s need for some drugs that are becoming almost unavailable.

S.E.A.

The State-Federal Relations Committee is very much aware of the tremendous strain placed upon the reduced personnel left to carry on the duties of one of the most important departments in USDA as far as contributing to a more healthful and productive agriculture; For without research the future remains very dim.

We are most disappointed to observe that agricultural research is the only service that experienced a reduction in actual dollars budgeted, when compared to the other major governmental department budgets. This curtailment and reduction of available funding does not appear
realistic when agricultural commodities are the major contributory factor in maintaining our balance of trade. In reality, our balance of trade would show a greater deficit without the availability of said commodities.

Surely, when this nation stops or retards the research of our most necessary industry, we are taking a short sighted approach to austerity.

S.E.A. administrators and staff are to be commended for their philosophical approach to the problem of a tremendous task, with fewer dollars and fewer personnel.

Due to recent outbreaks from some of the importing bird stations, we feel that the work in the vaccine field is of utmost importance and we would urge a rapid completion of this project.

This Committee commends S.E.A. on their practical approach to the Salmonella problem; like other problems in disease; best management practices for the industry often minimize the severity of the problem.

The two major areas of the research effort on brucellosis; that of the reduced dosage level of strain 19 required for immunity, and the one for differentiating between strain 19 and the field strain infection are two that are of utmost importance from a cost effective view point.

As mobility of cattle and sheep increases, the "Sleeping Giant" known as bluetongue, becomes even more of an economic as well as a disease problem, and we wish to commend those in this research effort.

The Denver laboratory has long carried on the bulk of this effort and now with the projected move to Fort Collins, we sincerely hope the effort will be increased and that an effective vaccine and rapid identification test will materialize.

APHIS

*National Veterinary Service Laboratory*

APHIS, is to be complimented on the new facilities soon to be dedicated at the National Veterinary Services Laboratory at Ames. It is noted that further consolidation of the diagnostic service laboratories is necessary to facilitate coordination of personnel and diagnostic service.

Since the primary function of this laboratory is to supplement state diagnostic services as a reference facility, the establishment and identification of this service as a line item in the budget should assure the proper recognition of need in the state-federal cooperative arena for continued support.

The recent purchase acreage to house the laboratory animals necessary for biologic testing is a purposeful, economic and progressive move which will enhance the necessary increased biological surveillance.

*Swine, Sheep, Goats and Horses*

The presence of African Swine Fever in the Dominican Republic and Haiti make the potential danger of its introduction into mainland U.S. a
real danger. This is emphasized by the volume of commerce and tourist traffic between these countries and the United States. Our Committee is gratified by the cooperative working arrangements that have been made between U.S. Agencies and these infected countries in an all-out cooperative effort to eradicate the disease before it reaches the mainland. The research regarding the economic impact of A.S.F., if introduced into the U.S. should get continued support.

The research conducted by the Plum Island staff and the training courses they offer in training federal and state diagnostic personnel are essential.

With the continuing presence and spread of pseudorabies in our swine population, this Committee recommends that the swine disease budget not be cut in the future and that a pilot eradication program involving a state or portion thereof where there is significant incidence of pseudorabies be initiated. The Committee senses that the present low incidence of the disease may allow the industry to be lulled with a feeling of complacency and that program guidelines and educational programs be maintained to prevent misdirection which would inadvertently result in unnecessary delay to the eradication goal. The Federal regulations relative to pseudorabies must be continually updated and revised to sustain and incorporate diagnostic and program improvements which will be revealed by supervised control programs now in progress.

Bluetongue continues to be a concern to the sheep, goat and cattle industry particularly as an economic barrier resulting in increased restrictions on exportations to certain countries. A continuing study of developing diagnostic methods should be made and a cooperative effort to accept these new techniques by all countries involved.

The equine industry and state and regulatory officials should be commended on the effective and efficient handling of the contagious equine metritis outbreak in our country. Hopefully, we can establish a continuity of research on this disease to provide more definitive diagnostic tools so that we can maintain our valuable international commerce in the equine industry.

This Committee urges the continuing effort of a strong industry educational program to maintain a high level of V.E.E. vaccination of horse equidae in those states bordering old Mexico.

**Poultry**

The Committee was appalled and expresses great concern for the lack of poultry disease budget funding. The poultry industry of this nation is a most important segment of our food producing system, and needs to have monies appropriated for assistance in those areas that can and do cause great economic losses which are ultimately reflected at the consumer's market place.

This Committee recommends that all steps be taken to insure and
provide adequate disease control programming for the poultry industry.

The Committee is of the opinion that the need exists to provide funding to continue the 3 year salmonellosis program which was presented in FY 1979. This disease is still the most wide spread in all species and causes the highest losses in human manpower losses.

The Committee is of the opinion that there is still a realistic need for Diagnostic Reference assistance for state laboratories. This service not only provides for uniformity, but in turn reduces the work load of the Veterinary Services Laboratory.

The Committee reviewed the position statements pertaining to the response of our Association's resolution #1 adopted at the Buffalo meeting. The Committee is in agreement with the response and continues to commend the California Turkey Industry for the progressive and far-sighted approach in an effort to protect its industry before the fact rather than waiting for another potential major outbreak of V.V.N.D.

F.S.Q.S., M.I.P.

The Committee reviewed the F.S.Q.S. M.I.P., program with the agency's state-federal coordinator. Budget, manpower ceilings, programs and policy were the major topics of discussion.

The Committee was informed that due to inflationary costs coupled with expanded residue protection expansions, the proposed budget request for both state and federal inspection programs exceeded the budget allocation by 3.2 million dollars. This discrepancy in available funding has caused great concern in both state and federal programs. The concern is most serious in those state programs that are totally dependent upon 50-50 funding from the Federal Government.

This Committee commends NASDA for the effort of naming an Ad Hoc Committee to evaluate the problem. As a result of the activity of the NASDA Committee, a joint USDA-NASDA Task Force Committee was formed to review the effort of reducing the total cost of the programs. The USDA-NASDA Task Force Committee reviewed data based on numbers of plants in relation to numbers of employees, and found insufficient data to support increases in budget. It was apparent to the joint task force that additional study was necessary. In a further effort to evaluate, a 5 state pilot review was initiated, coupled with a survey involving all states, in an attempt to capture information on cost increase from the respective states.

As a result of these efforts the joint task force has published 20 recommendations with the findings of the survey review. Significant items considered in this review include staffing patterns, supervisor-inspector ratios, cost of travel, training, laboratory and compliance activities; cross-utilization of state and federal inspection personnel and fiscal control procedures.

This Committee was most appreciative of the fact that the Secretary of
Agriculture has stated that an additional $1,000,000 will be placed in the budget as an adjunct to help in the discrepancies of allocation and requested budget. However, this Committee suggests that all Program Administrators in the state and federal programs review the recommendations of the Joint Task Force and that implementation of all suggested cost saving measures be given serious considerations.

The Committee was made aware of the expanded program involving residue surveillance. State Laboratories should take advantage of contractual monies available for the conducting of routine and residues testing. The Committee was informed that $1.1 million has been budgeted in that activity.

The STOP Program was discussed with this Committee. F.S.Q.S. is commended for its practical and realistic approach to safeguard against the introduction of antibiotics into our nation's food supply. The "Swab Test on Premise" (STOP) provides a program that does not cause undue stress to the meat packing industry and assures the consuming public of a product free of antibiotics.

**Veterinary Biologics**

The Committee continues to be alarmed at the apparent lack of control in the production and distribution of animal biologics marketed intrastate. It is hoped that new proposed legislation will be enacted in order that all animal biologics will be adequately supervised; both products marketed interstate and intrastate.

It is hoped the Veterinary Biologics Program can give adequate consideration to the need for lower volume, perhaps locally needed, veterinary biologics.

The Committee well appreciates the dire need for additional staff to enhance the effectiveness of Veterinary Biologics Service and recommends that Congress be encouraged to supply the needed support.

It is hoped the Veterinary Biologics Program will use the authority now available to enforce the compliance of the distribution and use of veterinary biologics as recommended by State and Federal Regulatory Officials, as are directed by the labeling of such products.

**Animal Care**

The Committee supports firm and fair enforcement of the Horse Protection Act. However, there is cause for concern that a disproportionate amount of attention is being focused on one breed. In the interest of preventing cruelty to horses and unfair competition, it is suggested that violations be dealt with wherever they occur, regardless of breed. Consideration should be given to testing for the use of drugs in show horses.

The Committee commends APHIS for making use of part time employees and work contracts to increase the efficiency of the Animal
Welfare Program. It is hoped that the popular appeal of this program, and competition for funds, does not result in the neglect of any of the animal disease eradication programs.

**Professional Development**

The Committee compliments APHIS on the quality of the animal health related courses offered to federal and state personnel. These should be continued, strengthened, and expanded to involve more state employees.

The Committee is concerned by the frequency of reports that federal employees are scheduled but training on short notice, or no notice, to state cooperators. On the surface, it appears that over training of some individuals has occurred. It is suggested that consideration be given to evaluating an employee’s past training and experience, present level of competency, and future prospects within the service before further training is given. In all cases, assignments of federal and state employees for training should be made well in advance and coordinated with other program activities within the states.

**Brucellosis**

This Committee is very gratified with not only the technical committee report, but also the manner of implementation given the newly proposed Brucellosis Program. The unbiased report will go a long way toward securing acceptance from the industry to work united toward the ultimate goal of eradication.

We urge that the time table be adhered to as closely as possible and we would like to reemphasize the importance of regulation and inspection of interstate movements as being vital to the control and spread of brucellosis.

The importance of the resolution of USAHA as to the controlled dissemination of Strain 19 antigen and vaccine must be stressed at this point in the Program.

We urge the continued use of brucellosis funds for the Brucellosis Program that was adopted several years ago. Also, the contracting of work with the various states to minimize the strain on APHIS limited personnel and to get maximum use out of tax dollars is, in this Committee’s opinion of great importance. Further, the hiring of animal health technicians for the vaccination and blood drawing is a good economic and personnel saving move.

**Screwworm and Darien Gap**

Due to the austere budgeting restrictions, this Committee feels that stiffer negotiating must be implemented when dealing with other nations where there is mutual benefit derived from cooperative animal health programs. The screwworm program is one case in point.

The other program we wish to call attention to is the cooperative
program with Columbia on FMD. We appreciate the recent development in negotiations which gives positive direction to participation; However, the concept of buying disease control or eradication without compensatory input from cooperating countries is not acceptable to this Committee.

_Tuberculosis_

The low level of infection that this disease is now in (only 27 cattle herds) reemphasizes the need for a special alertness on the part of F.S.Q.S. and the need for close cooperation and reporting between the two agencies. Considerable discussion and criticism has been heard about complacency in the alertness needed and dedication to duty from those depended upon to detect the last remaining infection in an eradication program.

The swine program reemphasizes the need for a better identification program and we are pleased to see the continuing effort being put forth by APHIS in this regard.

_Cattle Scabies_

Cattle Scabies, one of the oldest scourges of the livestock industry must be contained. It is imperative that a cooperative control and eradication program along the lines of the eleven points proposed by the N.C.A. be implemented immediately.

These points with added monitory support voted by Congress last year, will help a great deal, however without full support from those affected states, neither a program nor funding will accomplish the desired results.

_Regulation Compliance_

The apparent inability of APHIS officials to deal with the illegal interstate movements of livestock constitutes a threat to past and future progress of the nation's animal health programs. States which have been declared free of certain diseases are becoming infected. Unscrupulous violators of federal regulations governing the interstate movement of livestock operate with little risk of prosecution.

No attempt is being made to place the blame for lack of adequate enforcement at any level within the USDA or Department of Justice. However, members of the Committee would like to point out the fallacy of spending increasingly large amounts scarce appropriated funds on animal disease eradication programs while allowing progress already made to be eroded by the lack of enforcement of animal health regulations. It is strongly recommended that necessary action be taken to insure the prompt and vigorous prosecution of individuals who knowingly violate animal health regulations.

_Emergency Programs_

The Committee is most gratified to note that within the last 5 years,
the Regional Emergency Animal Disease Organization has been available to control and eradicate 18 different outbreaks of exotic animal diseases. (9 Hog Cholera — 9 Exotic Newcastle.) Without this expertise, which was contributed to by cooperative state-federal personnel under federal direction, the Swine and Poultry Industries might well be battling a new disease in The United States and 1 once eradicated. The 1979 Exotic Newcastle outbreaks while not completely eradicated appear to be under control due primarily to the ability to mobilize adequate, well trained forces, quickly and efficiently.

The training aids developed by the Emergency Program Technical staff plus efforts to aid neighboring countries to rid themselves of diseases exotic to, and threatening The United States Livestock Industry is most important and should receive the publicity and credit which is due.

Clearing the Darien Gap of Foot-and-Mouth disease will necessitate continued, active, cooperative participation by both Panama and Columbia. The Committee is cognizant that continued efforts to enlist this kind of program on the part of Columbia appears to be bearing fruit. Panama continues to contribute to the effort.

Import-Export

The Committee looks forward to the imminent completion of the Harry S. Truman Import Center at Fleming Key. We continue our objection to foreign animal imports by air for reasons previously expressed.

We concur wholeheartedly with the moratorium just now placed on bird importation from foreign countries. This should give time to review import procedures and to develop controls which hopefully will prevent future importation of exotic diseases.

The Mexican Border problems continue. We heard no suggestions for improvement and can contribute none that have not already been proposed. We urge that suggested remedies be pursued and that efforts be continued to prevent both overt and covert illegal importations across the border.

The conservative estimate of ($165,000,000), one hundred and sixty five million dollars loss to The United States economy by virtue of animal and poultry diseases which are either exotic to, or at least nauseous to foreign countries is fuel which should be used to encourage those people with power and influence to give all possible support to the control and eradication of 10 certain livestock diseases in The United States. Money appropriated for this purpose would not be inflationary. It would create employment and contribute to the general welfare of The United States. (Let's quit hiding our light under a barrel.)

BRUCELLOSIS

The SEA-AR program in brucellosis research includes "in-house" investigations at NADC and cooperative agreements with scientists
located at various universities. Extramural locations and titles of projects are:

Univ. of Alaska — Pathogenesis, diagnosis, and control of *Brucella suis* type 4 infection in ruminants.

Auburn Univ. — Biological control of bovine brucellosis by stimulation of cell-mediated immunity.

Univ. of California — Epizootiology of brucellosis: Evolution and taxonomy of *Brucella* organisms.

Colorado State Univ. — Brucellacidal capability of bovine phagocytes.

Univ. of Florida — Improved methods for diagnosis of bovine brucellosis.

Iowa State Univ. — Interaction of bovine phagocytic cells and *Brucella* organisms.

Louisiana State Univ. — Immunological and pathological responses of the bovine fetus to *Brucella abortus*.

Univ. of Minnesota — Brucellosis: Cell-mediated immunological mechanisms relating to diagnosis and pathogenesis in cattle.

Univ. of Missouri — Cell-mediated immune response by chemical modification of *Brucella abortus* antigen.

Montana State Univ. — Animal models for brucellosis research.

Univ. of Tennessee — Immunity to brucellosis at mucosal surfaces.

Texas A&M Univ. — Development of methods for diagnosis of bovine brucellosis.

Univ. of Vermont — Detection of early *Brucella abortus* infection in cattle.

Univ. of Wisconsin — Cellular interactions and immune response mechanisms in *Brucella* infected and immunized cattle.

Virginia Polytechnic Institute — Isolation and characterization of cellular components of *Brucella* (Strain 19) for diagnosis and immunization.
REPORT OF THE COMMITTEE ON FOOD ANIMAL HYGIENE

Chairman: Robert J. Lee, College Park, Md.
Co-Chairman: D. Bedell, Tifton, Ga.


The Food Animal Hygiene Committee met on Tuesday, October 30, 1979, from 1:30 p.m. to 5:00 p.m. There were 45 persons in attendance. The following subjects were discussed:

1. Condensation — Dr. Paul Friedman
2. Swab Test on Premises (STOP) — Dr. John Howder
4. Downer Cows in Wisconsin — Dr. Wm. Abbott
5. The Four-State Cooperative Residue Program — Dr. Wm. Leese and Morris Levy
6. Update on PCB’s — Dr. Wm. Leese
7. Proposed Quality Assurance Program — Wm. Dennis

The Committee adopted a resolution regarding condensation which was submitted to the resolutions committee.
REPORT OF THE COMMITTEE ON EPIZOOTIC ATTACK

**Chairman:** J. B. Young, Bryan, Texas.

**Co-Chairman:** H. A. McDaniel, Hyattsville, Md.


The chairman, Dr. J. B. Young called the meeting to order at 1:30 p.m. Approximately, 60 members and guests were present. Response on 2 resolutions passed last year were briefly discussed.

The first topic was the epidemiology of bluetongue (BT). All ruminants are susceptible to varying degrees. It is a non-contagious disease transmitted primarily by blood sucking insects, especially culicoides. Twenty serotypes of BTV are known throughout the world. Only 4 are known in the USA.

A major BT epizootic occurred in southeastern USA during 1979. Bluetongue virus seemingly overwinters in apparently infected cattle and feeding by culicoides provokes showering of large quantities of BT virus so subsequent culicoides feeding on these animals become infected and can transmit BT Virus to other ruminents.

Until 1974 only 1 serotype was known in USA, Serotype 10. Now we have determined that 4 serotypes are present in USA; 11, 13 and 17 in addition to 10.

A vaccine is available in USA for Serotype 10. Research results are promising for an inactivated vaccine against 11 and 17.

A plea was made for more solid morbidity-mortality and cost benefit data, a vital ingredient for any control or eradication program. However, additional technical methodology is also needed before reliable recommendations can be made for control and eradication. In addition better diagnostic tests are needed, especially to protect export markets and integrated pest management should be considered.

A slide-tape presentation depicting many problems and solutions developed during the National test exercise to assess state and federal preparedness to respond to an exotic disease outbreak. In response to the test exercise, assistance was requested from wildlife agencies and over 5,000 people plus bulldozers, draglines, trucks and other vehicles were committed. An addendum to this report describes the test exercise in more detail.

Vaccines against foot-and-mouth disease have been added to plans for combating an outbreak. The most economical and efficient slaughter plan will still be implemented first, but if expected progress is not made, a
stock pile of vaccine is being developed to be used as an adjunct to eradication, the ultimate goal.

An interesting illustrated discussion was held on *Hemophilus para-hemolytica* (*H. pleuropneumonia*) in swine. This problem has markedly increased in the last 4 years. In Mexico it is the number 1 problem in finishing swine (those over 120 lbs.) The incidence is also markedly increased in Canada and to a lesser extent so far in U.S.A.

Veterinarians and swine producers throughout the U.S. should be alerted on this emerging disease. It was reported that inoculated swine may die in 8 hours.

A resolution previously passed by the transmissible diseases of poultry committee was introduced. This resolution asked USDA to establish quarantine stations for imported pet birds, to not approve additional privately owned quarantine stations and phase out existing privately owned bird quarantine centers as rapidly as feasible. The motion was modified to add "the poultry" before industry in the 4th paragraph and passed unanimously.

A motion was also passed to support a motion previously passed in the import-export committee urging increased attention to inspection of baggage of people arriving from foreign countries.

**ADDENDUM**

During the last week of January 1979, a test exercise "Alpha 79" was conducted using a simulated exotic vesicular condition called "Nada." The Spanish word "Nada" was chosen in this instance because in Spanish it means "nothing" and hopefully, by the use of this term we were able to clarify that this was a test exercise.

NADA was confirmed on a farm in Mineola, Iowa, on January 28, 1979. It was described as the most contagious viral infection affecting cattle, swine, sheep, goats, and other cloven-footed domestic and wild animals. Once the existence of the disease was confirmed, it was essential that all pockets of the infection be quickly located and eliminated. Animals from infected herds were to be destroyed and exposed herds were to be quarantined and destroyed to prevent the spread of this highly communicable disease.

Due to the rapid spread of the disease to Nebraska, California, Texas, Illinois, and Georgia, all five Regional Emergency Animal Disease Eradication Organizations (READEO's) were activated to combat the epidemic. Actual READEO teams were mobilized in Omaha, Nebraska; Des Plaines, Illinois; Atlanta, Georgia; Ft. Worth, Texas; and Riverside, California, and were operational within 24 hours.

On Monday, January 29, 1979, Secretary Bergland's signature on the emergency declaration was simulated because of the existence and spread of NADA. That declaration authorized a program to "control and eradicate the disease wherever found" through the advance of Com-
It was estimated that approximately 500,000 animals, excluding wildlife, would be infected or exposed to NADA prior to full eradication of this simulated disease. Appropriated funds for fiscal year 1979 ($2,065,000) were clearly insufficient to deal with the outbreak. Once the outbreak of NADA was confirmed, all available contingency funds ($1.5 million) were released to initiate preliminary NADA activities within the infected States and to provide indemnity for slaughtered animals. These funds were quickly exhausted.

It is interesting to note that the last six outbreaks of foot-and-mouth disease (very similar to NADA) from 1884 to 1929 cost approximately $253 million to eradicate. A FMD epidemic in Mexico (1946-1954) in which USDA participated, cost approximately $136 million to eradicate.

A cost/benefit analysis, prepared at the University of Minnesota, indicated that there could be a $12 billion direct loss and a $36 billion indirect loss of domestic livestock over a 15-year period if FMD became endemic in the United States. An unpredictable loss would also occur in wildlife populations.

Based on this and the data available from each of the five regional READEO's, it was estimated during the test exercise that the simulated costs of dealing with the NADA outbreak would be $250 million.

Field operations (salary, travel, supplies, and equipment) $ 50 million
Laboratory support $ 50 million
Indemnity payments for animals, materials, and feed destroyed $150 million

Indemnity payments during the 60 simulated test exercise days included 489 positive and exposed herds involving 234,438 animals in 23 States, all of which were quarantined.

It is clearly evident that with this massive quarantine, many potential problems in disease eradication were evident—the proper control of milk and milk products, meat, and biologics, to name a few.

The test exercise also indicated our needs to improve our ability to communicate the information available. Not only within our READEO, but also between the READEO and other agencies, State and Local governments, the affected industry, as well as the consumer. In essence, during an emergency of this magnitude, everyone needs to be kept informed of the problem, what is being done to resolve the problem why this seems to be the best solution, as well as discussing the problems we anticipate and of course the benefits we hope to achieve.

On June 14, 1979, a critique of the test exercise was held. Many
problems, normally unnoticed, were brought to our attention. Personnel, supplies, vehicles, contracting, and of course day-to-day administration and coordination were just a few of the many items we reviewed a little closer.
REPORT OF THE ZOOLOGICAL ANIMAL COMMITTEE

Chairman: R. M. S. Temple, Bristolville, Ohio.

Co-Chairman: Keith Sherman, Hyattsville, Md.


The Chairman of the Committee, Dr. R. S. Temple, was not present, therefore Dr. K. C. Sherman, Co-Chairman, conducted the meeting. Only three members of the Committee were present, which did not constitute a quorum. There were seven non-members also in attendance at the meeting.

A report was given by Dr. D. E. Herrick, regarding actions taken by Veterinary Services, on the 1978 Resolutions presented by the Committee at the Buffalo, New York, meeting.

Regarding the Resolution for Veterinary Services to provide sufficient funding and personnel to permit at least two inspections per year at all USDA approved zoos was carried out by VS and such inspections were provided within the limitations of their currently allocated personnel and funds.

A Resolution was also presented last year which requested training courses be presented at the Plum Island Animal Disease Center to zoo veterinarians in the diagnosis of foreign animal diseases. This request was acted upon favorably last June when a course was presented to approximately twelve zoo veterinarians.

The other Resolution presented at last year's meeting was to recommend that the Tariff Act of 1930 be amended which would give the Secretary of Agriculture the discretionary authority with respect to determining the post-entry quarantine periods for zoological ruminants and swine. It was reported that a legislative proposal to take such action has been prepared by Veterinary Services and is currently in the Department's Congressional Liaison office before being submitted to the Congress.

Dr. Elmer Himes of the National Veterinary Services Laboratories, Ames, Iowa, gave a report on some imported fennec foxes, from which Mycobacterium bovis was recovered. Specimens were submitted to NVSL from these foxes which were located at the zoological parks in Duluth, Minnesota, Phoenix, Arizona and Cleveland, Ohio. Fennec foxes are native to the Sinai Peninsula and to northern Tunisia. These animals were apparently imported into this country at about the same time, but their complete epidemiological background is unknown. Dr. Himes reported this was the first instance of which he was aware where M. bovis has been isolated from wild captured canids.

In the ensuing discussions, Dr. Sherman recommended more suspicious
tissues be sent to NVSL for diagnostic purposes. Dr. Kenneth Hook, Director of NVSL, said such specimens could be processed on a low-priority basis as long as adequate reference assistance funds are available. A question was raised as to whether or not these foxes had been housed with primates, contrary to Department regulations. It was later determined this was not the case, but that possible exposure of primates may have occurred because of their exposure to waste material from the fox pens.

The matter of indifference in many zoological collections for the presence of Tuberculosis was discussed. It was brought out that in many cases isoniazid is used in an attempt to control Tuberculosis instead of following good husbandry practices to limit exposure. The potential of isoniazid to interface with the Tuberculosis test results was also cited.

The Committee discussed the importation of certain species of animals not regulated by USDA, APHIS. A recent instance was cited in which some wild rats were imported from the Sudan in northern Africa to a research facility in Denver, Colorado. Because of a significant die-off in these animals, the possibility of Rift Valley Fever was considered. This Committee wishes to support the Resolution of the Import-Export Committee which would require APHIS, VS, to work with other agencies to see that the responsibilities of each Agency are met, in all cases where a possibility exists of the introduction of livestock or poultry diseases with the importation of animals.
EFFICACY OF TERRAMYCIN/LA-200* ADMINISTERED DURING THE PREPATENT PERIOD OF ANAPLASMOSIS

W. P. Eckblad
Department of Veterinary Science
University of Idaho, Moscow, Idaho 83483

S. D. Lincoln
Caine Veterinary Teaching Center
University of Idaho, Caldwell, Idaho 83605

R. A. Magonigle
Animal Health Research Department
Pfizer Inc., Terre Haute, Indiana 47808

From the Agricultural Experiment Station, Department of Veterinary Science, University of Idaho, Moscow, Idaho 83843. Published with the approval of the director of the Idaho Agricultural Experiment Station, Moscow, Idaho as Research Paper No. 79815.

SUMMARY

The prophylactic efficacy of a long acting tetracycline formulation (Terramycin/LA-200) was determined in cows exposed to Anaplasma marginale. Each of 10 cows in three different treatment groups (T2, T3, T4) were prophylactically treated with intramuscular injections of 20 mg of Terramycin/LA-200 per kg of body weight. The T2 group was treated one time at one week post exposure (PE); the T3 group was treated two times at one and two weeks PE; and the T4 group was treated three times at one, two, and three weeks PE. Six cows (group T1) served as controls and were not prophylactically treated.

All animals developed clinical or subclinical evidence of disease. However, the principal groups had significantly longer (P < 0.001) prepatent periods than the non-prophylactically treated controls. Prophylactic treatment inhibited clinical signs of the disease in some of the animals in the principal groups. Subclinical signs of anaplasmosis were seen in 10 of the cows in the three prophylactic treatment groups (one in T2, three in T3, and six in T4).

INTRODUCTION

A concentrated oxytetracycline formulation, Terramycin/LA-200* (Pfizer, Inc.), has been developed to provide sustained plasma levels of the antibiotic for three to five days.1 Besides the reduction of total therapeutic volume and the number of treatments, the more slowly absorbed compound appears to enhance the animal's response against

*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the University of Idaho, and does not imply the approval to the exclusion of other products that may also be suitable.
anaplasmosis. These studies have shown the efficacy of this formulation in the treatment of acute anaplasmosis and the elimination of the carrier state.

To date in the United States, most prophylactic treatments for prevention of naturally occurring anaplasmosis have been oral administration as the method of drug delivery. The purpose of this research was to evaluate Terramycin/LA-200 as a parenteral prophylactic treatment program for anaplasmosis.

**Materials and Methods**

Thirty-six pregnant Angus cows (mean age = 28 months, mean weight = 370 kg) were acquired from an anaplasmosis-free herd. The animals were randomly placed into one of four treatment groups (Table 1). Each treatment group was then evenly divided into two replicates. One replicate of each group was transported to the Caine Veterinary Teaching Center, Caldwell, Idaho, while the other replicate remained at the Department of Veterinary Science, Moscow, Idaho.

All of the animals were negative to the plasma rapid card agglutination (RCA) test at the start of the experiment. Also, Wright’s-stained blood smears did not reveal evidence of parasitemia in a pre-exposure check for anaplasmosis. A 10-ml heparinized blood sample was taken from each animal in a treatment group, pooled, and subsequently inoculated into a splenectomized 8- to 12-month old Holstein-Friesian calf to assure that there had been no previous exposure to *Anaplasma marginale*. A different calf was used for each treatment group. The splenectomized calves were then monitored for 60 days after inoculation for clinical signs of anaplasmosis.

Each animal in the treatment groups was exposed on day 0 to *Anaplasma marginale*. Intramuscular inoculation of two blood aliquots (15 ml total) from samples collected on the same day from two documented anaplasmosis carrier animals were used.

Treatment with Terramycin/LA-200 was administered by deep intramuscular injection. Each treatment was given at the dosage of 20 mg/kg with no more than 10 ml of drug injected per site. Prophylactic treatment was initiated in the designated treatment group as described in Table 1. All of the animals received the first treatment on day 7 post-exposure (PE) and subsequent treatments at seven-day intervals thereafter. In total, group T2 received one treatment (day 7 PE), group T3 received two treatments (days 7 and 14 PE) and group T4 received three treatments of Terramycin/LA-200 (days 7, 14 and 21 PE). The control animals (T1) received placebo treatments (days 7, 14 and 21 PE).

Animals were observed three times weekly until day 20 and daily thereafter for clinical signs associated with anaplasmosis (elevated body
temperature, anemia, icterus, inappetance, dehydration, constipation). Packed cell volume (PCV), Wright-stained blood smears for the identification of the organism (percent parasitemia) and complement fixation (CF) or RCA tests were performed twice weekly until day 20 PE, three times weekly until day 40 PE, and then twice weekly through day 84 PE.

Besides low PCV and peak parasitemia, the severity of infection was also measured by the low percent of normal PCV. The low percent of normal value is the low PCV divided by the preinfection PCV. An animal's response to infection was categorized as severe when the PCV was 50% or less of normal, moderate when the PCV was 50-75% of normal, or no response when the PCV was 75-100% of normal.

RESULTS

All cows developed evidence of infection, as seen by a 0.5% or greater *Anaplasma marginale* parasitemia and a positive response to the anaplasmosis RCA and CF tests. RCA and CF tests became positive as early as 12 days PE. The prepatent period of anaplasmosis has been defined as the number of days between time of exposure and time that 1% of the erythrocytes in the animal contained marginal anaplasma bodies. By the use of this criteria, the control (non-prophylactic) cows had a mean prepatent period of 17 days PE while the principal groups that received prophylactic treatment (Terramycin/LA-200) averaged 44 (T2), 55 (T3) and 62 (T4) prepatent days PE.

The preinfection PCV, postinfection PCV, incubation period for infection as measured principally by PCV, and peak parasitemia levels are presented (Table 2). Each group's mean PCV and parasitemia levels through 84 days PE are also presented (Figure 1).

The time required after exposure to reach the low mean PCV for the control group was 26 ± 2 days. The mean low PCV for T2 was 59 ± 9 days PE, while for T3 it was 64 ± 5 days PE, and for T4 it was 72 ± 6 days PE. The principal groups (T2, T3, T4) which were on prophylactic treatment, had significantly longer (P < 0.001) prepatent periods as compared to T1 (Table 2). There was also a tendency for these groups to exhibit low PCV in successive one week intervals.

Cows in T1 which received no prophylactic treatment exhibited a mean low PCV of 19 ± 3% which was 50 ± 5% of normal. Their peak parasitemia was 15 ± 2%. All animals in this group received one *therapeutic* dosage of Terramycin/LA-200 when their percent of normal PCV exhibited a severe to low moderate response. This therapy was given to prevent possible death.

The 10 cows in T2 which received one prophylactic treatment at one week PE exhibited a mean low PCV of 24 ± 7%. This represented 61 ± 5% of normal PCV. Peak parasitemia for this group was 8 ± 8%. Two of these animals exhibited a severe to low moderate response (52 ± 7% of normal) which required therapeutic treatment (20 mg/kg of oxytetracycline HC1) to control clinical signs of the disease.
EFFICACY OF TERRAMYCIN/LA-200

Principal cows in T3 had two successive prophylactic treatments at one and two weeks PE. These cattle had a mean low PCV of 27 ± 7% at day 64 PE which was 71 ± 8% of normal and exhibited a parasitemia of 7 ± 8%. Additional therapeutic treatment was required in three animals when their average low percent of normal was 50 ± 7%.

Animals in T4 received three successive prophylactic treatments at one week intervals PE. The mean low PCV for the ten cows was 33 ± 8% at day 72 PE which represented 86 ± 20% of normal PCV and was significant (P < 0.001) when compared to the control group. Their peak parasitemia was 6 ± 10%. No therapeutic treatment was required for any of the animals in this group.

Subclinical infections were detected in 10 of the cows in the three prophylactic treatment groups (one in T2, three in T3, six in T4). Together, these cows exhibited no response to infection since their percent of normal PCV was 90 ± 5%. Their peak parasitemia levels were 0.8 ± 0.5%. They all were positive to RCA and CF tests. No obvious clinical signs associated with anaplasmosis were seen in these cows through 84 days PE.

DISCUSSION

Results would indicate that Terramycin/LA-200 may have limited application as a prophylactic drug for anaplasmosis based on this artificially induced study. The control animals (T1) exhibited typical clinical signs of anaplasmosis. When medicated therapeutically with Terramycin/LA-200, treatment was effective and the response was similar to that described in previous experiments.1,2,4

It has been previously shown that early oxytetracycline administration (before parasitemia has reached 1%) may result in prolongation of the incubation period.6 As seen in the present study, the prepatent period was extended in the principal groups by approximately the amount of time that the cattle were on prophylactic treatment. It would appear that the parasite has a privileged site or that the microbialstatic action of the drug was not sufficient to destroy the organism in situ. However, prophylactic treatment(s) were of value in minimizing the infection. The number of animals which did not exhibit clinical signs of disease increased as the number of prophylactic treatments increased. There was one animal in T2, three animals in T3, and six animals in T4 that had subclinical infections.

All of the cows had a humoral immune response to anaplasmosis as seen by positive reactions by the RCA and CF tests. These reactions were seen before clinical manifestations of disease were apparent. It has been stated that these antibodies have diagnostic value but their role in host resistance has not been established.8 Our data would suggest that these antibodies have little, if any, protective role in the prepatent period of the disease. The presence of both the early humoral antibodies and the antibiotic was also insufficient for clearance of the infective agent, since
clinical cases were seen in some animals in all three prophylactic treatment groups. As many as three doses of this antibiotic formulation were used in this study during the prepatent period, yet clinical cases were still observed. However, in another study only two doses were needed to clear carrier animals of anaplasmosis. This would suggest that an appropriate immune response in conjunction with the antibiotic treatment is usually necessary to eliminate the infection.

Although one (T2) or two (T3) doses of prophylactic treatment(s) reduced the incidence of clinical disease, it did not necessarily affect the severity of disease. There were animals in these principal groups which later required therapeutic treatment once signs of acute anaplasmosis were observed. However, disease severity was reduced in group T4 animals after they had received three prophylactic treatments as none of the animals required additional treatment.

It would, therefore, appear that if this formulation were to be used as a prophylactic agent, at least three doses would be necessary to inhibit expression of clinical signs and lessen the severity of disease. This conclusion should be weighed with respect to the large inoculum used in this study to establish clinical infection. In natural field situations, animals would probably receive a much smaller inoculum. Additional prophylactic studies whereby anaplasmosis is induced with a much smaller challenge dosage would be in order. Also, the relative importance of tetracyclines and the immune response in clearance of the organism needs to be examined.
Table 1. Experimental Protocol for Oxytetracycline Prophylaxis During Prepatent Period of Anaplasmosis

<table>
<thead>
<tr>
<th>Cattle Group</th>
<th>Treatment (Intramuscular Route)</th>
<th>Day of Treatment Post Exposure</th>
<th>Number of Animals Per Replicate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Nonmedicated placebo 0.1 ml/kg</td>
<td>7 14 21</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>T2</td>
<td>Terramycin/LA-200 20 mg/kg</td>
<td>7</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>T3</td>
<td>Terramycin/LA-200 20 mg/kg</td>
<td>7 14</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>T4</td>
<td>Terramycin/LA-200 20 mg/kg</td>
<td>7 14 21</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2. Results of Studies in Cows Exposed to *Anaplasma marginale* and Treated with Terramycin/LA-200 During the Prepatent Period

<table>
<thead>
<tr>
<th>Cattle Group</th>
<th>Number of Treatments</th>
<th>Packed Cell Volume (% PCV)</th>
<th>Time Required After Exposure to Reach Low PCV</th>
<th>% Peak Parasitemia</th>
<th>% Animals Requiring Additional Treatment</th>
<th>% Subclinical Infections</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preinfection</td>
<td>Low</td>
<td>% of Normal</td>
<td>Low PCV</td>
<td></td>
</tr>
<tr>
<td>T-1</td>
<td>0</td>
<td>39 ± 3</td>
<td>19 ± 3</td>
<td>50 ± 7</td>
<td>26 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>T-2</td>
<td>1</td>
<td>39 ± 4</td>
<td>24 ± 7</td>
<td>61 ± 15</td>
<td>59 ± 91</td>
<td>8 ± 8</td>
</tr>
<tr>
<td>T-3</td>
<td>2*</td>
<td>37 ± 3</td>
<td>27 ± 7</td>
<td>71 ± 18</td>
<td>64 ± 52</td>
<td>7 ± 8</td>
</tr>
<tr>
<td>T-4</td>
<td>3*</td>
<td>38 ± 3</td>
<td>33 ± 8</td>
<td>86 ± 20</td>
<td>72 ± 63</td>
<td>6 ± 10</td>
</tr>
</tbody>
</table>

1 Time required for 9 of 10 animals exhibiting clinical infection
2 Time required for 7 of 10 animals exhibiting clinical infection
3 Time required for 4 of 10 animals exhibiting clinical infection

* = Treatments at one week intervals
+ = Standard deviation
Weeks after **ANAPLASMA** inoculation

Hematocrit and percent parasitemia levels for principal and control groups. Lines represent hematocrit levels while bars indicate percent parasitemia.
The authors wish to thank Dr. Steve Parrish for performing the calf splenectomies and Patricia Tassinari, Nancy Toone, Kriss Hoffman and Judy McCain for technical assistance. The authors express gratitude to K. Kuttler and G. Amerault for making the Anaplasma inoculum available. The work reported herein was supported by funding from Pfizer, Inc.

REFERENCES


The Anaplasmosis Committee met in open session at 1:30 P.M., Wednesday, October 31, 1979, in the Town and Country Hotel, San Diego, California. Twenty-six persons were in attendance.

Dr. Gerald Buening, University of Missouri, discussed the “Immune Mechanisms In Anaplasmosis” comparing the disease response in cattle vaccinated with two doses of Anaplaz and with two doses of a chemically altered A. Marginale antigen. The disease responses exhibited following an experimental challenge with A. Marginale showed that the chemically altered vaccinated animals were less resistant to the disease challenge than the Anaplaz vaccinated animals. However, the chemically altered vaccinated animals exhibited substantially lower serum isoantibodies than the Anaplaz vaccinated animals. In addition the chemically altered vaccinated animals were not reactive to the anaplasmosis rapid card test or the CF test, but did elicit a significant cell-mediated response.

Mr. William Eckblad, University of Idaho, discussed the “Efficacy of Terramycin LA200 Administered During the Prepatent Period of Anaplasmosis.” This paper presented in the general session will be published in the proceedings.

Dr. Ed Richey, Oklahoma State University, discussed, “The Evidence of Neonatal Isoerythrolysis Following Vaccination of Heifers with Anaplaz.” This work involved the monitoring of two calving periods on 36 heifers subjected to six different vaccination programs. Thirty-four genetically potential neonatal isoerythrolytic (NI) calves and 26 non-potential NI calves were produced by the 36 heifers. Serological and hematological testing of the calves for 10 days after birth showed that eight of the 34 genetically potential NI calves possessed post nursing anti-calf red blood cell antibodies and exhibited mean minimum packed cell volumes and mean % drops in packed cell volumes significantly different than the remaining calves. All eight calves were born to dams vaccinated with Anaplaz vaccine.

Dr. Richard Searl, Fort Dodge Laboratories, discussed the results of a survey concerning Anaplaz usage and occurrence of NI. This survey involved the vaccination of 37,596 cows while nonpregnant, 34,373 cows with 50% being in early pregnancy. Only two cases of NI were reported in the 90,294 cows vaccinated. The two NI cases were reported in the
18,325 cows vaccinated when 50% of them were pregnant. Vaccination with Anaplaz should be given while breeding females are not pregnant and injections limited to a primary series (two vaccinations—four weeks apart) and one booster dose 12 months later.

Dr. Ed Richey, Oklahoma State University, presented evidence that four of 12 animals, which had received only one Anaplaz booster vaccination 12 months after the primary series, exhibited clinical anaplasmosis when challenged 24 months after the last vaccination.

In addition Dr. Richey presented reports on the use of low levels of Chlortetracycline (.5 mg per lb body weight) in clearing the carrier state of anaplasmosis, as an aid in the prevention of clinical anaplasmosis and as an aid in stopping an outbreak of anaplasmosis.

The Committee suggests that steps be initiated by Veterinary Services USDA to conduct a statistically valid survey to identify the current incidence of anaplasmosis in the United States. The last such survey was conducted in 1973.

The Committee agreed that efforts should be taken to accelerate research on vector identification, drug therapy, and immunity. Field studies should be conducted to evaluate methods of anaplasmosis control and eradication from infected herds and also methods of prevention in herds at high risk.
THE CURRENT STATUS OF RESEARCH ON AN EXPERIMENTAL INACTIVATED BLUETONGUE VIRUS VACCINE

J. L. Stott, B.S., M.S., B. I. Osburn, D.V.M., Ph.D.
and T. L. Barber, D.V.M., Ph.D.*

Department of Pathology, School of Veterinary Medicine
University of California, Davis, California,
*Arthropod-borne Animal Diseases Research Laboratory
SEA-AR, USDA, Denver, Colorado

Bluetongue (BT) is an infectious viral disease of domestic and wild ruminants. Bluetongue virus (BTV) is an Orbivirus, Family Reoviridae, and is antigenically related to epizootic hemorrhagic disease virus. There are 20 recognized serotypes of BTV on a worldwide basis, four of which are present in the United States. The insect vector transmits BTV among domestic and wild ruminants; BTV is also transmitted from persistently infected bulls to the dam and to the fetus.

There is a critical need for effective control measures either through reduction of insect vectors or with effective vaccines. Control measures for protection of sheep and cattle are needed because of the economic losses experienced in the United States in the past years. The only federally licensed vaccine currently available in the U.S. is a modified-live BTV, serotype 10, that is approved for use in sheep. Sheep vaccinated with one serotype have limited protection against reinfection with other serotypes.

Advantages of the use of an inactivated BTV vaccine would be as follows:

1. There would be no introduction of live virus into the susceptible host-vector pool and thus no possibility of reversion to virulence as might occur with modified-live virus strains. Reversion to virulence in either the animal host or insect vector has been a concern with modified-live arbovirus vaccines.

2. An inactivated vaccine is unlikely to cause problems when injected into pregnant ewes. Modified-live BTV vaccines were reported to cause abortions and the birth of dummy or deformed lambs as sequellae when ewes were vaccinated early in gestation.

3. There would be no possibility that new serotypes of BTV could develop from the recombination and/or reassortment among genomes of different BTV serotypes as might occur with the use of multivalent modified-live virus vaccines.

4. An inactivated BTV vaccine may provide a safe means of immunizing cattle. Before modified-live BTV vaccines are used in cattle, a number of questions must be resolved. There is good evidence that cattle can become persistently infected with field strains of BTV. Insufficient information is available to
determine whether modified-live BTV vaccines would: a) produce persistent infections in adults or newborn, b) produce persistently infected bulls which could transmit BTV through semen and c) affect the fetus resulting in abortion, deformed calves or neonatal death.

The problems stated above are valid concerns when considering the use of modified-live BTV vaccines. Inactivated BTV vaccine would circumvent certain of these problems, thus its development is highly desirable. As with any veterinary biological product, an inactivated BTV vaccine must meet standards for safety and efficacy.

Binary ethylenimine (BEI) was found to adequately inactivate BTV.8 The inactivant is inexpensive, safe and easy to store and handle. Chemical activity of BEI with proteins is well defined and the activity is neutralizable with sodium thiosulfate.1,2,3,14 Virus for vaccine preparation was extracted from infected monolayers of hamster kidney cells (BHK-21, clone 13). After inactivation of virus, several assay systems were evaluated to determine their reliability for residual live virus detection in experimental inactivated BTV vaccine: a) inoculation of monolayered cell cultures [green monkey kidney (VERO), fetal bovine bone marrow (FB,BM) and BHK cells], b) intravenous (i.v.) inoculation of 11 day old embryonating chicken eggs (ECE), and c) subcutaneous inoculation of susceptible sheep. The only test system found to be completely reliable was inoculation of susceptible sheep.

The scheme used to determine the efficacy of the inactivated BTV vaccine was as follows: a) sheep were given vaccine with or without adjuvant, b) four or six weeks after vaccination, sheep were given immunity challenge with virulent BTV, homologous in serotype to the vaccine virus, and were observed at least 21 days after challenge (DAC), and c) rectal temperatures were recorded and heparinized and clotted blood samples were obtained throughout the nine week experimental period. After immunity challenge, rectal temperatures and clinical signs of illness were found to be unreliable indicators of the presence or absence of protection. The most consistent correlate of protection was the titer and duration of viremia.

Humoral and cellular immune responses of sheep were determined after vaccination and subsequent immunity challenge. The presence of serotype specific neutralizing antibody was assayed by a plaque neutralization (PN) test, while non-neutralizing group specific antibody was assayed by the complement fixation (CF) and agar gel precipitin (AGP) tests.6,8,9,15 Lymphocyte stimulation by BTV antigen was employed as in in vitro correlate of cellular immunity.17 Blastogenesis was determined by incorporation of "H-thymidine in the acid precipitated fraction measured with a liquid scintillation counter. A summary of the various assay procedures is shown (Table I).

The inactivated BTV vaccine was initially tested in sheep as follows: a) without adjuvant, b) with Freund's incomplete adjuvant (FIA) or c) with
diethylaminoethyl dextran (DEAE) adjuvant. Sheep given BTV vaccine in either adjuvant were refractory to immunity challenge at six weeks; sheep given vaccine without adjuvant exhibited minimal protection. Due to the variable and high post-vaccinal rectal temperatures in sheep given BTV vaccine in DEAE adjuvant, subsequent vaccine tests were with FIA. In studies where challenge virus was given four or six weeks after vaccination, the greatest protection was in sheep challenged at six weeks.

The results of immunological and protective studies in sheep are summarized (Table 2). Vaccination of sheep with inactivated BTV vaccine in FIA resulted in the following:

1. After vaccination: a) sheep developed CF and AGP antibodies, and a strong cell mediated immune (CMI) response, and b) PN antibody was not detectable.

2. After virulent BTV immunity challenge the vaccinated sheep showed: a) minimal or no viremia, b) a strong CMI response at three to six DAC, c) little or no detectable PN antibody, and d) AGP antibody continued to be present and CF antibody increased.

3. After virulent BTV immunity challenge the non-vaccinated sheep showed: a) extensive viremia for up to three weeks, b) no detectable CMI within 14 DAC, and c) PN, CF and AGP antibodies closely followed viremias.

The fact that the inactivated BTV vaccine did not elicit a detectable neutralizing antibody response presents an interesting marker for the vaccine. Protection against challenge was demonstrated in the absence of neutralizing antibody. Thus vaccinated sheep could be differentiated from non-vaccinated sheep after natural infection.

Assessment of the vaccine experiments revealed two immune responses which correlated with protection, i.e., the non-neutralizing group specific antibody (CF and AGP) and CMI. To explore the possible role of the non-neutralizing antibody in protection the following experiment was conducted. Serum was obtained from vaccinated sheep 35 days after vaccination and pooled. This serum, which had AGP and CF antibody activity, but no detectable PN antibody, was infused i.v. into several non-vaccinated sheep. Immediately following infusion of antibody, the sheep were challenged with virulent BTV. The sheep were not protected, thus the non-neutralizing antibody alone was not protective.

Recent studies with aluminum hydroxide (AlOH) as adjuvant have shown that this adjuvant was not as effective as FIA. Sheep that were given BTV vaccine with AlOH had an AGP antibody response, similar to that seen with FIA, but after immunity challenge only partial protection was observed.

CONCLUSIONS

Preliminary investigations have shown the safety and efficacy of an
inactivated monovalent BTV vaccine for sheep. The vaccine elicited a CMI response when administered in FIA and provided protection against immunity challenge. After vaccination, sheep were more refractory to immunity challenge at 6 weeks than at 4 weeks. The development of a multivalent inactivated vaccine for sheep is currently underway.

The administration of inactivated BTV vaccine to cattle must be mentioned due to the economic impact BT is having on the beef and dairy cattle industries because of export and interstate shipping restrictions and reproductive failures. The vaccine is currently being administered to cattle to test its safety and efficacy. The fact that the vaccine elicits an AGP response, but no PN antibody, may prove to be a desirable attribute, as an immunological marker by which vaccinated animals may be differentiated from animals recovered from natural BTV infections. This is important because of our cattle population which presently may include persistently infected BTV carriers or recovered animals as well as those that have no contact with BTV.
Table 1. Assay procedures for assessing the safety and efficacy of experimental inactivated bluetongue virus vaccines

Safety

A. Test of choice for residual live virus in experimental inactivated vaccine

1. Subcutaneous inoculation of susceptible sheep followed by observation of rectal temperatures, assay of viremia and detection of humoral antibody, for 6 weeks

Efficacy

A. Vaccination

1. Subcutaneous inoculation of sheep with inactivated vaccine followed for 4 or 6 weeks by:
   a. Rectal temperature and viremia determinations
   b. Humoral antibody tests
      1) Plaque neutralization
      2) Agar gel precipitin
      3) Complement-fixation

2. Cell mediated immune test
   a. Lymphocyte stimulation

B. Immunity challenge

1. Subcutaneous and intradermal inoculation of virulent virus, followed for 3 weeks by:
   a. Rectal temperature and viremia determinations
   b. Humoral antibody tests
      1) Plaque neutralization
      2) Agar gel precipitin
<table>
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<th>Adjuvant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of doses</th>
<th>No. of sheep</th>
<th>Post-vaccination</th>
<th>Post-challenge</th>
<th>Protection&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>7</td>
<td>-</td>
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<td>4</td>
<td>+ + - I</td>
<td>+ + + I&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>25</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt; NA NA NA</td>
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<sup>a</sup> DEAE - diethylaminoethyl dextran; FIA - Freund's incomplete adjuvant; AlOH - aluminum hydroxide

<sup>b</sup> Indicates level of viremia; Yes - low level or no viremia; No - high level viremia; Partial - all animals had low level viremia

<sup>c</sup> ND - not done; NA - not applicable

<sup>d</sup> I - inconclusive due to poor lymphocyte viability

<sup>e</sup> S - slight; low levels in some sheep, none in others

<sup>f</sup> Negative to 14 days
ACKNOWLEDGMENTS

This research supported in part by the California Woolgrowers, California Cattlemen's Association, Livestock Disease Research Laboratory, and the Department of Food and Agriculture, State of California.

REFERENCES

Release of veterinary biological products to the market is conditioned upon their satisfactorily passing test requirements for purity, safety, potency, and efficacy. Test methods, procedures, and criteria established by Veterinary Services for the evaluation of veterinary biological products are published in Title 9, Animals and Animal Products, Code of Federal Regulations, Part 113 — Standard Requirements. These Standard Requirements cover only those veterinary biological products manufactured by three or more licensees. Test methods for veterinary biological products manufactured by less than three licensees are written into the Outline of Production for the specific product and are considered to be privileged communication.

The development and publishing of test methods in Title 9 CFR is a monumental task which requires the cooperation and exchange of scientific and technological expertise of biological manufacturers, veterinary services, and the scientific community. Untold scientific man-years of effort have been expended so far in developing and publishing our present standard requirements. Because of the continuing, dynamic advances in the development of better and more precise methods of evaluation in the field of biological sciences, a remorseless and unremitting effort must be maintained by all concerned to revise and update these standards to be certain they remain a reasonable and acceptable measure of the worth of veterinary biological products. The present standard requirements are in use by scientists in many parts of the world.

The consumer or livestock owner who uses USDA licensed veterinary biological products can rest assured that (1) samples from every serial of each biological product have been tested by the manufacturer for purity, safety, potency, and efficacy; (2) samples of every serial of biological product are retained by both the manufacturer and National Veterinary Services Laboratories (NVSL) in Ames, Iowa, for further testing should it be desired; (3) check testing of a substantial portion of the biological products manufactured is performed by NVSL prior to release to market; (4) valid statistical data to support any claims for the use of the product is on file with USDA; (5) each licensed manufacturer is inspected to determine whether the condition, equipment, facilities, and the like of the establishment and the methods used to prepare biological products are in conformity with the requirements in the regulations to insure the products accomplish the purpose for which they are intended.
These advantages are not available to the consumer or livestock owner who uses non-USDA licensed or "Intrastate Produced" veterinary biologics. Many of the non-USDA licensed or "Intrastate Produced" veterinary biologics carry a disclaimer statement on their labeling such as or similar to, "Buyers and users must assume all risk of use." USDA licensed manufacturers are responsible for the results obtained from the use of their products and such disclaimer statements are not permitted on USDA licensed veterinary biologics.

The following table shows a comparison of test results obtained from tests conducted by NVSL on USDA licensed product and non-USDA licensed product.

(SEE TABLE)

A direct comparison between the composite results of NVSL testing of USDA licensed and non-USDA licensed products has to be made with caution. However, the limited testing of non-USDA licensed products indicates they are of considerably lower quality or worth as measured by USDA standards. We have reliable information on the quality or worth of USDF licensed products as indicated by the confidence intervals on the percentage of unsatisfactory serials.

In comparing the composite results of the Firm's and NVSL's testing of USDA licensed product, it must be pointed out the Firm's tests are conducted on samples chosen at random while many of NVSL's tests are conducted on selected samples based on previous test history. The most important point, however, is the fact that all USDA licensed product that is found to be unsatisfactory, whether tested by the Firms or NVSL, is destroyed and never reaches the consumer or livestock owner. By contrast, all of the non-USDA licensed serials tested by NVSL were purchased from the open market.

Which products would you prefer to use? Which products can you afford to use?
## PRODUCTION AND TESTING
### FY 1978

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<th>Firms</th>
<th>Potency</th>
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<td>(3.2-4.7)**</td>
<td>(0.9-1.8)</td>
<td>(2.3-4.7)</td>
<td>(0.4-11.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| NVSL Test Results    | Unlicensed Products | | | | | |
|----------------------|---------------------| | | | | |
| No. Firms            | 14                  | | | | | |
| No. Serials          | 38                  | | | | | |
| No. Tests            | 36                  | | | | | |
| No. Tests Unsat      | 21                  | | | | | |
|                      | 55.3%              | | | | | |
|                      | (38.3-71.4)**      | | | | | |
| No. Available Tests  |                     | | | | | |
| with 9 CFR Reference | 108                 | | | | | |
| No. Available Tests  |                     | | | | | |
| without 9 CFR Ref.   | 22                  | | | | | |
| (Experimental, Develop- | 2       | | | | | |
| mental or Firm Test) |                     | | | | | |

*Malfunction of Equipment, Invalid Controls, etc.

**95% Confidence Interval
Any discussion of the regulation of veterinary biologics must begin with an understanding of the law which directs and authorizes such regulation. The 1913 law, known as the Virus-Serum-Toxin Act, has as its subject all viruses, serums, toxins, and analogous products intended for the treatment of domestic animals. These products are subject to the Act if they are prepared or sold in any place under the jurisdiction of the United States and if they are shipped or sold from one State, Territory, or the District of Columbia to any other state, Territory, or the District of Columbia, or if they are imported into the United States.

The purpose of the Act is to make it unlawful to prepare under United States jurisdiction or to ship interstate or to import biologics if they are worthless, contaminated, dangerous, or harmful. To achieve this purpose, the Congress authorized the Secretary of Agriculture to administer the Act and provided several instruments for administrative practices.

The Act broadly provides that biologics shall be prepared, sold, and shipped in compliance with regulations prescribed by the Secretary, and only by an establishment holding a valid license or imported under a permit. More specifically, the Act authorizes the Secretary to cause all imported biologics to be examined and inspected; to promulgate rules and regulations to prevent preparation, sale, or shipment of worthless, contaminated, dangerous, or harmful biologics; to issue, suspend, or revoke licenses and permits; and to issue licenses only on the condition that licensees shall permit inspection of the establishment, the products, and the preparation of the products. Penalties are provided for violators. The mission of the USDA, Veterinary Services Biologics Program, as delegated by the Secretary, is to carry out administration of the Act. Fulfillment of the program's mission requires activity in the general areas of inspection and regulation.

The functions of APHIS Veterinary Services include: (1) licensing of establishments and biological products; (2) inspection of the premises, production facilities, and production methods; (3) development of reference standards, reagents, and test methods to evaluate products; (4) examination of biological products for compliance with established standards; and (5) investigation of suspected violations of the Act or regulations.

Licensing requirements for veterinary biologics include, but are not necessarily limited to, the following three areas of consideration:
1. The needs of our livestock industry. (Regional and emergency needs are considered as well as the national need.)

2. The Virus-Serum-Toxin Act and related regulations.

3. The latest available knowledge and experience.

Biologics are needed for diagnostic, prophylactic, and therapeutic use in the control, prevention, treatment, and/or eradication of diseases of livestock, poultry, pet, fancy, and companion animals.

The need for each new biologic must be established by each licensee applicant with adequate data showing value, usefulness, and benefits to our livestock industries evaluated by valid epidemiologic studies.

The APHIS Deputy Administrator may issue a U.S. Veterinary Biologics Establishment License and U.S. Veterinary Biological Product License or Permit when in his opinion (1) the condition of the establishment and the methods of preparation of biological products are such as to reasonably ensure that the products will accomplish the purpose for which they are intended; (2) the establishment is operated under the direct supervision of a person competent by education and experience to handle all matters pertaining to the disease involved and the preparation and testing of the biological products named in the applications; and (3) written assurance has been received that the veterinary biological products to be prepared in the establishment will not be so advertised as to mislead or deceive the buyer or bear any statement, design, or device which is false or misleading in any particular.

It is a function of the Licensing and Standards Staff to recommend to the Deputy Administrator approval or rejection of applications for establishment and product licenses. Exercise of this function requires review of license applications, establishment blueprints, and legends, and the qualifications and experience of personnel. Each product license application must be supported by a detailed outline of production and testing and data establishing the purity, safety, potency, and efficacy of the product, as well as label copy showing the indications and directions for use and appropriate cautions and warnings. Review of these materials frequently results in correspondence and conferences leading to additional experimental work to resolve questions or clarify issues.

Veterinary Services Memorandum No. 800.57 sets forth the Basic License Requirements for Applications in detail and is inserted here for informational purposes.

Purpose

The purpose is to furnish information on requirements for obtaining an establishment license and a product license for preparation of biological products. References in Title 9, Code of Federal Regulations, are provided. Veterinary Services Memorandum No. 800.57, dated January 2, 1979, is hereby rescinded.
General Requirements

U.S. Veterinary Biologics Establishment License shall be issued for each establishment in which biological products are to be prepared and a U.S. Veterinary Biological Product License shall be issued for each biological product authorized to be prepared in the establishment.

Reference: Section 102.1

Establishment License

A. Application for the establishment license shall be made on VS Form 14-1 available from Biologics Licensing and Standards, Veterinary Services, Federal Building, Hyattsville, Maryland 20782. Supporting documents required to complete each applicable item on the form shall be attached, including Articles of Incorporation for the applicant and for each subsidiary, if any. Each applicant will be provided copies of pertinent interpretive and informational memorandums and notices.

Reference: Section 102.3

B. Plot plans and blueprints of the establishment shall be required. Legends keyed to the blueprints shall list the activities and equipment in each room or area.

References: Section 102.3 and Part 108

C. Licensed establishments shall be operated under competent supervision and by competent employees. A VS Form 14-7 available from Biological Materials Processing Section, National Veterinary Services Laboratories, P.O. Box 844, Ames, Iowa 50010, shall be submitted in triplicate for each supervisory employee responsible for essential steps in production, testing, and initial distribution.

References: Sections 102.4 and 114.7

D. An inspection shall be made before an establishment license is issued. If the facilities are not acceptable, recommendations for necessary corrections shall be made. Licenses shall not be issued until condition of the facilities is acceptable.

References: Section 102.4(a) and Part 108

E. A system of recordkeeping showing all phases of preparation, testing, and initial distribution of biological products to be produced in the establishment shall be required.

Reference: Part 116

F. A certification from the appropriate water pollution control agency that the establishment is in compliance with applicable water quality control standards pursuant to section 401 of the Federal Water Pollution Control Act, as amended (86 Stat. 877; 33 U.S.C. 1341), shall be filed with Veterinary Services for each licensed establishment.
Reference: Section 108.11

**Product License**

A. At least one product license shall be issued with the establishment license. A product license shall be issued for each additional product authorized to be prepared in the establishment.

Reference: Section 102.3

B. Application for a product license shall be made on VS Form 14-3 available from Biologics Licensing and Standards. The nature of the product shall determine the amount of supporting material required. Data may be limited to reports of laboratory and/or locally conducted tests. Field testing may be required. The product shall be proved pure, safe, potent, and efficacious before a license is issued.

References: Sections 102.3, 114.8, and 103.3

C. Each product shall be prepared according to an Outline of Production filed with Veterinary Services. A separate VS Form 14-15, available from Biologics Licensing and Standards, shall be used as the transmittal form when an Outline of Production is submitted to Veterinary Services for review.

References: Sections 102.3, 114.8, and 114.9

D. Labels and claims to be made on labels and in advertisements shall be reviewed by Veterinary Services before use. VS Form 14-15 shall be used as the transmittal form when labels are substituted.

References: Sections 102.3, 102.4, and 112.5

E. At least three consecutive serials will be subject to prelicensing evaluation by Veterinary Services. Each serial must be comprised of fractions which have been prepared from separate batches of medium cells, serum, eggs, diluting fluid, and stabilizer in accordance with a filed Outline of Production. Seed from the same production seed lot may be used to prepare the three consecutive serials provided that a separate container of seed be used for each serial. The minimum volume of each serial must be approximately equal to one-third that of an average serial as stated in the Outline of Production. The applicant must complete all required tests and submit summaries on VS Forms 14-8 available from Biological Materials Processing Section. These tests shall include all applicable Standard Requirements and special tests written into the filed Outline of Production.

References: Sections 113.4, 114.8, and 114.9

F. When notified to do so, the applicant shall submit samples to Veterinary Services for testing. VS Form 14-20, available from Biological Materials Processing Section, shall be used as the transmittal form for these samples.
For more than half a century, USDA has, in cooperation with industry, conducted a national program of surveillance over veterinary biologics in interstate commerce. The biologics industry has responded to needs of farmers, ranchers, zoological gardens, fanciers, pet dealers, and owners by developing and improving its products to meet increasingly higher standards.

Licensing requirements for purity, safety, potency, and efficacy are based upon the latest scientifically proven and accepted available knowledge applied diligently but in a practical manner to avoid causing unnecessary disruption of production or a shortage of indispensable biologics.

The Deputy Administrator has in the past and will continue in the future to authorize the production, distribution, and evaluation of biological products prior to licensing for the convenience of license applicants and to permit and encourage important research projects which may lead to the licensure of new biological products. Occasionally, when the need for a product and available data warrant such action, a license may be issued under the condition that additional data will be obtained and submitted for review.

All the risks inherent to biological products must be investigated by laboratory and field tests in significant numbers of laboratory and host animals under conditions to be found in the field. Risks which cannot be eliminated, but which would not preclude licensure, are required to be clearly indicated in label warnings and contraindications with appropriate prophylactic recommendations. Unforeseeable safety problems with a specific product may arise after licensure which require a complete or partial revision and updating of safety standards for the product in order to eliminate or minimize these hazards. If the degree of risk warrants such action, the licensee may be required to withdraw the product from sale or recall the product from the user pending appropriate corrective measures.

Strict adherence to good production and laboratory procedures aids immeasurably in minimizing risks inherent in and peculiar to biological production and testing, and manufacturers are urged to institute mandatory rules to assure safety of personnel and product.

The responsibility of all biological producers and USDA to assure purity, safety, potency, and efficacy for all biologics produced under Federal license is limited only by the vigilant and practical application of all available knowledge and experience. The producers and regulatory agencies are all doing their best to dispatch their responsibilities in a manner which will meet the technological standards of the scientific community and continue to deserve the public trust.

Over the past several years, technological advances in the life sciences have accumulated at an unprecedented rate. Under these rapidly
changing conditions, both the administration of and compliance with the Act have become occupations of infinitely greater complexity than in the relatively uncomplicated environment of 60 years ago. We must all live with the truth, however, that the purposes of the Act become ever more relevant as our knowledge increases. We cannot apply the techniques which lead to the manufacture of more numerous and more complicated biologics and ignore those which permit more precise measurements of product worth and safety. Furthermore, our sources of knowledge must go beyond those of an industry and an agency and encompass the entire scientific community.

In addition to the scientific environment, we all function in a social environment which is also a dynamic force. The days of "caveat emptor" are, of course, far behind us. No longer can the consumer be expected to judge the quality of a product by personal inspection. Instead, the public must depend upon the integrity of the manufacturer and, in many cases, upon the rules and standards under which the product is manufactured. The strength and momentum of the consumerism movement is too evident to require emphasis here. What should be emphasized is that our responsibility under the Act demands that we be fully responsive to the needs of consumer protection. We are aware that regulations may restrict consumer choice and may have economic impact, but such considerations must be secondary to our obligation to our assigned responsibility. We recognize that the animal health industry is making its own adjustments within the social environment. We look forward to continued cooperation with the animal health industry in our efforts.

In summary, our regulatory duties require that we recognize an environment of increased technical knowledge and complexity. Application of this knowledge results in a decrease in our need and prerogatives to make assumptions or empirically based decisions about product quality.

We must also recognize an environment of increasing concern for consumer protection which will focus public attention on both industry and regulatory agencies. More attention will be given to the use of official rather than informal channels of communication.

What I have said does not mean that there will be no occasions for policy decisions or exercise of judgment. In fact, development of new products and advancing technology will increase the need for decisions based on judgment and reasonableness. The law and the environment, however, will increasingly dictate and define limited areas of maneuver.
REPORT OF THE BIOLOGICS COMMITTEE

Chairman, Dr. Robert F. Kahrs, Florida

Donald E. Baldwin, NE; William H. Beckenhauer, NE; Allen C. Braemer, CA; Earl L. Drake, NV; John Finnell, IL; Donald A. Fuller, IA; B. B. Hancock, IA; Robert E. Horton, NJ; Werner P. Heuschele, OH; Majon Huff, CO; Gerald L. Johnson, KS; Donald Kahn, NJ; Peter Langer, Can; Lloyd Lauerman, CO; Raymond W. Loan, TX; Gerald V. Peacock, MD; Robert J. Price, MD; Jeffery L. Stott, CA; Al Strating, IA; Kenneth E. Thayer, OR; J. Donald Todd, KS; George B. E. West, CA

A paper entitled "Regulations and Standards for Licensing Veterinary Biologics," by Dr. Gerald V. Peacock, Director, National Program Planning, Veterinary Services, USDA, Hyattsville, Maryland, was presented and discussed by the Committee and guests.

A paper entitled "Summary of Veterinary Services Test Results on Biologics, 1978-79", by Dr. Robert J. Price, Senior Staff Veterinarian, Veterinary Services, USDA, Hyattsville, Maryland, was presented and discussed by the Committee and guests.

The entire meeting was devoted to discussion of the proposed Animal Biological Products Act of 1979 (HR 4853), introduced to the House of Representatives on July 18, 1979, by Representative William Wampler of Virginia and referred to the House Committee on Agriculture. This act is intended to regulate, through the Secretary of Agriculture, the production, sale, and shipment of animal biological products and to repeal the Serum, Virus, Toxin Act of 1913.

The Committee and guests represented various constituencies and consensus was difficult to obtain. The Committee agreed that some control of veterinary biologics is needed but could not agree on a method of implementing this philosophy to best serve the consumer, the livestock producer, the veterinary profession, and the biologics industry while preserving rights of the states to meet their individual needs.

By motion, the Committee adopted the following statement to preface a critique of the proposed legislation. "Some regulation of the biologics industry is necessary to assure pure, potent, safe, and effective products. The Committee supports licensing all veterinary biologicals and encourages non-licensed producers to obtain Federal licenses. We feel a non-regulated industry cannot be tolerated and some regulation is necessary to insure uniformly safe and effective biological products based on reasonable and acceptable standards of quality."

However the Biologics Committee does not support the Wampler Bill (HR 4853). The Committee, by vote, approved two specific objections. The specific prohibition of hog cholera vaccines and limitation of funds for implementation of the Act were unanimously opposed by the Committee.
Many other specific concerns were voiced and a five-member subcommittee prepared a list of these. It is attached to this report.

REPORT OF SUBCOMMITTEE TO CRITIQUE HR4853

These concerns regarding HR4853, The Animal Biological Products Act of 1979, were expressed (usually by split vote) by the Subcommittee of B. Eller, D.C.; M. Huff, CO; R. Loan, TX; G. Peacock, MD; and G. West, CA. They were not submitted to the entire Committee for approval.

GENERAL CONCERNS

There should be provision to exempt diagnostic antigens used in research and in diagnostic laboratories at the discretion of the Secretary when this use is not in conflict with organized disease control activities.

There should be provision for limited or special product licensing to meet exceptional area needs, temporary situations, minor species requirements, or to expedite introduction of new products.

The reason for need of new legislation as opposed to enforcement and modernization of existing laws was questioned. The legislative finding failed to clearly answer this question.

The need to regulate export was questioned.

The significance and relevance of the term "on the record" was questioned.

The means by which individual states are judged capable of administering biologic product control legislation must be elaborated.

SPECIFIC RECOMMENDATIONS FOR DELETION OR REWRITING

The following specific concerns identify specific sections and lines of HR 4853 as presented to the House of Representatives on July 18, 1979, and require copy of that document for appreciation.

Section 2 (Legislative Finding), Lines 19-25 on page 2, are objectionable generalizations, as are Lines 1-6 on page 3.

Section 2 (Legislative Finding) Lines 7-15 on page 3, must be rewritten to clarify the concern over products exported.

Section 3 (Definitions) on page 7, Line 9, the modifier whether or not should be replaced with if harmful. In the definition of "safe" or "safety", the phrase when used according to label directions should appear in Line 13 and be deleted from Line 16.

Section 4 (Prohibited Acts), Lines 2-6, represent an apparent oversight in drafting, as it takes away states' prerogatives granted in Section 20. Suggest elimination. The term on the record appearing throughout the Bill (Line 14 on page 11; line 25 on page 12; line 3 on page 14; and line 6 on page 17), implies a degree of formality and inflexibility which
suggests the elimination of informal proceedings, negotiations, and reasonable communication prior to formal hearings.

Section 9 (Suspension and Revocation and Licenses and Permits): The word indefinitely should be removed from Line 12 on page 14. Lines 1-3 on page 16 should be deleted because advertisements and promotions should be treated separately from labels. The entire Section (8) is unreasonable and includes characteristics unrelated to biological production.

Section 10 (Judicial Review), Lines 9 and 10 on page 18, should be deleted because they define judgments which can only be made by the Courts.

Section 20 (States' Prerogatives) should be rewritten, cleaned up, and clarified to remove ambiguities.
THE EFFECTS OF ADULT CATTLE VACCINATION WITH STRAIN 19 ON THE INCIDENCE OF BRUCELLOSIS IN DAIRY HERDS IN FLORIDA AND PUERTO RICO

Paul Nicoletti, DFM, MS

Studies on adult cattle vaccination using Strain 19 were initiated in Florida in 1975 and preliminary results reported. There was a reduction of brucellosis in 5 experimental herds regardless of vaccinal method in a modified test and slaughter program. The dose of vaccine and site of inoculation influenced the results of postvaccinal diagnostic procedures.

In 1977, the subcutaneous inoculation with a reduced dose (0.2 ml) of Strain 19 (approx. $3 \times 10^8$ cells) was approved for problem infected herds. The following is to report on the effects of the reduced dose vaccination of 153 dairy herds in Florida and Puerto Rico. Comparisons are made in numbers of cattle culled due to brucellosis preceding and following vaccination. Results of serologic and bacteriologic studies are also reported.

MATERIALS AND METHODS

Each herd was examined by the card test and reactors slaughtered. The remaining cattle were inoculated subcutaneously with the reduced dose of Strain 19 within 10 days. The next herd test was conducted 3-4 months after vaccination in Puerto Rico and 4-6 months after vaccination in Florida. Herd tests were subsequently conducted at intervals of 2 months in Puerto Rico and 4 months in Florida. When possible, specimens from the udder of cattle which aborted were cultured and serologic tests performed on blood prior to herd tests. Culture positive cattle were immediately culled.

Following vaccination, the card test was performed on all serums as a screening procedure. Serums which were positive and those collected at the time of specimens for bacteriologic examinations were further tested by the rivanol (RIV) and complement fixation (CF) tests. Bacteriologic studies were conducted to evaluate serologic tests and to determine the extent of persistent Strain 19 infections. The card, RIV and CF tests were performed and results classified as previously described except that dilutions to 1:640 and 3 units of complement were used in the CF test. Cultures were performed on milk or non-lactating udder secretions according to the previous report.

Cattle were usually sold if positive on the CF test unless Strain 19 was isolated. Most cattle from which organisms identified as Strain 19 were

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Professor, College of Veterinary Medicine, University of Florida, Gainesville 32610. Previously, epidemiologist, United States Department of Agriculture.

The author wishes to acknowledge the contributions of Ms. M. Perdue for laboratory assistance and Dr. K. Thompson, USDA, San Juan, Puerto Rico, for data.

USDA, Uniform Methods and Rules for Brucellosis Eradication, 1977.
isolated remained in the herds for repeated serologic and bacteriologic studies. Replacement cattle were vaccinated following a negative card test and prior to entering the herds. Following the first parturition, replacements were included in the herd tests regardless of date of vaccination. However, recent vaccination was considered in evaluations of laboratory findings.

RESULTS

Table 1 compares the numbers of vaccinated cattle and size of herds in Florida and Puerto Rico. The numbers of cattle sold per month as reactors during a 6 month prevaccination period and postvaccination intervals are also shown. The percent reduction was least on the first postvaccinal herd test. It is assumed that these reactors largely represent cattle which were in the incubative stage of infection when vaccinated. Most of these were removed from the herds and there was a larger reduction on the second herd test. By the third herd test there was an 87 and 86 percent reduction in reactors sold from Florida and Puerto Rico herds respectively. This had increased to 95 percent in Puerto Rico by the fifth herd test.

The percent of serums which were positive to the card, RIV and CF tests at three postvaccinal intervals among Florida cows is shown in table 2. These data include all cows whether considered infected or positive from vaccination. Nearly all CF test positive cows were sold and replacements added to the herds. It was not possible to always ascertain dates of vaccination of the replacements. Therefore, the percent of positive serums to card and RIV tests on succeeding herd tests was not reduced by the percent of those which were CF test positive on the preceding herd test.

On all occasions the percent of card test positive serums was higher than with other tests and was lowest in the CF test. Thirteen months after herd vaccination 8.5 percent of the serums were card positive while only 0.7 percent had CF titers of 1:40 or greater.

An objective of these studies was to determine the relationship of serum titers to shedding of brucella from the udder. Specimens for bacteriologic examinations were mostly selected from cattle considered most likely to be infected; i.e., those with positive blood titers and in which abortions suggested possible brucellosis. The results of comparisons of serologic and bacteriologic studies are shown in table 3.

Specimens from 956 card positive cattle were cultured and 481 (50.4%) were shedding brucella. Of the card test positive cattle, 93.7% were also RIV test positive and 74.8% were CF test positive. Brucella abortus was cultured from 481 (53.6%) of the 896 RIV test positive cattle. With the CF test, 66.3% of the positive cattle were shedding brucella organisms.

Two cows were negative on all tests when brucellae were isolated. One had aborted and was not retested. The owner suspected impending
abortion in the other cow and organisms were recovered from the udder. Following a normal calving within a few days, the cow's serum was positive on all tests. An additional 6 cows were culture positive and had less than a 1:40 CF test titer. Three of these were Strain 19 infected cows.

Strain 19 was identified in 66 (13.7%) of 483 isolations. Most of these cattle remained in the herds and were negative on subsequent culture attempts.

DISCUSSION

Prior to adult cattle vaccination, the number of infected dairy herds and reactors culled in Florida and Puerto Rico increased. It must be assumed that cattle were more susceptible to brucellosis due to decreased use of calfhood vaccination in replacements. In Puerto Rico, Strain 19 vaccination was not permitted from 1965 to 1977. The dense concentration of cattle results in a high exposure potential to infectious agents. Management practices to prevent introduction of brucellosis or reduce its impact are difficult and seldom followed. Test and slaughter methods alone are often unsuccessful in eliminating infection in large intensive cattle enterprises.

The large reduction in brucellosis in the dairy herds in these areas after adult cattle vaccination can mostly be attributed to the effectiveness of vaccination since few other changes were made. The herd vaccination and subsequent identification of infected cattle and their removal resulted in apparent elimination of brucellosis in many herds in Puerto Rico within one year. The larger herd size in Florida presented more difficulties in total elimination of infection. The probability of initial infection and that it will persist is related to herd size. In these studies herd size did not appear to affect the percent of reduction of infection after vaccination (table 1).

The recommendation of a reduced dose of Strain 19 for adult cattle followed evidence that there was no decrease in protection when compared to a standard dose and significant decreases in postvaccinal titers. However, in adult vaccinated herds supplemental tests are necessary to reduce a false positive reaction on sensitive procedures such as standard agglutination or card tests. The CF test is superior to other procedures as it is negative more quickly after vaccination and results in fewer false positive reactions. Adult cattle vaccination is a procedure which emphasizes the necessity of properly conducted and interpreted diagnostic tests in brucellosis.

Cattle in all stages of gestation were vaccinated and there were few reports of abortions suggestive of vaccine induction. No Strain 19 isolations were made from specimens submitted from cows which aborted at any time during these studies. These observations again emphasize that the abortifacient effects of Strain 19 are minimal. Strain 19 was isolated from 66 cows on the first postvaccinal test (3-5 months) but these were less than one percent of the populations represented in
the serologic and bacteriologic surveillance. It can again be concluded that permanent Strain 19 infection of the udder is rare and that there is no proven public health significance.

CONCLUSION

The effects of adult cattle vaccination using a reduced dose of Strain 19 was studied in 153 dairy herds in Florida and Puerto Rico. There was a reduction of greater than 85% in infected cattle removed from the herds by the third postvaccinal test.

Card, rivanol and complement fixation tests were used in the studies. The card test was a satisfactory herd screening procedure but was too sensitive for the only basis of identifying infected cows. The complement fixation test was least sensitive and failed to properly classify a few infected cattle.

Strain 19 organisms were isolated from 66 cows on the first postvaccinal test but these represented less than one percent of the surveyed population.

It is concluded that adult cattle vaccination with a reduced dose of Strain 19 combined with proper diagnostic tests and interpretations, provide a very practical, economic and often necessary means to control brucellosis in large dairy herds.
Table 1 - Comparison of Dairy Herds Before and After Adult Cattle Vaccination for Brucellosis

<table>
<thead>
<tr>
<th>No. Cows Vaccinated</th>
<th>Florida 54393</th>
<th>Puerto Rico 10854</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Herds</td>
<td>85</td>
<td>68</td>
</tr>
<tr>
<td>Ave. Cows per Herd</td>
<td>640</td>
<td>160</td>
</tr>
<tr>
<td>Reactors*/Month-Prevaccination</td>
<td>707</td>
<td>218</td>
</tr>
<tr>
<td>Reactors**/Month-Postvaccination (Percent Reduction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Test</td>
<td>490 (31)</td>
<td>138 (32)</td>
</tr>
<tr>
<td>Second &quot;</td>
<td>204 (71)</td>
<td>35 (84)</td>
</tr>
<tr>
<td>Third &quot;</td>
<td>95 (87)</td>
<td>30 (86)</td>
</tr>
<tr>
<td>Fourth &quot;</td>
<td>-</td>
<td>18 (92)</td>
</tr>
<tr>
<td>Fifth &quot;</td>
<td>-</td>
<td>10 (95)</td>
</tr>
</tbody>
</table>

* Card Test  
** Complement Fixation Test

Table 2 - Results of Three Serologic Tests at Intervals Following Vaccination of Infected Dairy Herds With Strain 19.

<table>
<thead>
<tr>
<th>Percent Positive</th>
<th>Card</th>
<th>Rivanol</th>
<th>Complement Fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 months</td>
<td>13</td>
<td>6.1</td>
<td>4.5</td>
</tr>
<tr>
<td>9 months</td>
<td>11.3</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>13 months</td>
<td>8.5</td>
<td>1.5</td>
<td>.7</td>
</tr>
</tbody>
</table>
Table 3 - Comparison of Serologic Results and Cultures of Milk Three to Five Months Postvaccination

<table>
<thead>
<tr>
<th>Test</th>
<th>Number Cultured</th>
<th>Number Positive(%)</th>
<th>Strain 19 Isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Card P</td>
<td>956</td>
<td>481 (50.4)</td>
<td>66</td>
</tr>
<tr>
<td>N</td>
<td>41</td>
<td>2 (4.9)</td>
<td>0</td>
</tr>
<tr>
<td>Rivanol P</td>
<td>896</td>
<td>481 (53.6)</td>
<td>66</td>
</tr>
<tr>
<td>N</td>
<td>101</td>
<td>2 (2.0)</td>
<td>0</td>
</tr>
<tr>
<td>Complement Fixation P</td>
<td>716</td>
<td>475 (66.3)</td>
<td>63</td>
</tr>
<tr>
<td>N</td>
<td>281</td>
<td>8 (2.8)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>997</td>
<td>483 (48.4)</td>
<td>66 (13.7)</td>
</tr>
</tbody>
</table>

REFERENCES


USE OF AUTOMATED COMPLEMENT FIXATION SCREENING TEST
FOR SERODIAGNOSIS OF BOVINE BRUCELLOSIS

Margaret E. Meyer, Ph.D.
School of Veterinary Medicine,
University of California, Davis, California

INTRODUCTION

The complement fixation test (CFT) is considered to be the most specific and most sensitive of the tests for the serodiagnosis of bovine brucellosis. Use of this test for mass screening of animal populations for the detection of brucellosis or other diseases, or even its extensive use as a supplemental and/or confirmatory test has not been feasible. The costs stemming from the amount of manual labor required to conduct this test and the high degree of training and technical skills required to perform, read, and interpret the results of the CF tests have all served to preclude its routine, volume use when performed manually. Use of automated equipment for the mechanical performance of the CFT enables the use of this test for mass screening by overcoming the disadvantages associated with having to perform the operations manually. This is not a new idea—the technology and methodology for performance of an automated CF test have been available and in use for twelve plus years.

Using a Technicon AutoAnalyzer, Joubert et al. developed both an automated CF screening test (ACFST) and an automated seroagglutination test (ASAT) and applied both systems, on a small scale, to the diagnosis of bovine brucellosis. Since that time the ASAT system has been refined and can be performed on the AutoAnalyzer either as a qualitative (one dilution) screening test or as a semi-quantitative Both the flexibility and diagnostic precision of the automated system can be increased when necessary by using one channel of a two channel system for the ASAT and simultaneously use the second channel for the ACFST.

The possibility of using Technicon AutoAnalyzers and the automated CF screening test for inclusion in a brucellosis control and eradication program was thoroughly evaluated by the New Zealand Department of Agriculture. The equipment system and the screening CF test was successful, New Zealand used this test as the definitive screening test in its eradication program. By using 30 AutoAnalyzers, 5 million cattle were mass screened for detection of brucellosis between 1971 and 1977 and some 300,000 tests are still performed per month to keep 50,000 herds under CF surveillance. The automated CF test also has been used for detecting Brucella ovis infection in sheep, and the system is currently under evaluation for use in the Canadian brucellosis eradication program.

Last year the opportunity arose to have a Technicon AutoAnalyzer placed in the Brucellosis Laboratory of the School of Veterinary
Medicine, University of California at Davis. This paper reports the results of the use of the equipment and of the automated CF screening test on several cattle herds in California.

MATERIALS AND METHODS

*Automated equipment.* The equipment used for automating the CF-test was a Technicon AutoAnalyzer II. Diagrams of this equipment and the flow of serum and reagents through its component parts have been pictured and described in great detail.

*Method of preparing reagents for performing ACFST.* The reagents for the ACFST were prepared according to the Technicon Company protocol in “Complement Fixation for Brucellosis.” A more detailed description of the CF system is given in the Technicon Instruction Manual on “Automated Complement Fixation Testing.” Briefly, the reagents were prepared as follows:

1. Antigen — A 1:200 dilution of concentrated standard tube test antigen in veronal buffer was used throughout this work.

2. Complement. A 3% solution (2 units) was prepared in veronal buffer 20 to 30 minutes prior to use and maintained in an ice bath from then until the completion of the day’s work.

3. Hemolysin. Baltimore Biologics 50% glycerinated rabbit anti-sheep hemolysin was used at a 1:100 dilution. The amount needed for a day’s use was made up in veronal buffer and kept at room temperature.

4. Sheep red blood cells. Blood was obtained from the same animal approximately every 21 days. Fifty ml of freshly drawn blood was added to 50 ml of Alsevers solution, the cells washed three times by centrifugation and stored under refrigeration as a 10% suspension in Alsevers solution. The cells were washed again in one volume of Alsevers on the day of use and used as a 2% suspension.

The serums for testing were inactivated at 60°C for 40 minutes. Known positive and negative serums were routinely included as controls. The positive control serum was obtained from Hyland Laboratories. It has a titer of 1:160 on the standard tube test.

Except for the antigen, each reagent was titrated when freshly made and retitrated when any one of the reagents in the system was replenished. The antigen concentration was maintained at 1:200, in accordance with the method used by Samagh and Boulanger.

The equipment was operated a speed of 120 tests per hour, incubation time was 32 minutes, and the time for the first samples to flow through the system was 50 minutes, and thereafter samples came through every 30 seconds.

During the course of the 9 to 10 months in which the automated equipment was in operation, serums from a variety of herds were examined. Four of these herds are reported herein. Two of the herds
were examined initially to serve as controls for the level of sensitivity of the test system. The first herd was an entirely non-vaccinated 75 cow dairy herd. The second control herd was a 100 cow dairy herd with a long history of calfhood vaccination. Both of these herds have long been free of brucellosis.

The two infected herds detailed herein include a 525 cow, chronically infected calfhood vaccinated dairy herd in Southern California that has been quarantined for over 5 years. The second infected herd was a 525 cow beef herd, essentially non-vaccinated, that was in the midst of an explosive outbreak of brucellosis. Both herds were already on an approximately 30 day testing schedule when the ACFST became functional.

The modus operandi followed during the course of this study was that serums were first examined by the California Bureau of Animal Health Laboratories by their routine testing procedures. That is, serums from infected herds are screened by the standard plate agglutination test at the 1:50 dilution. Serums positive at 1:50 then are tested at 1:100 and 1:200 dilutions and also are card and rivanol tested. To be classified a reactor, an animal usually must be both card and rivanol positive although, depending upon the herd history, a card positive, rivanol negative animal may also be considered a reactor.

After being tested by the Bureau of Animal Health Laboratory, the serums were delivered to the University of California at Davis and they were then examined by the ACFST and the records of the testing procedures were compared. After at least 4 consecutive herd tests were examined by ACFST, no further CF screening was done on that particular herd, but it was followed retrospectively for 6 months to ascertain the outcome of the status attained by animals wherein the results differed between the routine testing procedures and the ACFST. The two infected herds detailed herein are the only herds in which sufficient time has elapsed from the initiation of this work to allow for a completed herd follow up. Several additional infected herds are in various stages of the retrospective aspect of this study.

RESULTS AND DISCUSSION

It has been demonstrated repeatedly that the CF test is the test of choice for the serodiagnosis of bovine brucellosis. It also has been demonstrated repeatedly that Technicon AutoAnalyzers are flexible and efficient systems for automating serodiagnostic tests. In addition to flexibility, automation offers speed, diagnostic precision, replicability of results and removal of subjective reading of results, none of which can be fully achieved with manual operations—especially when examining the high volume of serums that accrue during mass testing of large populations of animals. Thus, the issue addressed in this study is the suitability and applicability of an automated CF test for use as a herd screening test to detect suspect and reactor animals.

Results on the two control herds. No suspect or reactor animals were
detected by the conventional testing procedures or the ACFST in either the non-vaccinated or calfhood vaccinated, non-infected control herds. Thus, the level of sensitivity of the CF test was such that it avoided detecting any low level titers in the non-vaccinated herd or any residual, low titers in the calfhood vaccinated herd, and the known infected herds then were examined.

Results on the two infected herds. The comparative results of the conventional tests performed by the laboratories of the California Bureau of Animal and of the ACFST, and the details of the retrospective examination of herd records on the two infected herds are shown in Tables 1, 2, and 3.

In the chronically infected calfhood vaccinated dairy herd, serums from the herd tests were examined comparatively in November and December 1978 and January and February 1979. In the course of these tests, 2,100 serums were examined. Twenty-six animals were detected simultaneously as reactors by the conventional tests and positive on the ACFST. That is, the two testing procedures were in agreement on the same serum sample. These results are shown in Table 1.

In addition to the 26 animals wherein the test results were in simultaneous agreement, there were 22 animals in which the tests did not agree. Each of these animals was positive on the ACFST, negative on the card test (hence the rivanol test was not done), and plate titers ranged from negative to positive at 1:200. The details on these animals are shown in Table 2, including the outcome of a 7 month follow up of the records on these animals (through September 1979). Of these 22 animals, 2 were culled by the owner and no follow up was possible. Among the remaining 20 animals, 13 became reactors by the conventional tests between March and September 1979 and have been slaughtered. At the time during November through February that each of these animals was positive on the ACFST, each was card negative and many also were negative (less than 1:50) on the plate agglutination test. In short, each of these animals was considered non-infected and remained in the herd from 30 days to 4 months when each, in fact, was infected.

Among the remaining 7 animals that were ACFST positive but negative by conventional tests at the time of simultaneous testing, 5 either have retained their original plate agglutination titer or had a rise in that titer, and 2 still remain negative on the card and plate tests. Each of these animals is still being followed.

Results on the 525 cow infected, non-vaccinated beef herd. The simultaneous testing of this herd was done on the herd tests for the period from February 5, through April 24, 1979, and the herd records retrospectively examined through August 1979.

During this 3 month testing period, the conventional tests revealed 47 animals that were classified as reactors (plate, card, and rivanol tests were positive) and removed from the herd. On each of these 47 animals,
the ACFST also was positive. During this same testing period, an additional 14 animals were positive on ACFST, but were negative on the conventional tests. The details on these 14 animals are shown in Table 3. The left half of this table shows the results at the time of the initial, simultaneous testing. The right half shows the results of the retrospective examination of the herd records. These records revealed that each of these 14 animals subsequently had a rise in titer on the plate agglutination test and also became card and rivanol test positive, all were classified as reactors, and have been slaughtered. In this group of animals, the ACFST detected infection from 12 to 55 days in advance of the conventional tests. During the course of this 3 month test period, one animal that was 1:50 on the plate test, card positive, and +25 on the rivanol test, and classified as a reactor by the Bureau of Animal Health, was ACFST negative. During this 3 month period of simultaneous testing, 1,478 serums were examined.

**Summary of results.** In the 5 month period of testing from December 1978 through April 1979, 3,753 serums were simultaneously examined. One hundred animals, (39 in the chronically infected dairy herd, 61 in the beef herd) were classified as reactors by the California Bureau of Health on the basis of the conventional tests and sent to slaughter. All of these animals were also ACFST positive, but 27 (13 in the dairy herd, 14 in the beef herd) were detected by the ACFST from 12 days to 120 days (animal 288, Table 2) in advance of the conventional tests. Thus, in these two herds alone, 27% of the infected animals were retained in the herds for varying periods up to 4 months. Seven conventional test negative, ACFST positive animals are still being followed; one ACFST negative animal, conventional test positive animal was slaughtered and obviously could not be followed.

**Present status of this study.** In addition to the herds reported herein in detail, several other of the chronically infected large dairy herds in Southern California also have been examined simultaneously by the conventional tests and the ACFST. In fact, just under 10,000 serums have been examined comparatively.

In these herds, 41 animals were classified as reactors by the Bureau of Animal Health conventional tests and the animals sent to slaughter. The ACFST was simultaneously positive on each of these animals. An additional 64 animals were positive on the ACFST but negative on the conventional tests at the time of simultaneous testing. There herds and the 64 animals involved are in various stages of elapsed time in the retrospective aspects of this study.

These data clearly show that the automated CF screening test is suitable as a herd screening test for detecting suspect and reactor animals and is applicable to a brucellosis control and eradication program. In just a 5 month testing period on only two infected herds with a combined total of 1,050 animals, 27% of the infected animals were detected in advance, and some were well in advance, of the decision af-
forded by the tests conventionally used. Thus, the CF screening test affords to the control program the obvious advantage of earlier detection. Automation of this test makes feasible and practical the use of the CF test and imparts to the user the speed, replicability, and relief from subjective interpretation, and enables the use of the CF test on large populations of animals.

Comments on the use of the AutoAnalyzer. During the 9-10 months that this equipment was in routine operation, we incurred but one work stoppage due to equipment problems. One piece of tubing became plugged with some congealed serum and less than 20 minutes were required to clean and replace the piece of tubing.

The equipment was set to perform 120 tests per hour and we frequently were able to do 700-800 tests per day in 6½ hours, day after day.

Personnel requirements to achieve this were minimal. We had 1 full time technician, who was trained by representatives from Technicon to operate the equipment. The only assistance this technician had was part time high school students who worked in the Bureau of Animal Health Laboratories and decanted serum for shipment to Davis from Southern California. While the initial purchase price of equipment for the automation of serodiagnostic procedures may seem high, the savings in cost of manpower and reagents over manual procedures for mass screening rapidly repays this initial cost.

The AutoAnalyzer that is now in use at the University of California is not only being used for the serodiagnosis of brucellosis, it is also being used for research and development of rapid screening tests for Q-fever and Johne's Disease and we anticipate using it for mass screening of various diseases of poultry.
### TABLE 1. Results of ACFST in a Chronically Infected Calfhood Vaccinated Dairy Herd of 525 Animals

<table>
<thead>
<tr>
<th>Dates of tests</th>
<th>Animals wherein conventional tests and ACFST agreed</th>
<th>Test Results (Plate Card ACFST)</th>
<th>Disposition of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACFST and conventional tests done simultaneously on 30 day herd testing in Nov. and Dec. 1978; Jan. and Feb. 1979. Total of 2100 serums.</td>
<td>N-134</td>
<td>1:100 + +</td>
<td>Slaughtered</td>
</tr>
<tr>
<td></td>
<td>N-374</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OR-485</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-213</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3239</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OR-448</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>326</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>447</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>348</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>157</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>225</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>386</td>
<td>1:50 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>376</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3239</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>445</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1114</td>
<td>1:50 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>235</td>
<td>1:200 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>109</td>
<td>1:50 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>385</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>383</td>
<td>1:100 + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>104</td>
<td>1:100 + +</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2. Results of ACFST in a Chronically Infected Calfhood Vaccinated Dairy Herd of 525 Animals

<table>
<thead>
<tr>
<th>Date of tests</th>
<th>Animals wherein conventional tests and ACFST did not agree</th>
<th>Test Results</th>
<th>Status attained on conventional tests March through Sept 1979</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov., Dec. 1978</td>
<td>N-306 - - +</td>
<td>1:100 + -</td>
<td>ACFST in March</td>
</tr>
<tr>
<td></td>
<td>N-53 1:50 - +</td>
<td>1:50 + +</td>
<td>Slaughtered Jan. 9</td>
</tr>
<tr>
<td>Jan., Feb. 1979</td>
<td>N-23 1:200 - AC</td>
<td>1:200 +</td>
<td>Slaughtered Feb. 28</td>
</tr>
<tr>
<td></td>
<td>N-383 (Dec.) - - +</td>
<td>1:50 +</td>
<td>Slaughtered Feb. 28</td>
</tr>
<tr>
<td></td>
<td>541 1:50 - +</td>
<td>1:100 -</td>
<td>Still in herd.</td>
</tr>
<tr>
<td></td>
<td>518 1:50 - +</td>
<td>1:52 -</td>
<td>Still in herd.</td>
</tr>
<tr>
<td></td>
<td>473 1:100 - +</td>
<td>1:100 + +</td>
<td>Slaughtered July 16</td>
</tr>
<tr>
<td></td>
<td>410 1:50 - +</td>
<td>- -</td>
<td>Still in herd.</td>
</tr>
<tr>
<td></td>
<td>106 - - +</td>
<td>culled - no follow up possible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>254 - - +</td>
<td>culled - no follow up possible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 - - +</td>
<td>1:50 + +</td>
<td>Slaughtered in March</td>
</tr>
<tr>
<td></td>
<td>580 - - +</td>
<td>1:100 + +</td>
<td>Slaughtered in April</td>
</tr>
<tr>
<td></td>
<td>185 1:200 - +</td>
<td>1:200 - -</td>
<td>Still in herd.</td>
</tr>
<tr>
<td></td>
<td>3991 - - +</td>
<td>1:50</td>
<td>Still in herd.</td>
</tr>
<tr>
<td></td>
<td>31 - - +</td>
<td>1:200 + +</td>
<td>Slaughtered in June</td>
</tr>
<tr>
<td></td>
<td>51 1:100 - +</td>
<td>1:200 + +</td>
<td>Slaughtered in April</td>
</tr>
<tr>
<td></td>
<td>359 1:100 - +</td>
<td>1:200 + +</td>
<td>Slaughtered in April</td>
</tr>
<tr>
<td></td>
<td>389 - - +</td>
<td>negative in July</td>
<td>Still in herd.</td>
</tr>
<tr>
<td></td>
<td>288 - - +</td>
<td>1:100 + +</td>
<td>Slaughtered in Sept.</td>
</tr>
<tr>
<td></td>
<td>295 - - +</td>
<td>1:100 + +</td>
<td>Slaughtered in April</td>
</tr>
<tr>
<td></td>
<td>3583 1:50 - +</td>
<td>1:50 + +</td>
<td>Slaughtered in April</td>
</tr>
<tr>
<td></td>
<td>501 1:50 - +</td>
<td>1:100 + +</td>
<td>Slaughtered in April</td>
</tr>
</tbody>
</table>
### TABLE 3. Comparative Results of Conventional Serodiagnostic Tests and ACFST in Infected Beef Herd.

<table>
<thead>
<tr>
<th>Date tested</th>
<th>Animal No.</th>
<th>Plate Card Riv.</th>
<th>ACFST</th>
<th>Date positive</th>
<th>Plate Card Riv.</th>
<th>ACFST CF days ahead</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/5/79</td>
<td>54</td>
<td>1:100</td>
<td>- -</td>
<td>+</td>
<td>2/27</td>
<td>1:100 + +100 +</td>
</tr>
<tr>
<td>2/5/79</td>
<td>A-152</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>3/14</td>
<td>1:200 + +200 +</td>
</tr>
<tr>
<td>2/26/79</td>
<td>157</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>3/26</td>
<td>1:200 + +50 +</td>
</tr>
<tr>
<td>3/14/79</td>
<td>119</td>
<td>-</td>
<td>- -</td>
<td>+</td>
<td>4/23</td>
<td>1:200 + +50 +</td>
</tr>
<tr>
<td>3/14/79</td>
<td>153</td>
<td>-</td>
<td>- -</td>
<td>+</td>
<td>4/23</td>
<td>1:50 + +100 +</td>
</tr>
<tr>
<td>3/26/79</td>
<td>B-46</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>5/14</td>
<td>1:100 + +25 +</td>
</tr>
<tr>
<td>3/26/79</td>
<td>137</td>
<td>-</td>
<td>- -</td>
<td>+</td>
<td>5/14</td>
<td>1:100 + +50 +</td>
</tr>
<tr>
<td>4/23/79</td>
<td>B111</td>
<td>-</td>
<td>- -</td>
<td>+</td>
<td>5/14</td>
<td>1:100 + +50 +</td>
</tr>
<tr>
<td>3/14/79</td>
<td>176</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>3/26</td>
<td>1:200 + +100 +</td>
</tr>
<tr>
<td>4/23/79</td>
<td>76</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>6/5</td>
<td>1:200 + +200 +</td>
</tr>
<tr>
<td>4/23/79</td>
<td>133</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>6/5</td>
<td>1:200 + +200 +</td>
</tr>
<tr>
<td>2/26/79</td>
<td>C-96</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>3/26</td>
<td>1:100 + +25 +</td>
</tr>
<tr>
<td>3/26/79</td>
<td>140</td>
<td>1:50</td>
<td>- -</td>
<td>+</td>
<td>4/15</td>
<td>1:100 + +50 +</td>
</tr>
</tbody>
</table>

* - = negative  
+ = positive
ACKNOWLEDGMENTS

The Technicon AutoAnalyzer was made available to the University of California by courtesy of the Technicon Company who also trained the personnel in its use.

The USDA Regional Office in Sacramento made available funds for the salary for the technician to operate the equipment.

The technician in charge of operating the equipment and coordinating serum deliveries, etc. was Mr. Ken Tudor.

Serums, herd records, and invaluable assistance was provided by personnel of the California State Department of Food and Agriculture, Bureau of Animal Health, especially by Dr. L. C. Vanderwagen.

REFERENCES


EFFECT OF REDUCED DOSAGES OF BRUCELLA ABORTUS STRAIN 19 IN CATTLE VACCINATED AS YEARLINGS

B. L. Deyoe, DVM, PhD; T. A. Dorsey, DVM; Kathryn B. Meredith; and Linda Garrett, BS

National Animal Disease Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture
P.O. Box 70, Ames, Iowa 50010

SUMMARY

One-hundred-six yearling crossbred beef heifers were utilized in an experiment to determine the postvaccinal serological responses and immunogenicity of reduced doses of Brucella abortus Strain 19 vaccine. Five groups of heifers (17 per group) were vaccinated with the following doses: Group A = 1 x 10^7 colony forming units (CFU), Group B = 1 x 10^8 CFU, Group C = 1 x 10^9 CFU, Group D = 1 x 10^10 CFU, Group E = 9 x 10^10 CFU (standard dose). The remaining 21 heifers served as non-vaccinated controls (NVC). At 8 months postvaccination, during midgestation, the immunity of all heifers was challenged by conjunctival exposure to 6 x 10^8 CFU of B. abortus strain 2308. The serologic responses were measured by the Card test, standard tube agglutination, mercaptoethanol, rivanol, and complement fixation tests. Immunogenicity of the vaccine was evaluated by bacteriological examinations conducted at parturition on the dam and fetus or calf and at necropsy on various lymph nodes, uterus, udder, and spleen.

Postvaccinal serologic responses were minimal in Groups A and B, with all animals receding to negative status on all tests in less than 8 weeks after vaccination. In comparison, the higher vaccine doses elicited serologic responses that persisted throughout the experiment in some cattle. Analysis of postchallenge bacteriologic, serologic, and clinical data showed that all vaccine doses used produced significant immunity. Heifers in Group A which were vaccinated with 1/5,000 of the current minimum recommended dose, were fully as resistant to challenge exposure as those vaccinated with higher doses.

INTRODUCTION

Vaccination of cattle with Strain 19 has been a long-standing and important tool for increasing resistance to brucellosis, and thereby controlling the disease. However, during the past decade vaccination with Strain 19 has not been utilized widely enough to have a marked impact on the nationwide control of brucellosis. Reviews of the literature and field studies have suggested that vaccination of cattle at any age, using reduced dosages, would provide significant resistance to brucellosis. Consequently, utilizing reduced dosages should alleviate many of the undesirable side effects of Strain 19 vaccination and promote more widespread usage of vaccination.

To test the hypothesis that reduced doses of Strain 19 would provide
serviceable immunity with negligible undesirable side effects, a 2-phase controlled experiment was initiated to determine the effects of reduced doses in cattle vaccinated as yearlings and as calves. This is a preliminary report on results of the first phase of the experiments.

MATERIALS AND METHODS

The yearling age group was selected for the first trial because: (1) the immunologic response would be anticipated to be similar to that of adult cattle, (2) it would provide a postvaccinal period of at least 8 months, and thus provide some measure of persistence of immunity, and (3) 10-14 months of age is a time when heifers are frequently selected as herd replacements and would be an appropriate age for vaccination.

The cattle utilized in this experiment were 106 crossbred heifers acquired from the Meat Animal Research Center, Clay Center, NE. These heifers were purchased when 11 to 13 months of age and held until they were acclimated for approximately one month prior to vaccination. The median age at the time of vaccination was 13 months. Breeds represented are tabulated (Table 1). All the heifers were sired by Red Poll or Short-horn bulls. There was 1 two-breed cross, 49 three-breed crosses, and 56 four-breed crosses.

The heifers were randomly divided into 6 groups (5 groups of vaccinated, and 1 nonvaccinated control group) and vaccinated on April 26, 1978. The vaccine used was a lyophilized product prepared by the Diagnostic Reagents Section, National Veterinary Services Laboratory (NVSL), Ames, IA. The same lot of vaccine was used in a prior experiment conducted by NVSL, in this experiment, and in another reduced dosage vaccination experiment currently in progress.

All dosages were injected in 2 ml volume by the subcutaneous route. The lyophilized vaccine was reconstituted, pooled, and viability counts conducted 4 days prior to vaccination. The dense suspension of Brucella abortus Strain 19 was held until the day of vaccination under refrigeration (4°C). At that time appropriate dilutions, based on results of the viability counts, were made to achieve the desired dosages. Phosphate buffered saline (pH 7.1) was used as a diluent. Additional viability counts were performed on each vaccine dilution both before and after the cattle were vaccinated. The average of these counts was taken as the number of colony forming units (CFU) of Strain 19 that the cattle actually received (Table 2). During the vaccination process, the vaccine was kept in an ice water bath to minimize loss of viability, particularly in the more dilute suspensions. The $9.0 \times 10^{10}$ dose was considered to be equivalent to the average standard dose of vaccine. However, the vaccine lot used was outdated and viability was lower than anticipated. Consequently, the vaccine as used contained approximately 1.9 times the antigen mass of that contained in the product as prepared.

Following vaccination, blood samples for serologic testing and other purposes were collected at weekly intervals for the first 8 weeks post-
vaccination, biweekly for the next 8 weeks, then at 20, 26, and 33 weeks postvaccination.

All the cattle were maintained together in open lots until approximately 3 weeks before challenge when they were transferred to individual isolation facilities. On July 3, 1978, four Red Poll bulls were placed in the lots for breeding purposes. At this point, the median age of the heifers was 15 months. The bulls remained with the heifers for 10 weeks. During this time the cattle were observed for breeding activity at least twice daily and breeding dates were recorded.

At 34 weeks postvaccination and when all pregnant heifers were in the middle trimester of gestation, challenge exposure with \( B. abortus \) strain 2308 was performed. The challenge culture was derived from the fetal stomach contents from the 3rd abortion of cow 2308 in 1942. The lyophilized material was reconstituted and passaged twice on potato infusion agar prior to final harvesting and standardization of the actual exposure material. The diluent was 1% peptone in saline. The exposure was administered by dropping 0.045 ml of the \( B. abortus \) suspension into the conjunctival sac of each eye of each animal, then manually holding the eye shut for at least 30 seconds to allow absorption and distribution. The exposure dose administered was \( 6.0 \times 10^8 \) colony forming units, determined by viability counts before and after the cattle were exposed. Two heifers suffered traumatic injuries during acclimation to the individual isolation units and were euthanized, thus 104 were challenged.

Following the challenge exposure, blood samples were collected at weekly intervals for 8 weeks, then biweekly for the remainder of the experiment.

At abortion or parturition the following samples were collected and cultured for \( Brucella \): aborted fetus — spleen, liver, lungs, stomach contents; live calf — blood and rectal contents; dam — uterine contents and quarter milk samples.

Necropsies of the heifers commenced at 14 weeks postchallenge, beginning with nonpregnant heifers and those that had aborted prior to that time. Necropsy procedures from beginning to end covered a span of 4 months. At necropsy the following tissues were collected and cultured for \( Brucella \): spleen, uterine washings, each quarter of the udder, and parotid, mandibular, retropharyngeal, bronchial, hepatic, prescapular, prefemoral, popliteal, internal iliac, and supramammary lymph nodes. Media used for cultural examinations was tryptose-serum agar and/or a standard selective antibiotic-containing media. All \( Brucella \) isolated were examined for characteristics of \( B. abortus \) Strain 19 and \( B. abortus \) strain 2308.

Serologic tests done routinely were the Brucellosis card test (card), standard tube agglutination (STT), mercaptoethanol-tube agglutination (ME), Rivanol precipitation-plate agglutination (RIV), and a microtiter complement fixation test (CF) (Wisconsin procedure). In this study, for
purposes of analyzing the data, the following reactions were considered to indicate reactor status:

Card - any degree of readily visible agglutination
STT - + 100 or higher
ME - + 25 or higher
RIV - + 25 or higher
CF - 3+ at 1:10 or higher

No titer tolerances for vaccination were considered, because the ultimate goal is to develop vaccinal procedures wherein such allowances are unnecessary.

In an attempt to provide a measure of severity of infection, an index of infection was used to score the extent of infection in each individual animal. The index calculated (see Table 4) was the mean of individual scores of all cattle in each group. Individual scores were determined by the following cumulative scale: Seroconversion - 1 or 2 points, depending on persistence; Isolation of \textit{B. abortus} from lymph nodes - 1 to 3 points, depending on number of nodes, location, and number of organisms isolated; Udder infection - 1 or 2 points, depending on distribution and number of organisms; Uterine infection - 1 or 2 points, depending on number of organisms; Abortion, stillbirth, or weak calf caused by brucellosis - 3 points; (total points possible = 12).

RESULTS

The postvaccinal serologic response was directly dose-related, as illustrated by the comparison of agglutinin responses between groups of heifers (Figure 1). A more complete analysis of the response to various serologic tests confirmed this dose-related response (Table 3). By 4 and 8 weeks postvaccination, there were no longer any reactors in Groups A and B, respectively. In contrast, the postvaccinal responses in other groups persisted at reactor levels for long periods of time. Titers tended to remain at borderline levels in Groups D and E so that fluctuations in the number of reactors were observed on consecutive tests. There were 11 cattle among Groups C, D, and E classified as reactors by the Brucellosis Card test at a week before challenge.

The heifers were confined in open lots after vaccination, therefore individual measurements of body temperature or other parameters of toxemia were not practical. However, several heifers were observed to be anorexic during the first 72 hours postvaccination. Anorexia was confined to heifers in Groups D and E.

After challenge exposure to \textit{B. abortus} 2308 the mean seroagglutinin titers of cattle in all vaccinated groups increased slightly, while those in the nonvaccinated control group (NVC) increased dramatically (Figure 2). The relationship between vaccinated groups observed during the postvaccinal period, in terms of serologic test results, were maintained throughout the postchallenge period also.
A total of 22 heifers were found to be infected at parturition and/or necropsy; 11 of these were in the nonvaccinated control group (Table 4). The abortion rate in the control group was 33%, whereas abortions occurred only in one animal each from 2 groups of vaccinates. A number of heifers from which *B. abortus* 2308 was not isolated at necropsy, seroconverted, thereby indicating a transient infection with subsequent spontaneous recovery. Animals were classified as having seroconverted when they became reactors to any test or had a 4-fold or greater STT titer rise and remained so for at least a 30 day period.

Statistical analysis of the results indicated that on infection rate (isolation of *B. abortus* 2308) alone, Groups A, D, and E were significantly more resistant than the control group. When infection plus seroconversions were taken into account, all groups of vaccinates were significantly different than the control group. Group comparisons of the index of infection data revealed that differences between the control group and vaccinated groups were significant (Group C) or highly significant (Groups A, B, D, and E).

The results of bacteriologic examinations at the time of parturition and necropsy revealed a combination of sites of infection (Table 5). Only 5 cattle had infection, detectable by isolation of *Brucella*, in the uterus, mammary glands, as well as lymph nodes. Eight heifers had uterine infection, while 8 also had udder infection. Remaining animals had lymph node infection only. Rates of infection were higher in nonpregnant heifers (10 of 13) than in pregnant ones (12 of 91). However, necropsies on nonpregnant animals were conducted at 3 1/2 to 4 1/2 months postchallenge, whereas necropsies on pregnant cattle were conducted postpartum in the general range of 4 to 7 months postchallenge. Thus, it was not possible to determine whether nonpregnant cattle were in the process of spontaneous recovery or were in incubative stages of disease. All *B. abortus* recovered were consistent with the characteristics of strain 2308; organisms typical of *B. abortus* strain 19 were not isolated from any animal.

Serologic tests at or near parturition and necropsy failed to identify many infected cattle as reactors (Table 6). This was apparently related to the severity and extent of infection, as animals with infection in lymph nodes only were seldom serologic reactors. There were a number of serologic reactors, particularly on Card and STT tests, among vaccinated noninfected cattle. Examination of serologic results throughout the experiment indicated that these "false positive" reactions were due to persistent postvaccinal responses in addition to slight anamnestic responses caused by challenge with virulent *B. abortus*.

DISCUSSION

The results of this experiment indicate that all doses of Strain 19 used provide significant protection against challenge exposure. Furthermore, the lowest vaccine dose (1 x 10⁷ CFU or 1/5,000 of the current minimum
DOSES OF *BRUCELLA ABORTUS* STRAIN 19 IN YEARLINGS 97

Recommended dose) was fully as protective as the standard dose. Moreover, persistent postvaccinal responses in the lower doses were negligible in comparison with doses of $1 \times 10^{10}$ CFU or above. These results are in accord with field observations\(^2\) and with results of a recent experiment by Alton et al.\(^3\) where pregnant cows were vaccinated with reduced doses.

The interval between vaccination and challenge was 8 months in this experiment. Whether reduced doses tend to have a diminishing effect with longer postvaccination intervals is speculative at this point. The 2nd phase of this experiment, currently in progress, is designed to partially answer that question. In the 2nd phase, the same vaccine doses have been used, but the vaccine was administered to 5 month old heifer calves and the interval between vaccination and challenge will be 15 months.

Another important observation was that the lowest 2 doses, which were diluted in PBS or saline, consistently lost viability even though maintained in an ice water bath. Viability loss was generally negligible in suspensions containing 10 billion organisms or more. Before widespread field use of reduced doses is recommended, more research on methods for maintaining the stability of Strain 19 in dilute suspensions is indicated.

The number of cattle that became infected, by bacteriological evidence, after challenge exposure to *B. abortus* 2308 was considerably less than anticipated. One cannot assume, however, that the challenge organism has lost its virulence. Actually, the challenge culture was also titrated in guinea pigs and the calculated $\text{ID}_{50}$ was less than 10 CFU. Furthermore, the first abortion occurred within 5 weeks after the challenge exposure, indicating a high degree of virulence of the challenge. The most likely explanation would seem to be an unusually high level of natural resistance to brucellosis among cattle utilized in this experiment.
Table 1. Breeds Represented in Crossbred Cattle used for Reduced Dosage Vaccine Study in Yearlings

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. of cattle*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Poll</td>
<td>88</td>
</tr>
<tr>
<td>Hereford</td>
<td>1</td>
</tr>
<tr>
<td>Angus</td>
<td>-</td>
</tr>
<tr>
<td>Shorthorn</td>
<td>18</td>
</tr>
<tr>
<td>Brahman</td>
<td>-</td>
</tr>
<tr>
<td>Pinzgauer</td>
<td>-</td>
</tr>
<tr>
<td>Devon</td>
<td>-</td>
</tr>
<tr>
<td>Sahiwal</td>
<td>-</td>
</tr>
<tr>
<td>Holstein</td>
<td>-</td>
</tr>
<tr>
<td>Tarentaise</td>
<td>-</td>
</tr>
<tr>
<td>Jersey</td>
<td>-</td>
</tr>
<tr>
<td>Simmental</td>
<td>-</td>
</tr>
<tr>
<td>S. Gertrudis</td>
<td>-</td>
</tr>
<tr>
<td>So. Devon</td>
<td>-</td>
</tr>
<tr>
<td>Brown Swiss</td>
<td>-</td>
</tr>
<tr>
<td>Charolais</td>
<td>-</td>
</tr>
<tr>
<td>Limousin</td>
<td>-</td>
</tr>
<tr>
<td>Gelbvieh</td>
<td>-</td>
</tr>
<tr>
<td>Maine Anjou</td>
<td>-</td>
</tr>
<tr>
<td>Brangus</td>
<td>-</td>
</tr>
<tr>
<td>M. Shorthorn</td>
<td>-</td>
</tr>
<tr>
<td>Red Dane</td>
<td>-</td>
</tr>
<tr>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>1/8</td>
<td></td>
</tr>
</tbody>
</table>

*Fractions represent proportion of designated breeds represented, e.g. 88 were 1/2 Red Poll, 1 was 1/4 Red Poll, and 6 were 1/8 Red Poll.

Table 2. Experimental Design of Reduced Dosage of Strain 19 Experiment Conducted in Yearling Cattle

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cattle</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume (ml)</td>
</tr>
<tr>
<td>A</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>NVC</td>
<td>21</td>
<td>None</td>
</tr>
</tbody>
</table>
Table 3. Numbers of Postvaccinal Serologic Reactors to the Various Tests at Selected Intervals after Vaccination

<table>
<thead>
<tr>
<th>Test</th>
<th>Weeks postvaccination</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>26</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group E (9.0 x 10^10 CFU)</td>
<td>Card</td>
<td>17</td>
<td>17</td>
<td>13</td>
<td>13</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>STT</td>
<td>17</td>
<td>17</td>
<td>13</td>
<td>11</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>ME</td>
<td>16</td>
<td>17</td>
<td>15</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>RIV</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>16</td>
<td>15</td>
<td>11</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group D (1.1 x 10^10 CFU)</td>
<td>Card</td>
<td>16</td>
<td>17</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>STT</td>
<td>17</td>
<td>17</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ME</td>
<td>13</td>
<td>15</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>RIV</td>
<td>14</td>
<td>16</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>12</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group C (1.1 x 10^9 CFU)</td>
<td>Card</td>
<td>14</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
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<td>STT</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ME</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>RIV</td>
<td>9</td>
<td>10</td>
<td>7</td>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>5</td>
<td>4</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B (1.0 x 10^8 CFU)</td>
<td>Card</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>STT</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td></td>
<td>ME</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>RIV</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CF</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group A (1.9 x 10^7 CFU)</td>
<td>Card</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>STT</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>ME</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>RIV</td>
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</table>
Table 4. Comparative Resistance of Vaccinated and Control Heifers to Challenge with *Brucella abortus* 2308 in Midgestation

<table>
<thead>
<tr>
<th>Group*</th>
<th>No. of cattle</th>
<th>No. of abortions**</th>
<th>No. infected† at necropsy</th>
<th>No. infected &amp; seroconverted</th>
<th>Index of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVC</td>
<td>20</td>
<td>15</td>
<td>5</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>A</td>
<td>16</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>17</td>
<td>13</td>
<td>0</td>
<td>4</td>
<td>5</td>
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<tr>
<td>C</td>
<td>17</td>
<td>16</td>
<td>1</td>
<td>4</td>
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<td>D</td>
<td>17</td>
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<td>1</td>
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</tr>
<tr>
<td>E</td>
<td>17</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

*See Table 2 for identity of groups.

**Abortions include premature expulsion of fetus, stillbirths, or birth of weak live calf that died; i.e. any loss of a calf due to brucellosis.

†Infected = isolation of *B. abortus* 2308.
Table 5. Results of Bacteriologic Examination at Parturition and/or Necropsy; Numbers of Cattle with Infection in Various Sites

<table>
<thead>
<tr>
<th>Group*</th>
<th>Cattle infected/total</th>
<th>Location** of Brucella infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UT + UD + LN</td>
</tr>
<tr>
<td>NVC</td>
<td>11/20</td>
<td>4</td>
</tr>
<tr>
<td>A</td>
<td>1/16</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>4/17</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>4/17</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>1/17</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>1/17</td>
<td>0</td>
</tr>
</tbody>
</table>

*See Table 2 for identity of groups.

** UT = uterus; UD = udder; LN = lymph nodes; numbers in parentheses = nonpregnant cattle.

† Heifer (#96) had low level uterine infection and live normal calf at parturition, with no subsequent isolation of B. abortus from dam or calf.
Table 6. Numbers of Reactors with Various Serologic Tests at Parturition and at Necropsy

<table>
<thead>
<tr>
<th>Test</th>
<th>Bacteriologic classification of cattle</th>
<th>Nonvaccinated infected</th>
<th>Vaccinated infected</th>
<th>Nonvaccinated noninfected</th>
<th>Vaccinated noninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>at parturition</td>
<td>necropsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cattle</td>
<td></td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>70</td>
</tr>
<tr>
<td>Card reactors</td>
<td>4</td>
<td>3</td>
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<td>5</td>
<td></td>
</tr>
<tr>
<td>STT reactors</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>ME reactors</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RIV reactors</td>
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<td>3</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CF reactors</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>at necropsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cattle</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>73</td>
<td></td>
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<tr>
<td>Card reactors</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>9</td>
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<td>CF reactors</td>
<td>8</td>
<td>3</td>
<td>0</td>
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</table>
DOSES OF *BRUCELLA ABORTUS* STRAIN 19 IN YEARLINGS

Figure 1
GEOMETRIC MEAN AGGLUTININ TITERS - POSTVACCINATION
See Table 2 for group identity

![Graph showing geometric mean agglutinin titers postvaccination.]

Figure 2
GEOMETRIC MEAN AGGLUTININ TITERS - POSTCHALLENGE

![Graph showing geometric mean agglutinin titers postchallenge.]

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Table 2
See Table 2 for group identity.
REFERENCES


STATUS OF THE COOPERATIVE STATE-FEDERAL
BRUCELLOSIS ERADICATION PROGRAM

Billy G. Johnson, D.V.M. Hyattsville, MD

This has been a year of change in the Brucellosis Eradication Program. The revisions to the Uniform Methods and Rules, recommended by the United States Animal Health Association at the meeting last year, have now been incorporated effective September 4, 1979. Some of the original provisions were altered based on comments received from a broad section of the livestock industry.

These changes were made after nearly 3 years of extensive review and evaluation of the program. We will all be involved during this coming year and several years in the future implementing these changes and the success or failure of the program will probably be based on our diligence in making these changes.

The status report being given today is primarily a numerical gauge of the program compared with activities of previous years. It does not measure changes in interests, opinions, or willingness to move forward toward eradication. These changes should be reflected in future status reports.

Data in this report is estimated using 11 months of actual information with projected counts for the last month. The final totals for the year may vary slightly.

Figure No. 1

Brucellosis Eradication
Blood Testing: Cattle

<table>
<thead>
<tr>
<th>Millions Cattle Tested</th>
<th>Thous Reactors Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970 11.8 11.8 13.6 14.6</td>
<td>1971 17.7 20.8 20.8 17.9</td>
</tr>
<tr>
<td>1972 10.3 11.8 13.6 14.6</td>
<td>1973 17.7 20.8 20.8 17.9</td>
</tr>
<tr>
<td>1974 10.3 11.8 13.6 14.6</td>
<td>1975 17.7 20.8 20.8 17.9</td>
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<tr>
<td>1976 10.3 11.8 13.6 14.6</td>
<td>1977 17.7 20.8 20.8 17.9</td>
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<tr>
<td>1978 10.3 11.8 13.6 14.6</td>
<td>1979 17.7 20.8 20.8 17.9</td>
</tr>
<tr>
<td>1980 10.3 11.8 13.6 14.6</td>
<td>1981 17.7 20.8 20.8 17.9</td>
</tr>
</tbody>
</table>

*Estimated Fiscal Year
The total number of cattle tested in all areas of the program fell from 20.8 million in FY 1978 to 17.9 million during FY 1979. Most of this reduction was due to less cattle being sampled at slaughter while the number tested on farms is only slightly less than was tested last year. The number of reactors disclosed was down by 42,000 from 241,000 in FY 1978 to 199,000 in FY 1979. The reactor rate for all cattle tested was 1.11 compared to 1.16 in FY 1978 (1.14 in FY 1977, 1.29 in FY 1976, 1.46 in FY 1975, and 1.34 in FY 1974).

Figure No. 2

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>At Packing Plants</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>58.2%</td>
<td>41.6%</td>
</tr>
<tr>
<td>1972</td>
<td>62.2%</td>
<td>37.8%</td>
</tr>
<tr>
<td>1973</td>
<td>63.8%</td>
<td>36.2%</td>
</tr>
<tr>
<td>1974</td>
<td>68.6%</td>
<td>31.4%</td>
</tr>
<tr>
<td>1975</td>
<td>70.0%</td>
<td>30.0%</td>
</tr>
<tr>
<td>1976</td>
<td>69.8%</td>
<td>30.4%</td>
</tr>
<tr>
<td>1977</td>
<td>67.1%</td>
<td>32.9%</td>
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<tr>
<td>1978</td>
<td>62.2%</td>
<td>37.8%</td>
</tr>
<tr>
<td>1979*</td>
<td>60.1%</td>
<td>39.9%</td>
</tr>
<tr>
<td>1980</td>
<td></td>
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</tr>
</tbody>
</table>

*Estimated Million Cows Blood Tested

The drastic sell off of breeding cattle for slaughter that resulted in from 13.5 to nearly 15 million cattle being sampled at livestock markets and slaughter during each of the previous 3 years appears to have ended. There were only 11.1 million cattle tested under the MCT program this past year which is the lowest coverage since 1974. The percent of that total that was tested at livestock markets went up slightly to 39.9 percent, a trend that has also occurred since 1974. The number of reactors found through the MCI program dropped from approximately 74,000 in 1978 to 55,000 this year. The MCI reactor rate is .50, a slight decrease from .54 last year (0.53 in FY 1977, 0.66 in FY 1976, and 0.71 in FY 1975). There were 16,598 herds tested as a result of MCI traces, 5,995 which were found infected. The five high incidence States which have not accelerated their programs had 65.7 percent of the total MCI reactors.
There were 778,628 milk samples collected during this year of which 2,034 were found suspicious to the brucellosis ring test. This represented .261 percent of the total samples collected. Of those found suspicious, 1,838 were blood tested with infection found in 348. This is down from 435 infected herds found during 1978 and 601 in 1977. This is the lowest number of herds found infected as a result of BRT screening since the program began. This is an encouraging sign that infected herds are being handled more effectively and that the exposure potential of dairy cattle to beef cattle is being reduced. Even though the infection level is being lowered, continued attention must be given to the BRT procedures. During the late 1960's the ratio of total BRT suspicious herds to infected herds found was approximately 6 to 1. During the early 1970's when less attention was being given to all phases of the brucellosis eradication program, this ratio went down to as low as 3.7 to 1. It is now back to 6 to 1. This is an inexpensive but highly effective surveillance procedure for the dairy population, providing all herds are sampled regularly, samples are handled and tested properly, and suspicious herds are investigated and tested.
The number of infected herds in the 50 states and Puerto Rico dropped by about 600 herds in 1978. There was continued improvement in most certified free States since there were 360 infected herds in 30 States during FY 1979 compared with 364 in 27 States last year. Exceptions to this were Connecticut, Virginia, Nevada, and Washington, all of which had increases over the previous year.

During FY 1979, there were 10 States that had disclosed no indigenous field strain infection for a period of 12 months. Two States, Connecticut and Oregon, which were in this category found that brucellosis had been reestablished within their borders.

Connecticut which had no affected herds since 1972 found infection in three dairy herds. Implicated in this outbreak was a New York cattle dealer who was also licensed in Connecticut. At the time the affected herds were disclosed in Connecticut, this dealer was under investigation by the State of New York for possible illegal activities. In September 1979 he pleaded nolo contendere to the charges levied against him, was fined $50,000 and his New York dealer's license was suspended for 2 years. It should be noted that the name of this dealer appeared in epidemiological reports as the possible source of infection of many affected herds in New York and other States.

Previous to the recent outbreak in Oregon, the last known brucellosis affected herd was released from quarantine in May of 1978. A cattle
company with feedlots and other cattle holdings in Oregon and Washington is involved in the current outbreak. Although still under investigation, there is a possibility that cattle were removed from a feedlot and placed into breeding herds owned by this company. The full extent of this outbreak will not be known until a relatively large number of contact and adjacent herds are tested.

These two cases illustrate very well why the Uniform Methods and Rules (UM&R) changes recommended by the U.S. Animal Health Association at last year's meeting were needed.

Georgia, Tennessee, and Alabama, which have for the most part completed the area testing portion of their accelerated programs were down approximately 800 infected herds from 1978. This statistic combined with the significant drop in the MCI reactor rates are good indicators that the intensified programs have been a success in reducing the level of infection over a short period of time. Both Arkansas and Kentucky which were in the first year of accelerated programs saw an increased number of infected herds as is expected with high level of area testing. The totals for both States should level off during 1980 and then begin to fall rapidly thereafter.

Except for Oklahoma, the number of infected herds in the five high incidence States not accelerated has remained about the same or increased over the previous year; Oklahoma had a small decline.

The area of most concern is the Midwest States where a general increase in both the number of infected herds and MCI rates was seen. These include the States of Missouri, Kansas, Nebraska, and South Dakota. Based on information contained in the epidemiological reports, movement of exposed and infected animals is accounting for much of this increase. Many of the herds are being found through quarantine and retest procedures, but considering that the MCI rates have increased in all four States, there is also evidence that additional spread is occurring.
Texas is now accounting for 35.1 percent of all infected herds found. Mississippi, Arkansas, and Louisiana each had over 1,000 infected herds and account for 25.2 percent. There were six States with 300 to 1,000 infected herds. These include Alabama, Florida, Kentucky, Tennessee, Oklahoma, and Missouri, and total 31.8 percent. These 10 States account for 92.1 percent of all infected herds found in the United States. Forty States account for the remaining 7.9 percent.
The level of calfhood vaccination increased by over 1.1 million calves this year to 5.2 million. The largest increases were in Texas, Mississippi, and Oklahoma where approximately 1 million calves were vaccinated. These are States where State and Federal funds were used for fee-basis payments to practicing veterinarians for this activity. Although the largest increases were in those States, there were general increases throughout the country in the number of calves being vaccinated.
The number of certified free States increased from 27 to 30. The number of certified free counties had increased from 2,016 to 2,039 by June 30, 1979. An additional eleven counties are being listed as certified free during the last 3 months of the year bringing the total to 2,050. This leaves 20 States and Puerto Rico as modified certified. Two counties and the Yellowstone National Park are noncertified.

The swine brucellosis program made significant progress in FY 1979, due largely to the May 1978 amendment to 9 CFR Part 78 that required identification of sows and boars in interstate commerce. Better identification for tracing reactor swine provided an incentive for some States to initiate market swine testing and for others to expand existing programs to include a greater number of slaughter plants.
Figure No. 8

Swine Brucellosis

**Animals Blood Tested**

![Graph showing the number of animals tested for swine brucellosis from 1973 to 1979. The total number of animals tested increased by 77% from 1.94 million in FY 1978 to 3.44 million in FY 1979. Included in this total are 2.85 million sows and boars tested under the Market Swine Testing (MST) program and 590,000 tested on farms.](image-url)

The total number of swine tested during the year increased by 77 percent from 1.94 million in FY 1978 to 3.44 million in FY 1979. Included in this total is 2.85 million sows and boars tested under the Market Swine Testing (MST) program and 590,000 tested on farms.
The expansion of the program resulted in previously untested herds and areas being brought under surveillance in FY 1979 and caused a predicted increase in the animal reactor rate. The MST reactor rate for the year was 0.04 compared to 0.03 in FY 1978. The reactor rate on farm tests also increased in FY 1979 to 0.30, up from 0.19 the previous year. The rate on all tests was 0.08 in FY 1979 compared to 0.07 last year.
The number of Validated Brucellosis-Free States increased from 14 up to 16 during the year with the validation of Pennsylvania, Puerto Rico, and the Virgin Islands being partially offset when one State, Oregon, lost its status for failing to carry out requirements for revalidation. The newly validated States joined Colorado, Iowa, Maine, Minnesota, Montana, New Hampshire, Nevada, South Dakota, Utah, Vermont, Washington, Wisconsin, and Wyoming on the list of States holding free status at the end of FY 1979.

The number of Validated Brucellosis Free counties increased from 711 in FY 1978 to 821 at the present time. In addition to all counties in the 16 validated States, there were free counties in California — 53; Connecticut — 6; Hawaii — 3; and Massachusetts — 8.
There was a decrease in validated herds with 4,944 holding that status at the end of FY 1979 compared with 5,262 a year ago.

During FY 1979, the major program emphasis was directed toward collecting blood samples at the 72 largest packing plants which collectively handle 90 percent of the sows and boars slaughtered annually in the United States. Thirty-nine of these were testing eligible swine at the end of the year. In addition, swine were being sampled at over 700 small volume plants, usually by State and Federal meat inspection personnel. At the present time, 60 percent of the eligible swine slaughtered are tested under the market swine testing program.

Efforts will be made to increase this percentage in FY 1980 by emphasizing blood collecting at all plants slaughtering sows and boars but particularly at those with volumes of over 1,000 swine monthly.

Brucellosis research and field trials supported in whole or in part by USDA funding are being conducted at many different Universities as well as by scientists within USDA. Most of the projects are supported by Science and Education Administration (SEA) and Cooperative Research (CR) but several large efforts continue to receive support from APHIS. At the present time, Veterinary Services (APHIS) is supporting investigations on latent brucella infection in calves, development of new serologic tests, evaluation of methods to distinguish residual vaccinal titers from infection response, evaluation of a new vaccine, studies on
brucella infections in wildlife, improvements in surveillance tests and procedures and other aspects of brucellosis. There are five veterinarians from VS assigned to projects at various Universities to gain indepth knowledge on brucellosis epidemiology. These individuals upon completion of their assignments will be qualified to serve as highly trained Regional Brucellosis Epidemiologists in resolving some of the complex situations in brucellosis eradication.

Plans for a national, comprehensive brucellosis information system have been approved by the Department of Agriculture and must now be approved by General Services Administration. The data management system is to be developed by a contractor. Information on herd tests, market cattle tests, brucellosis vaccinations, brucellosis ring tests and brucellosis epidemiology will be computerized to provide monthly reports, and ad hoc retrieval capability at local and national levels. File security provisions will restrict retrieval of information to officials with a need to know. Five States have been selected for the initial implementation and evaluation of this system. A calendar is presently being prepared for scheduling the various steps required in approving the plan, selecting the successful contractor, developing the data base management system and evaluating and improving the data base. Expansion of the system to other States could be started as soon as the evaluation and acceptance of the data base management system has been completed in the five pilot States.
REPORT OF THE COMMITTEE ON BRUCELLOSIS

Chairman: Dr. A. J. Roth, Virginia

Co-Chairman: Mr. John Armstrong, Texas

Dr. J. A. Acree, FL; Dr. H. Neil Becker, FL; Mr. Richard Nelson, VT; Dr. John Cobb, GA; Mr. Jack Dahl, ND; Dr. Paul Doby, IL; Dr. Frank Drazek, NY; Mr. Burton Eller, DC; Dr. D. E. Flagg, ND; Mr. Bill Gallagher, SD; Dr. R. L. Hartin, OK; Mr. Bert W. Hawkins, OR; Dr. Billy Johnson, MD; Mr. Alfred Keating, IL; Mr. T. A. Kincaid, Jr., TX; Dr. W. C. Ray, MD; Mr. Larry Schaffer, NE; Mr. Raymond Schnell, ND; Mr. Walker Stemler, IL; Dr. L. C. Vanderwagen, CA; Dr. David Walker, VT; Dr. Taylor Woods, MO; Dr. Clint Jewett, AR; Mr. William Knox, WI; Dr. Harvey McCrory, MS; Mr. J. O. Pearce, FL; and Dr. Willis Lyle, WI.

There were 29 members in attendance. The Committee met on Sunday afternoon in general session, and Monday and Tuesday afternoons and Wednesday morning in executive session.

The Brucellosis Committee was called to order at 1:30 P.M. Sunday, October 28, 1979, by the Chairman. Approximately 200 people, including Committee members, were in attendance.

Dr. Frank Mulhern was called on to discuss brucellosis funding. He expanded his topic to briefly review eradication achievements in other programs and to relate the need for a change in attitude regarding brucellosis.

Three years ago, people were saying the Brucellosis Program would not work; it wasn't cost beneficial; more research was needed; the entire program should be studied.

The need for more research was confirmed and is now receiving additional attention. Efforts to control brucellosis were found to be highly cost beneficial. With changes to improve epidemiology and to encourage individual assumption of responsibility, the program can work effectively.

The National Brucellosis Commission's report has been out for 14 months. Now is the hour, said Dr. Mulhern to move ahead with a new program—a new thrust. This meeting is crucial, he said. He noted that the federal commitment is now at the $75 million-per-year level, with states contributing an equal amount. This amounts to $150 million in taxpayers' money.

Dr. Mulhern said he is convinced that an acceptable program can be reached by people of good will reasoning together. He noted the importance of stopping the movement of exposed animals. In the marketing area, this calls for biting the bullet. Industry must accept more responsibility.
Congress looks at the economics of it, observed Dr. Mulhern. Its members want to know how long it will take. He went on to trace the rise in federal brucellosis spending from $23 million in FY 1974 to $77.7 million in FY 1980. Resources are not the problem; research is not the problem; the problem is essentially attitude.

Dr. Paul Becton next discussed the changes which were put into effect by the UM&R, issued with the effective date of September 4, 1979. Your recommendations have been taking shape over the past year, he noted. States have been reacting positively to the changes. Industry involvement began early, with the holding of hearings and public meetings.

The first draft of proposed changes was published on January 20 this year, with a 60-day period for comments and suggestions. Meanwhile, the draft was reviewed by APHIS, with the Technical Commission, to assure adherence to sound epidemiological principles. It became evident that the 60-day comment period would not be long enough. This was extended to April 30, just after the last of four regional meetings was held to explain and discuss changes with program administrators in the various states. Another draft was prepared and reviewed with the Technical Commission. This draft was presented at the July 11 meeting in Chicago.

Dr. Becton next discussed the results of intensified programs in Georgia and Tennessee.

Georgia went from about 500 infected herds in 1977 to under 200 today. Its MCI reactor rate has gone from .380 to .165.

Tennessee went from about 1,250 infected herds to 500. Its MCI reactor rate has dropped from .70 to .30. Both states have made considerable progress representing actual reduction in disease.

All states are responding to requests for a resume of their planned actions on UM&R changes. High prevalence states are showing very positive attitudes.

The Chairman next called upon Mr. Roy Wheeler and Dr. Payne of the Texas Independent Cattlemen's Association, to discuss some points of concern.

Next on the program was Mr. Ray Schnell, representing the Livestock Marketing Association.

Mr. Schnell said his organization only seeks workable solutions. He called for an aggressive vaccination program to be implemented through a system of incentive to producers. He also called for greater flexibility regarding feeder cattle and more options for individual states.

Mr. Jack Dahl of the National Cattlemen's Association spoke next. Mr. Dahl said he endorsed Mr. Schnell's comments on the need for greater flexibility. He then spoke to other areas of concern.

Mr. Dahl also faulted the S-branding provisions as they apply to quarantine feeders.
Dr. Ed Johnson of the Idaho Cattle Feeders' Association next presented proposals on several of the UM&R changes.

Dr. Johnson favors changing the new states' classifications to allow areas within states to be classified as A, B or C. He also favors national adoption of his state's "Registered Feedlot Program," which assures one-way cattle without having to S-brand all cattle entering quarantine feedlots.

Dr. Johnson said his organization is opposed to dealer licensing but favors a concept of accountability termed "proof of merit."

Dr. Billy Deyoe of the National Animal Disease Center, Ames, Iowa, next presented data on a controlled experiment with reduced dosage of Strain 19 vaccine, as applied to a group of yearling heifers. The result of this study was reported earlier this morning.

Mr. John Armstrong of the Texas Animal Health Commission discussed the Texas multi-area plan dated September 28, 1979. The plan calls for a line separating the relatively disease-free western counties from the higher incidence counties to the east. The aim is to allow the western area to achieve Class A or B status through intensive testing, while emphasizing vaccination in eastern counties. The line would move progressively east as cleanup is achieved. (Appendix Pages 124-128).

Dr. Clarence Campbell, Florida State Veterinarian, next presented Florida's plan to eradicate brucellosis. It involves dividing Florida into a clean area northwest of the Suwanee River and a high-prevalence area southeast of the river.

He asked the Committee to consider providing a practical method of quarantine release in adult-vaccinated herds, to allow freer movement of AV cattle. (See Appendix Pages 129-131)

Dr. Bert Lewis of Canada's Ministry of Agriculture, next discussed the eradication program of his country. He described the Canadian program in some detail, noting steady progress toward the goal of complete eradication of the disease. Dr. Lewis noted that, in addition to the areas listed as brucellosis-free last year, British Columbia and part of Northern Alberta have now qualified. All other areas except Ontario are listed as low incidence regions. As of September 30, 241 herds are under brucellosis quarantine in Canada, compared with a peak of 1,043 in 1974. (Appendix Pages 132-133)

Dr. James H. Whittem of the Australian Embassy next presented a report describing progress in his country during 1978-1979.

According to Dr. Whittem, Australia now has all brucellosis records in a database system. The system provides the capability of identifying important factors in the program. It also allows the evaluation of alternate strategies for future years. He pointed out that there is evidence of continued progress in reducing the prevalence of brucellosis. The number of known infected herds decreased from 10,750 to 7,850 during
the year. Herd prevalence remained high at seven percent but individual animal prevalence decreased from 0.65 to 0.21 percent. Use of Brucella vaccine continued to decline in Australia. (Appendix Pages 134-135)

Dr. Frank Drazek presented a report entitled "Brucellosis: A New York Appraisal of a National Program.

His report indicates that New York has made remarkable progress with little reliance on vaccine. The 1977 outbreak in that state resulted from cattle brought in by four dealers. Without a dealer registration law, the outbreak would have been far more costly. Early detection by a traceback was a saving factor. Dr. Drazek noted that registration also has served to protect dealers falsely accused of selling diseased animals. This leads to an increase in business confidence.

Dr. Bryan Espe presented a report entitled "An Eight-Year Study of MCI Program in Oklahoma." The period covered was 1971 through 1978.

Dr. James Alexander presented a report titled "The Cost of Brucellosis to Two California Dairies." The report attempted to use dairy production figures to determine the effect of brucellosis infection upon milk production.

Dr. L. F. Woodward presented a report titled "Immunogenic Properties of Soluble Antigens or Whole Cells of Brucella abortus, Strain 45/20, Associated with Mycobacterial Adjutants."

Dr. Mort Sanders presented a report titled "Field Study on Reduced Strain 19 Dosage in Calves of Various Ages."

The Chairman proceeded to assign two or three person subcommittees to prepare recommendations on some of the more controversial topics.

Dr. Willis Lyle of Wisconsin presented a report favoring a two-level or multi-level system of indemnities. He recommended the formation of a subcommittee to give the matter further study.

Dr. Billy Johnson commented on procedures now underway to develop an indemnity plan keyed to the replacement value of infected animals. An impact statement has been prepared. The proposed rulemaking will call for a 60-day comment period. The target date for comments is December 1, 1979. The target date for implementation is April 1, 1980.

In response to a question, Dr. Paul Becton said APHIS needs input from the Committee on the question of scaling down indemnity payments in states with low quality programs.

Dr. Margaret Myer, Chairperson of the Scientific Advisory Committee, reported out two suggested resolutions to the Brucellosis Committee as requested by the Chairman, one on movement of AV-branded cattle (See Appendix Pages 136) and the other on reduced dosage of Brucella abortus, Strain 19. (See Appendix Page 136) Both resolutions were given to subcommittees for review and action.

The Committee then took up the attached Motion on Identification of
Adult-Vaccinated Animals, presented by Dr. Harvey F. McCrory of Mississippi. The motion passed. (See Appendix Page 137)

Mr. Jack Dahl presented a motion requesting APHIS to further evaluate permanent livestock identification systems and report their findings to the Brucellosis Committee at the 1980 yearly meeting. Motion passed. (See Appendix Page 137)

A motion by Dr. John Cobb was seconded and passed. It proposes that the Public Health Subcommittee re-examine the public health aspect of brucellosis at the annual meeting of the USAHA in 1981.

Dr. J. F. Hudelson presented a motion on continuing education for all veterinarians doing regulatory work; seconded and passed. (See Appendix Page 137)

Dr. Clint Jewett presented a motion urging USDA, APHIS to make fuller use of veterinary practitioners' services in states where fee-basis involvement exists. The motion, as approved, also offers a fee-basis structure guide. (See Appendix Page 138)

Mr. Ray Schell's motion on states requiring that buyers and sellers of cattle be given an educational statement on brucellosis when change of ownership occurs; passed as amended, including an attached example. (See Appendix Pages 138-140)

Dr. David Berman of the Scientific Advisory Committee discussed epidemiological principles involved in allowing freer movement of adult-vaccinated animals.

Dr. Jim Acree presented a motion to modify current UM & R provisions on Dealer Registration and Recordkeeping. This motion, as amended, was passed. (See Appendix Page 141)

Dr. R. L. Hartin presented motions on S-branded cattle and quarantine feedlots which call for modifications of UM & R provisions to allow certain classes of cattle to enter quarantined feedlots and move to slaughter when finished, without being S-branded. (Appendix Page 142)

Dr. L. C. Vanderwagen presented a motion to allow quarantined pastures in Class A states. The approved motion calls for modification of existing UM & R provisions. (See Appendix Page 142)

A motion by Dr. Willis E. Lyle on Indemnity Levels was approved. (See Appendix Page 142-143)

A motion on reduced dosage by Dr. Willis Lyle will be reviewed at the midyear meeting. Chairman Roth requested APHIS, if at all possible, to have recommendations for adoption at that time. (Appendix Page 143)

A subcommittee will be appointed to study the matter of testing requirements for animals going into feedlots. Dr. Becton told the Committee that such a study was in the planning stage.

A motion on Multiple Area Plan presented by Mr. John Armstrong was passed. (Appendix Page 143)
A motion on Special State-Federal Brucellosis-Quarantined Area presented by Dr. L. C. Vanderwagen passed. (Appendix Page 144-145)

A motion titled "Standards for State and Federal Regulatory Performance" presented by Mr. Bert Hawkins passed. (Appendix Page 145)

A motion for movement of AV Cattle presented by Dr. Taylor Woods passed as amended. (Appendix Page 146-146)

A motion on Adjacent Herd and Epidemiologically Traced Herd Testing presented by Mr. Jack Dahl was passed as amended. (Appendix Page 147)

A motion by Mr. Bill Knox on Research passed. It calls for the timely presentation of scientific papers and other data to the Committee members. (Appendix Page 147-148)

A motion by Chairman Jack Roth to have present Brucellosis Subcommittees established as permanent standing committees passed. (Appendix Page 148)

A motion by Chairman Jack Roth calling for standardization of Strain 19 Vaccine passed. Referred to Subcommittee for July, 1980 meeting.

Mr. Bill Gallagher's motion to allow only official vaccinates to move in interstate commerce. To be reported out at 1980 mid-year meeting.

Mr. Clint Booth's report on Brucellosis Education, containing several recommendations, was accepted by the Committee. (Appendix Pages 149-150)

A motion of the Swine Subcommittee, presented by subcommittee chairman Dr. Paul Doby, passed. It calls for a study of the entire Swine Brucellosis Program. Dr. Doby also presented his Swine Committee Report. (Appendix Pages 150-151)

Dr. David Berman said work is in progress on the evaluation of complement-fixation testing, but there are no conclusions to report at this time.

At the invitation of Dr. Taylor Woods, the July, 1980 midyear meeting will be held in Kansas City.

Meeting adjourned.
MOTION ON MULTIPLE AREA PLAN FOR BRUCELLOSIS REGULATION IN TEXAS

I. Introduction
   A. USAHA recommendation one state one area.
   B. Large, relatively clean West Texas Area.
   C. Synopsis of proposal.
   D. Recognize three segments of beef cattle industry (differing operations and regulatory impacts).
      1. Cow-calf
      2. Stocker
      3. Feeder
   E. Producer support—necessary ingredient.

II. Regulatory Aspects
   A. USAHA recommendation for 2 component state—July and October meetings.
   B. Attorney General's opinion—no statutory changes needed (to be verified).
   C. Implement through TAHC regulations (not earlier than January 1, 1980).
   D. General principle—operate test area as though separate state.
   E. See attached map.

III. Area of Intensive Testing (West Texas Test Area)
   A. Area adjacent to New Mexico border to West and line to East representing area where clean-up and test believed feasible to achieve Class A status. (Original line)
   B. Recognition of infection West of the line to be cleaned up.
   C. Moving line West to East—growing contiguous area(s).
   D. Establish first Class A area at 12 months (if possible)—based on contiguous area with no known infection for 12 months. Add adjacent areas as demonstrated clean for 12 months.

IV. Testing
   A. Unvaccinated female cattle (except spayed) over 12 months of age, all vaccinates to be tested over 20 and 24 months of age for dairy and beef cattle, respectively.
   B. First point of concentration.
      1. Market.
      2. At sellers premise.
         a. Private treaty.
         b. Direct shipment to slaughter.
      3. The first test (at origin or first point concentration) is official—not MCI.
   C. MCI—back up only until reliability demonstrated—increased use when reliable—area testing a check on MCI.
D. Routine use supplemental tests on card positive reactors. (Including culture)
E. Card positive — supplemental negative.
   1. Epidemiological investigation.
   2. Test if indicated.
   3. Recognize potential for infection and delayed clean-up.
F. Regional lab support. (CF and rivanol)
   1. Amarillo
   2. San Angelo (?)
G. Adjacent pasture testing (of herds of different ownership).
   1. Initially test pasture adjacent to infected herds.
   2. Test remainder of adjacent herd as indicated.
   3. Epidemiologic judgment.
H. Possible use of area testing in higher prevalence areas which want to assure Class A status.
   1. Accelerated program for federal funds.
I. Post purchase testing.
   1. As required by UM&R’s for imports to area.
   2. Recommend after all sales (60-120 days).
J. Resources.
   1. Seek additional federal funds to reduce cost to producer.
   2. Maintain equity with producers in other parts of state.
   3. TAHC resources analysis—needed urgently.
   4. Adequate lab support.
K. Tests for imports to area (see Section VII).

V. Vaccination
A. Continue calfhood vaccination per UM&R’s.
B. Minimum use of adult (whole herd) vaccination.
C. Adequate records.
D. Imports (see Section VII).

VI. Clean-up
A. TAHC analyze/locate infection.
B. Begin clean-up now (no regulatory change needed to assure achieving Class A status).
C. Adjacent pasture (herd if indicated) testing (for herds of different ownership).
D. Depopulation in problem herds.
E. Accelerated program funds as necessary.
F. Assure fat cattle to slaughter unless tested clean.

VII. Entry Requirements
A. Use same rules as Class A states.
B. Test requirements.
   1. Unvaccinated animals: test requirements same as for Class A state or certified free area per the UM&R’s current at the
time of import with testing required for females 12 months of age or older.

2. *Unvaccinated animals:* may be imported to quarantined feedlot or pasture (see below).

3. Post purchase testing of all females strongly encouraged.

C. Vaccination

1. All eligible females to be official vaccinates or tested negative (in accordance with UM&R's) if over 12 months of age or sent to quarantined feedlot or quarantined pasture.

2. Avoid late vaccination.

D. Quarantined feedlots.

1. No entry test (on imports from outside the test area).

2. No vaccination required.


4. Sell only to slaughter.

5. UM&R's apply.

E. Quarantined pasture.

1. To provide for import of unvaccinated, untested stocker (winter pasture) animals.

2. S-Branded on/before arrival.

3. Sell to slaughter/quarantined feedlot.

4. Seek revised UM&R's to allow quarantined pasture.

5. Rationale—avoid releasing untested imported animals under 18 months into clean area.

6. Could be stopped after calfhood vaccination rates increase into other parts of state.

VIII. *Establishing/Maintaining Demarcation Line*

A. Initial line — as proposed by West Texas Committee.

1. Along county lines.

   a. Presently understood by truckers, etc., as regards certified free counties.

   b. Clean-up programs organized on county basis.

   c. Producers understand division.

   d. If part of a ranch/farm in test or clean area — all adjacent premise also in area.

B. Animals entering area without permit to be delivered directly to specified markets or slaughter for TAHC (or USDA) processing.

C. Permits for all cattle entering the test area other than those going directly to market or slaughter.

D. Road surveillance, by TAHC and law enforcement officials.

E. Inspection and surveillance at markets, slaughter plants.

F. Producer and market operator support and participation.

IX. *Area Commitment to Program*

A. Support of key leadership.

B. Massive education (regulatory, producer, veterinarian).
C. Involvement of individual producers at local level (Extension).
D. Local or area Brucellosis Committees.
E. Maximum legal action on violators.
F. Report violators in news media.
G. Infected herd list published periodically.
H. Sound epidemiologic support.
I. Private veterinarian involvement.
J. Report success, create incentive, show progress.

X. Determining the Class A Area After the Test Period
A. Duration of test period to be consistent with National Plan.
B. All certified free counties in which no further infection found.
C. All known infected herds cleaned up and free from quarantine.
D. Imported infection shown to be isolated and cleaned up.
E. Clean tests (supplemental) on movement for sale for 12 months (?)
F. Demarcation along county lines.

XI. Maintaining Class A Status
A. Class A freedom same as Class A state.
B. Use improved MCI for monitoring.
C. Ring test for dairy operations.
D. Continue border surveillance and import procedures.
E. Resources and motivation to maintain Class A status.
F. In case of reintroduction of infection, Class A state rules apply.

XII. Methods for Enlarging the Class A Area
A. Test and clean-up adjacent areas to initial West Texas area.
B. On producer initiative, consider other areas for clean-up and establishment of Class A status.

XIII. Review and Approval
A. Initial review by USAHA with objective of modifying UM&R's to allow multiple areas in a state.
B. Review of progress and proposal for area Class A' status — Brucellosis Committee of USAHA, recommendation to USDA.
C. Monitoring of progress by USAHA and USDA.

XIV. Summary
A. Propose multiple areas in state initial Class A area in West Texas.
B. Avoid undue burden on relatively clean area.
C. Credible demonstration of clean area.
D. Intensive testing, clean-up and continued monitoring.
E. Assure protection to states importing from Class A area(s).
F. Adequate resources available.
G. Producer and industry support — the key ingredient.
H. Operate in accordance with prevailing UM&R's.
Texas Brucellosis Eradication Program
September 1, 1976
Infected Herds Under Quarantine
1,284

There are 100,000 farms and ranches in Texas that keep cattle.
BRUCELLOSIS

FLORIDA'S BRUCELLOSIS ERADICATION PROGRAM

Florida is a state surrounded on all sides, but one, by water. Therefore, it can be seen that it is vulnerable to the introduction of brucellosis from other states by way of its northern boundary which is common with Alabama and Georgia. Of this approximate 480 mile land border, 175 miles, or 36% of this total, abuts with certified counties in these adjoining states. The remaining 1200 coastal mile border is threatened by brucellosis introduction only through such unlikely hosts as sea cows. As this committee is aware, the states of Georgia and Alabama are currently engaged in an accelerated brucellosis program, augmented materially by federal funds, which is directed toward complete state certification. This, of course, will improve Florida's posture with regard to lessened introduction of the disease into the state.

However, I wish to add another ingredient to this positive approach toward eliminating brucellosis in our area. For the first time in my recollection, and I have been with Florida’s animal disease regulatory division since 1945, the Florida cattlemen's association is of a singular mind in the eradication of the disease. To this end, the association this year introduced and had passed by our legislature a bill which provides as follows:

Senate Bill No. 967

An act relating to the animal industry; renumbering s. 585.155(3), (4), Florida Statutes, and adding a new subsection (3) to said section; requiring each owner of a cattle herd to enroll such herd in a program of whole herd brucellosis vaccination upon funding and approval of such a program by the United States Department of Agriculture; exempting certain herds; authorizing the Department of Agriculture and Consumer Services to make rules; providing an appropriation; providing an effective date.

Be It Enacted by the Legislature of the State of Florida:

Section 1. Present subsections (3) and (4) of section 585.155, Florida Statutes, are renumbered as subsections (4) and (5), respectively, and a new subsection (3) is added to said section to read:

585.155 Whole herd and calf vaccination

(3) Upon approval and funding, by the United States Department of Agriculture, of a program of whole herd vaccination for brucellosis, each owner of a cattle herd in this state shall enroll such herd in a program of whole herd brucellosis vaccination. Dairy cattle herds owned by a person operating a dairy farm as defined in section 502.012, F.S., shall be exempt from provisions of this subsection. Those cattle herds that are in the process of being certified and qualified or which are certified and qualified as of October 1, 1979, may also be exempt from provisions of this subsection. In granting such exemptions to a certified or qualified cattle herd or a cattle herd that is in the process of being certified or qualified,
the Department of Agriculture and Consumer Services shall give con-
sideration to the establishment of areas of low brucellosis incidence
which can be recognized by the United State Department of Agriculture
as Class "A" or "B" status under the Uniform Methods and Rules for
Brucellosis Control and Eradication. The Department of Agriculture and
Consumer Services may make all necessary rules to carry out provisions
of this subsection.

Reaching such an accord on the part of our industry was no simple
matter, and I would be less than honest if I thought that the realization of
our goal would be easy sledding from here on. However, we may never
again be given such an opportunity as that which we now have with our
industry's support in launching an eradication effort. Congress has acted
to provide the funding for the initiation of the project; APHIS is
receptive to our plan of attack; so the ball is now in your court, Gent-
tlemen.

Our request is:

1. That this committee recognize the concept of at least a two-area
   state in determining A, B or C status after January 1, 1982; and
2. That this committee provide for a practical method of quarantine
   release in adult vaccinated herds which would allow for a freer
   movement of these premium AV cattle which have been conferred a
degree of immunity not found in unvaccinated animals.

Florida is on the threshold of moving off of a "dead center" position
which has heretofore precluded making serious headway toward
eradication of brucellosis but, strapped by such non-realistic mangles of
12 month or longer quarantine periods on AV herds as I understand is
being proposed by one of your sub-committees, those heavily infected
portions of this nation simply will not take advantage of this most ef-
factive tool which would properly prepare them for the final stages of the
eradication.

We have made significant inroads in reducing brucellosis incidence
through the use of this procedure since this association approved the AV
plan at Miami Beach in 1976. And I would implore this committee to
maintain this progressive attitude and to develop a safe practically
structured plan for adult vaccinated herd quarantine release so that we
can continue this progress.

In addressing the point of your adopting the concept of at least a two-
area state in determining A, B or C status after January 1, 1982, I feel
that this would be justified in those states meeting the following criteria:

1. That regulatory authority exists to maintain separate areas within
   the state.
2. That legislative authority for maintaining separate areas within the
   state will be available.
3. That resources be committed to maintaining separate areas; and
4. That creditable methods exist for the constraint on movement of animals between areas.

Through this last slide, I have shown you that legislative and regulatory authority exists in Florida to establish such areas. Now let me demonstrate the practicality of such a move in Florida.

At the present time Florida can be divided into two parts — by the Suwannee River which flows from its northern Georgia boundary to the Gulf of Mexico. The northwestern or "Panhandle" area contains 21 counties and the southern area 46.

Let me point out a few comparisons of the two areas.

<table>
<thead>
<tr>
<th></th>
<th>TOTAL</th>
<th>SOUTH OF RIVER</th>
<th>WEST OF RIVER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test eligible cattle</td>
<td>1,158,657</td>
<td>1,049,064 (91%)</td>
<td>109,543 (9%)</td>
</tr>
<tr>
<td>Cattle herds in State</td>
<td>19,479</td>
<td>14,359 (74%)</td>
<td>5,120 (26%)</td>
</tr>
<tr>
<td>Average Herd Size</td>
<td>60</td>
<td>73</td>
<td>21</td>
</tr>
<tr>
<td>Florida Counties</td>
<td>67</td>
<td>46</td>
<td>21</td>
</tr>
<tr>
<td>Quarantined Herds</td>
<td>663</td>
<td>638 (96%)</td>
<td>25 (4%)</td>
</tr>
<tr>
<td>Adult Vaccinated Herds</td>
<td>250</td>
<td>244 (98%)</td>
<td>6 (2%)</td>
</tr>
</tbody>
</table>

(135 Dairy  
115 Beef) (130 Dairy  
114 Beef) (5 Dairy  
1 Beef)

| Brucellosis Certified |                      |                |               |
| Counties              | 19                    | 6 (32%)        | 13 (68%)      |
| % of Total Counties certified | 28%       | 13%            | 62%           |

It is a natural, then, to divide the state as shown since we have 11 truck stop stations on all bridges crossing the Suwannee River which check health certificates to determine that all livestock movements are properly qualified to enter either area. You state veterinarians on this committee are aware that we are currently looking at health certificates on all livestock coming into Florida through these stations as to their propriety.

Pursuit vehicles are utilized by these road guards in the event any truck or trailers attempt to "run" the stations.

So even now our operation is established to implement such a program, if approved.

As stated, Gentlemen, Florida is now ready to move out with this task — the only deterrent remaining being practical enabling action by this committee.

I seriously request your most conscientious consideration of these recommendations.
REPORT OF THE COMMITTEE

TOTAL NUMBER OF NEWLY INFECTED HERDS FOR BRUCELLOSIS IN CANADA FOR FISCAL YEAR 1974-75 TO FISCAL YEAR 1979-80 (SEPT. 30, 1979)

TOTAL NUMBER OF HERDS DEPOPULATED FOR BRUCELLOSIS IN CANADA FOR FISCAL YEAR 1974-75 TO FISCAL YEAR 1979-80 (SEPT. 30, 1979)
BRUCELLOSIS SITUATION IN CANADA ON SEPTEMBER 30, 1979
FOR EACH PROVINCE

TOTAL NUMBER OF HERDS UNDER QUARANTINE
TOTAL NUMBER OF HERDS
PERCENTAGE OF HERDS UNDER QUARANTINE
241
259,141
.09

NON DESIGNATED REGION
LOW INCIDENCE REGION
BRUCELLOSIS FREE REGION

SOURCE: Brucellosis Monthly Statistics
Agriculture Canada
### TABLE 1

AUSTRALIAN BRUCELLOSIS CAMPAIGN - SELECTED STATISTICS 1976-79

<table>
<thead>
<tr>
<th></th>
<th>1976-77</th>
<th>1977-78</th>
<th>1978-79</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of breeder cattle</td>
<td>18.5 million</td>
<td>18 million</td>
<td>10.3 million</td>
</tr>
<tr>
<td>No of herds</td>
<td>200,000</td>
<td>193,000</td>
<td>179,000</td>
</tr>
<tr>
<td>No of females slaughtered</td>
<td>4 million</td>
<td>4.5 million</td>
<td>4.3 million</td>
</tr>
<tr>
<td>No of blood samples from slaughtered cattle</td>
<td>1.2 million</td>
<td>1.9 million</td>
<td>1.8 million</td>
</tr>
<tr>
<td>Reactor Rate</td>
<td>2.7%</td>
<td>2%</td>
<td>1.3%</td>
</tr>
<tr>
<td>No of herds</td>
<td>14,000</td>
<td>42,500</td>
<td>46,500</td>
</tr>
<tr>
<td>No of blood samples tested</td>
<td>1.2 million</td>
<td>4.6 million</td>
<td>8.9 million</td>
</tr>
<tr>
<td>No of reactors detected</td>
<td>35,000</td>
<td>82,000</td>
<td>62,000</td>
</tr>
<tr>
<td>No of reactors slaughtered</td>
<td>33,000</td>
<td>76,000</td>
<td>60,000</td>
</tr>
<tr>
<td>Total No of blood tests carried out</td>
<td>5 million</td>
<td>9.8 million</td>
<td>12 million</td>
</tr>
<tr>
<td>No of known infected herds</td>
<td>18,000</td>
<td>10,750</td>
<td>7,870</td>
</tr>
<tr>
<td>Herd Prevalence</td>
<td>11%</td>
<td>6.5%</td>
<td>7.01%</td>
</tr>
<tr>
<td>Individual Prevalance</td>
<td>1.7%</td>
<td>0.65%</td>
<td>0.21%</td>
</tr>
</tbody>
</table>
BRUCELLOSIS

FIGURE 1
PREVALENCE OF BRUCELLOSIS BY STATE - 1978/79

B = No of Breeders
H = No of Herds
HP = Herd Prevalence
CP = Cattle Prevalence

FIGURE 2
REPORT OF THE COMMITTEE

SUGGESTED RESOLUTION TO THE BRUCELLOSIS COMMITTEE ON MOVEMENT OF "ADULT VACCINATED (AV)" BRANDED CATTLE BY THE BRUCELLOSIS SCIENTIFIC ADVISORY COMMITTEE

Previously infected, adult vaccinated beef herds shall not be released from quarantine for a period of one year following removal of last reactor animal and shall contain no reactor animals at the time of release from quarantine. Such a herd is one wherein all animals in the herd have less than $1^+$ at 1:40 on the CF Test or less than $\frac{25}{2}$ on the rivanol test for two consecutive tests during the one year period.

If sufficient data are accumulated to show that a one year period between removal of the last reactor animal and the last negative test is too long, this period will be reduced.

In all herd tests, a presumptive test shall be performed on each test eligible animal. The card, CF, and/or rivanol tests shall be performed on all presumptive test positive animals. Animals positive to any of these tests, that are not identifiable as official vaccinates, shall be classified as reactors.

Animals positive on the card test, and having a titer of $2^+$ at 1:10 or $+4$ at 1:20 on the CF test shall be classified as suspects and be quarantined until they are card negative or less than $+1$ at 1:10 on the CF test.

Beef herds of unknown history, but containing no reactor animals at time of adult vaccination. If the first herd test is conducted within 120 days post vaccination and reveals no reactors, and second herd test is conducted not less than 90 days following the first tests and it reveals no reactor animals, the herd will be released from quarantine.

This same time and testing schedule shall also apply to "certified free" beef herds.

Suspect animals in herds certified free or in herds or unknown history will be handled the same as individual suspect animals in previously infected, AV beef herds.

DAIRY HERDS

All of the foregoing shall apply to AV dairy herds. In addition, dairy herds shall be negative to the last milk ring prior to release from quarantine.

For movement of individual AV dairy animals, other than movement to slaughter, animals shall have less than a 1:16 titer by the milk ring test on quarter samples.

SUGGESTED RESOLUTION TO THE BRUCELLOSIS COMMITTEE ON REDUCED DOSAGE OF BRUCELLA ABORTUS, STRAIN 19 VACCINE

Whereas: The Brucellosis Scientific Advisory Committee recognizes that the effectiveness of reduced dosage of Strain 19 Vaccine for cattle
above calfhood age has been established in field and controlled trials in the last three years and

Whereas: acting on the recommendations of the Brucellosis Technical Advisory Committee and

Whereas: the U.M. and R. includes recommendations for whole herd vaccination with reduced dosage of Strain 19 Vaccine, and

Whereas: the present label of Brucella abortus, Strain 19 Vaccine does not permit its appropriate use at reduced dosage, and

Whereas: there is a critical need for data on stabilizers, lyophilization temperatures, shelf life, and production methods which will ensure distribution of proper dosage levels for field use.

Therefore: Be it resolved that the USDA give highest possible priority to providing funds and personnel for working cooperatively with universities and industry to develop appropriate data and to present such findings to the National Brucellosis Committee at its July, 1980 meeting.

A

MOTION ON IDENTIFICATION OF ADULT-VACCINATED CATTLE FOR BRUCELLOSIS

It is moved that the Uniform Methods and Rules be corrected to permit or allow:

1. States desiring to use a heat brand, restrict this to an open-ended V on the jaw only, or

2. States not desiring to use the heat V, to use a special metal tag and tattoo in the ear, similar to the calfhood vaccination tags and tattoo.

B

MOTION ON IDENTIFICATION SYSTEMS

Because implementation and widespread use of electronic identification systems are at least several years away, it is moved the USAHA Brucellosis Committee request APHIS to analyze and compare currently used identification systems and methods and to report their findings back to the Committee.

A

MOTION ON CONTINUING EDUCATION

It is moved that all veterinarians involved in any official Brucellosis work at state or federal expense be required to attend a continuing education program on Brucellosis no later than December 31, 1981 and that this training be recommended for other veterinarians doing official Brucellosis work. These programs to be designed and guidelines developed by the Brucellosis staff at APHIS with advice from a member of the Association of Teachers of Veterinary Public Health and Preventive Medicine (ATVPHPH). The programs to be developed in a uniform man-
REPORT OF THE COMMITTEE

ner in order to be presented by state and federal personnel, University personnel and other qualified persons in each state. We further move that Brucellosis programs be offered each year at local, state, regional and national veterinary meetings and that each veterinarian performing official Brucellosis work be required to attend one of these programs at least once during each three-year period following the initial program.

B

MOTION ON VETERINARY PRACTITIONERS AND FEE BASIS STRUCTURE

It is moved that the Animal and Plant Health Inspection Service of the United States Department of Agriculture utilize the services of veterinary practitioners in the Brucellosis-eradication effort in those states where fee-basis veterinary professional involvement does not exist and where there is justification so to do.

It is further moved that where such justification is present, the following fee-based structure be used as a guideline:

Animal No. 1: ........................................ $10.00
Animals Nos. 2 thru 10, per head: ...................... 2.50
Remainder of Animals, per head: ..................... 2.00
Fee per hour for development of plan for eradication of Brucellosis from an infected herd: .................. 40.00/hr.

MOTION ON EDUCATIONAL DOCUMENT FOR CHANGE OF OWNERSHIP

It is proposed that the following Motion be adopted by the USAHA Brucellosis Committee and recommended for incorporation into the UM & R.

Motion: Each state should have regulations which require that an educational statement on Brucellosis:

1. be given to buyers/sellers at the time of change of ownership of cattle:
   a. To create an awareness that Brucellosis is a contagious disease.
   b. to reduce the risk of buying/selling Brucellosis-infected cattle;
   c. to encourage post-purchase testing of cattle 45-120 days after change of ownership;
   d. to encourage owners to vaccinate calves;
   e. to motivate buyers/sellers to take positive actions to prevent the spread of Brucellosis.

2. be designed to meet varying conditions on a state by state or regional basis according to the needs for effective education.
3. be written in plain English and have the approval of the responsible state-federal officials.

4. be printed in large, easily readable type on paper which is a contrasting color that will attract the attention of buyers/sellers.

5. become a part of the necessary papers involved in any livestock transactions such as brand release, bill of sale, health certificate, etc., with copies for both buyers and seller.

EXAMPLE — Next page
BRUCELLOSIS IS A CONTAGIOUS DISEASE
Learn How To Reduce Your Risk of Buying Brucellosis Infected Cattle
$SAVE DOLLARS$ — READ THIS

*BRUCELLOSIS exposed cattle may be blood test negative at the time of sale because they are in the incubation stage of infection and still developing into blood test reactors.

*TO REDUCE RISKS of spreading disease from cattle that are in the incubatory stages of brucellosis at the time of sale AND TO SAVE DOLLARS, follow these recommendations:

1. Ask the seller to give you information on the BRUCELLOSIS status of the herd or herds of origin of any cattle you purchase.

2. Take appropriate precautions to protect these cattle from exposure to BRUCELLOSIS while in your ownership.

3. Have post-purchase blood tests for BRUCELLOSIS conducted between 45 and 120 days following purchase to check for previously incubating infection.

4. If the blood tests indicate BRUCELLOSIS, request your D.V.M. and a D.V.M. from the State Animal Health Agency to evaluate the situation and provide assistance to save you dollars that otherwise could be lost.

FOR FURTHER INFORMATION
CALL Telephone Number 000-000-0000
Name and Address of State Animal Health Agency

INCREASE PROTECTION WITH VACCINATION

Vaccination with Strain 19 *Brucella abortus* vaccine is recommended to increase protection for calves that may be sold or exposed to BRUCELLOSIS in the future.
BRUCELLOSIS

MOTION ON DEALER REGISTRATION AND RECORDKEEPING

Dealer
1. Any person engaged in the business of buying or selling livestock in commerce either on his own account or as the employee or agent of the vendor and/or purchaser, or
2. Any person engaged in the business of buying or selling livestock in commerce on a commission basis.
3. The term shall not include a person who buys or sells livestock as part of his own bona fide breeding, feeding or dairy and/or beef operations and is not engaged in the business of buying, selling, trading or negotiating the transfer of livestock, nor a person who receives livestock exclusively for immediate slaughter on his own premises.

Dealer Registration and Recordkeeping

Any dealer who purchases, deals in, or sells cattle, or acts as a commission representative or broker, and any dealer who operates and conducts an auction where cattle are sold must be registered or licensed with the appropriate state agency of his primary place of business, and maintain required records.

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, suspend or cancel the registration: (a) where there is adequate evidence to establish intent to violate or circumvent animal health regulations; (b) where there is a demonstrated history of repeated inability to trace back to the point of origin those positive-reacting animals handled by the dealer.

2. Records Required — Each registered or licensed person, firm or corporation shall keep sufficient records, for a minimum of two years, of all test-eligible animals purchased for resale to enable the state agency to satisfactorily trace back such animals to their herd of origin.

3. Violations, Remedies — Provisions 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 this part. This shall include the authority to subpoena persons and/or records in violation of these minimum standards as well as 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 which accomplishes the purposes of Section U of this section will be considered an acceptable alternative. Acceptance of this alternative will be based on a review of the provisions and accomplishments (effectiveness of tracing MCI reactors to their herd of origin) of such law or regulation on an individual state basis.
A
MOTION ON "S"-BRANDED CATTLE

Cattle which have been identified by branding with a hot iron the letter "S" (at least 2x2 inches), placed on the left jaw or high on the tailhead (over the fourth to the seventh coccyeal vertebrae). "S" branding is required for exposed cattle and for untested test-eligible cattle, originating in modified certified and non-certified areas, to move in marketing channels from farms or markets to quarantined feedlots (Part I, I), to quarantined pastures (Part I, J), or to immediate slaughter. Testing of test-eligible cattle is recommended in lieu of "S" branding. Finished fed heifers moving in marketing channels to slaughter will be exempted from the "S" branding requirement.

B
MOTION ON QUARANTINED FEEDLOT

A quarantined feedlot shall be a confined area under official state quarantine and shall be approved jointly by the state and federal animal health officials. All animals in a quarantined feedlot shall be classified as exposed to Brucellosis. The quarantined feedlot shall be maintained for finish feeding of animals in drylot with no provision for pasturing or grazing. There shall be no breeding or "two-way" cattle allowed in the quarantined feedlot. All negative exposed animals (and all untested test-eligible animals) must be permanently identified with a hot iron "S" brand, either on the left jaw or high on the tailhead, either prior to or upon entering such feedlots. All cattle leaving such feedlot must go directly to slaughter; or, may be moved directly to another quarantine feedlot with a permit from the state animal health official; or may be "S" branded at the feedlot and move through a market to be sold to move direct to another quarantine feedlot or move direct to slaughter with a 1-27 permit being issued at the market to cover the movement. The animal health officials shall establish procedures for accounting of all animals entering or leaving such quarantined feedlots. Community notifications shall be made of the presence of a quarantined feedlot.

A
MOTION ON QUARANTINED PASTURES PERMITTED — CLASS A STATES

It is moved that the sentence in Paragraph J, Quarantined Pasture, Part 1 of Definitions that denies the use of quarantined pastures in Class A States be deleted.

MOTION ON VARIABLE LEVEL INDEMNITY

It is moved that: USDA-Aphis should:

1. Indemnities should remain at a fixed level but be reviewed at least annually to reflect the market price for the animal.
BRUCELLOSIS

Facts:

a. Indemnity based solely on the market value would be preferable but administrative problems of appraising all animals in high incidence states would be an unreasonable hardship, both in time and in cost.

b. The difficulty of finding slaughtering facilities for Brucellosis reactors will enter into future indemnity considerations.

2. An increased decision-making authority should be given the VIC's regarding indemnity payments for depopulation procedures.

C

MOTION ON REDUCED DOSAGE OF BRUCELLA ABORTUS, STRAIN 19 VACCINE

It is moved that the USDA give highest possible priority to providing funds and personnel for working cooperatively with universities and industry to develop appropriate data and to present such findings to the National Brucellosis Committee at its July, 1980 meeting.

MOTION ON MULTIPLE AREA PLAN

It is moved that the UMRs be modified to allow for the division of individual states into two or more areas of differing status as a means of facilitating eradication. USDA approval of the state proposals for such areas is to be based on review and recommendation of a special committee of USAHA members. These members will be knowledgeable about Brucellosis and shall come from states other than the one under consideration.

The USAHA advisory committee will base its recommendation on the following:

1. Assessment of the commitment of the producers involved.
2. Plan to assure sufficient testing to determine status claimed.
3. Entry requirements of the area to maintain status.
4. Methods for establishing and maintaining the demarcation line.
5. Ability to maintain status after achieving it.
6. (Where appropriate) Methods for changing the size or status of multiple areas.

Specific Proposed Change to UMRs:

III. TERMINATION OF PART V AND PART VI AND INSTITUTING CLASSIFICATION OF STATUS BY STATES OR AREAS — Area status of Modified Certified Brucellosis Areas and Certified Brucellosis-Free Areas will be terminated as soon as possible but not later than January 1, 1982. Part V will be amended in its entirety to establish standards for entire states or areas to be classified as Class "C" — Higher Risk of Brucellosis States. Part VI will be amended in its entirety to establish standards for entire states or areas to be classified as Class "B"
REPORT OF THE COMMITTEE

- Intermediate Risk of Brucellosis States. A new Part VII will be written to amend the Uniform Methods and Rules, establishing standards for entire states or areas to be classified as Class “A” — Brucellosis-Free States.

The USAHA Advisory Committee will assure that in light of the above, the following specific criteria will be met as conditions of a recommendation for approval:

1. Regulatory authority exists to maintain separate areas within the state.
2. Legislative authority for maintaining separate areas within state will be available.
3. Commitment of resources to maintain separate areas.
4. Creditable method for constraint on movement of animals across the boundary.

MOTION ON SPECIAL STATE-FEDERAL QUARANTINE AREA

It is moved that the UM & R be amended to provide for a state-federal quarantine of the circumscribed area or population without loss or denial of Class A status for the state.

Procedures

Exception to standards for States to qualify for Class A status and exception to standards to maintain Class A status

The Chairman of the United States Animal Health Association (USAHA) Brucellosis Committee may appoint a committee of five members to review an application from cooperating State and Federal animal health officials for retention of status as Class A, or advancement to that status when data indicates that State has an area that no longer qualifies as Class A or does not qualify for advancement to Class A status. No State may have more than two such areas. All members appointed to the committee shall be knowledgeable about brucellosis. Members shall represent one of each category: the cattle industry, a university, a State and Federal animal health official from two nonadjacent states, and a representative of the USAHA Brucellosis Committee. No members shall be residents of the State making the application. The committee, after review of the application and interview of witnesses, as may be deemed necessary, shall recommend approval or disapproval to USDA.

A. Format for application for exception

States making a request for exception will prepare a cooperative State-Federal agreement. This agreement will include the following specific issues and define the responsibility of each agency:

1. The procedures and responsibilities for placement and enforcement of quarantines both on the area and herds within.
BRUCELLOSIS

2. The procedures to permit movement for specific purposes both within the defined area and from the defined area.

3. The legal description of geographic boundaries of the area to be quarantined.

4. The specific time period for elimination of all foci of infection.

B. Requirements for submission of application

1. The application shall be accompanied by evidence that the majority of the industry members involved in the area participated in development of the proposed plan, are aware of restrictions it will require, and will assist in monitoring and enforcing the plan.

2. Prior to submission, the State shall be certain it provides for movements to slaughter, identified with "B" or "S" brand under a permit (VS Form 1-27 or equivalent) or for other purposes with a certificate showing a negative test on each animal covered within 30 days of movement.

3. Appropriate legal authority and adequate funding must be available.

4. The application shall not be for areas larger than two counties with clearly defined boundaries providing geographic barriers compatible with monitoring and restricting movement of cattle from and into the area.

5. The time period to eliminate all foci of infection shall be no longer than two years.

C. Monitoring and Reporting

1. Semiannual progress reports shall be submitted to VS.

2. The reports will be reviewed and forwarded to Committee members together with appropriate comments.

3. The Committee may request additional reports at more frequent intervals and/or more detail on some components of the report.

D. Termination

1. The area will be terminated and restored or advanced to Class A status when all requirements for Class A have been met.

2. The area may be terminated and the entire State reclassified a Class B at any time the review committee recommends such action, or at the expiration of the time period approved by the committee.

MOTION ON STANDARDS OF STATE AND FEDERAL REGULATORY PERFORMANCE

Specific areas of concern include: MCI program, laboratory capability,
performance of personnel, especially epidemiologists, management information system, research, adequate funding and milk ring test.

It is moved that the Brucellosis Committee appoint from its membership a Subcommittee which will draft standards of performance for state and federal regulatory agencies, along with methods for measuring this performance.

**MOTION ON MOVEMENT OF AV VACCINATED ANIMALS AS ADOPTED BY THE USAHA BRUCELLOSIS COMMITTEE**

**Beef Herds of Unknown History**

(But containing no reactor animals at time of adult vaccination.) If the first herd test is conducted within 30 to 120 days post vaccination and reveals no reactors, and the second herd test is conducted not less than 90 days following the first test and it reveals no reactor animals, this herd will be released from quarantine.

**Certified Free Beef Herds**

This same time and testing schedule shall also apply to certified free beef herds if no reactors are identified on the post vaccination tests.

Suspect animals in herds certified free or in herds of unknown history will be handled the same as individual suspect animals in previously infected AV beef herds.

**Previously Infected Beef Herds**

Recent history shows that it usually takes from 12 to 18 months to release an infected beef herd from quarantine. The use of adult vaccination to produce whole herd immunization has been shown to reduce the amount of testing needed to be released from quarantine and to reduce the number of animals lost to slaughter as reactors.

"States asked to allow import of adult vaccinated animals from previously known infected herds want assurance that they are importing animals as free from disease as are those they are importing from previously infected non-vaccinated herds released from quarantine. The proposal is that previously infected adult vaccinated herds shall be released from quarantine on two negative tests, the first not less than 30 days following removal of all reactors and the second not less than 120 days following removal of the last reactor, as is currently provided in the Uniform Methods and Rules for release of quarantine in infected non-vaccinated herds. Such herd shall be considered negative if all animals in the herd show titers of less than 1+ at 1:40 on the CF test or less than +25 on the rivanol test. An additional herd test is required not less than 6 months after release of a vaccinated herd or a non-vaccinated herd from quarantine to make this movement on a state-option basis. A post-scale retest on the cattle moving between 120 and 180 days after arrival should be required."
In all herd tests, a presumptive test shall be performed on each test-eligible animal. The card, and CF and/or rivanol tests shall be performed on all presumptive test positive animals. Animals positive to any of these tests, that are not identifiable as official vaccinates shall be classified as reactors.

Official vaccinates positive on the card test and having a titer between \(+2\) at 1:10 to \(+4\) at 1:20 on the CF test in herds eligible for release from quarantine shall be classified as suspects and be quarantined individually (not the whole herd) until they are card negative or less than \(+2\) at 1:10 on the CF test.

**Dairy Herds**

All of the foregoing shall apply to AV dairy herds. In addition, dairy herds shall be negative to the last milk ring test prior to release from quarantine.

For movement of individual AV dairy animals, other than to slaughter, animals shall have less than a 1:16 titer by the milk ring test on quarter samples.

**MOTION ON “ADJACENT HERD TESTING”**

Adjacent herds, or herds sharing common pasture, or having other contact with the affected herd, and herds containing previous purchases from or exchanges with the affected herd **shall have an adjacent herd plan developed within 30 days**. If a disagreement occurs; consultation between the herd owner, chief State animal health officer, epidemiologist, and owner's veterinarian, if requested, will be held to resolve the situation. These findings may involve enforcement of the plan up to the level of an infected herd.

**MOTION ON RESEARCH**

Because the Brucellosis Committee has the obligation to base its recommendations and decisions on current, accurate data and well-documented research, it is requested that research papers presented to the Committee and/or the USAHA General Session be made available in written format, or prior to the opening session of the Committee. Sufficient copies should be provided for members of the Committee, the Scientific Advisory Committee and the Industry Advisory Committee. We also ask that the USDA-APHIS Brucellosis Status Report, traditionally presented to the USAHA Thursday General Session, be made available in written form to the Brucellosis Committee at or prior to the opening session.

It is also requested that the Scientific Advisory Committee report, no later than the opening of the second (Monday) session of the Brucellosis Committee, its counsel on how recently reported research could, or should, impact the UM&R.

The Scientific Advisory Committee is also invited to identify specific
areas of research needed to help expedite the attainment of the national goal of Brucellosis eradication.

Recent brucella research has demonstrated the benefits of increased funding. While producers may be the immediate beneficiaries, it is appropriate to conclude that future eradication program costs will be tempered as the research findings are incorporated into the eradication effort. Thus, we are convinced that increased funding of brucella research is warranted by any cost-benefit analysis. We urge continued emphasis on improved diagnostic tests and immunization.

It is moved that the present brucellosis subcommittees be established as permanent standing committees. The sub-chairman would report at the yearly meeting or as directed by the USAHA Brucellosis Committee Chairman.

Recommended Subcommittees:

Swine Brucellosis
Adult Vaccination Procedures, Herd Procedures, Identification and Movement of Adult Vaccinates from non-quarantined herds.
Practitioner Involvement in the National Brucellosis Eradication Program
Epidemiological Services
Educational Document for Change of Ownership
Animal Identification in all phases of the National Brucellosis Eradication Program
Public Health
Quarantine Areas
Chairman: Clint Booth

Dr. James A. Acree, Dr. D. T. Berman, Mr. John S. Cargile, Dr. Harry Geyer, Mr. W. D. Knox, Mr. Sid Moore, Dr. Paul Schnurrenburger.

At the second meeting of this Subcommittee, it was recognized that a number of valuable information efforts have been carried out, both on the state and federal levels. The Subcommittee heard reports on several current information efforts. The Extension Service has prepared an information piece on Brucellosis and will issue this through State Extension Veterinarians. The Texas Agricultural Experiment Station is in the final stages of preparing a slide-tape presentation which can be used in local Brucellosis meetings. Several favorable comments were expressed on the APHIS radio public service announcements featuring Grandpa Jones.

The Subcommittee recognized that it is particularly appropriate to update and issue additional information items at this time.

"We face a special challenge in information at this time. Now is when we need a comprehensive information and education effort by everyone involved in the Brucellosis Eradication Program."

The Subcommittee made the following recommendations:

1. The State Veterinarian and APHIS Veterinarian-in-Charge in each State should designate a Brucellosis Information Officer to coordinate information efforts. Further, these regulatory officials should be responsible for developing a comprehensive statewide Brucellosis information plan, which should involve the Extension Service, practicing veterinarians, Farm Bureau and other farm organizations, industry organizations, both beef and dairy, market interests, and any other agricultural elements with a stake in Brucellosis eradication.

2. Regulatory officials should establish better communications with veterinary practitioners, directly and through their organizations. In many instances in the past, the practitioner has been left out of the program. To the extent the practicing veterinarian is made a part of the program, he will be more inclined to be a positive source of information about the disease and the eradication program.

3. Regulatory officials should work through cooperating markets to provide suitable information on Brucellosis.

4. We commend APHIS for its training courses for federal veterinarians, epidemiologists and some state veterinary medical officers. However, this effort needs to be expanded to encourage more personnel to participate. A series of orientation and training sessions on Brucellosis should be held within the next 12 months to as-
surve that all state-federal field personnel are well informed and prepared to offer meaningful information on Brucellosis to producers. This effort should be designed to go all the way down to animal health technicians and livestock inspectors involved with Brucellosis field work. Further, there needs to be a systematic review to assure that everyone who needs the training gets it.

5. Similarly, we commend APHIS for its program to check the accuracy of laboratory tests and encourage the continuance of this effort. In addition, they should make sure that laboratory personnel are well trained in new techniques and standard procedures.

6. This Association should issue a press release on Brucellosis vaccination to the farm press sometime following the annual meeting.

7. APHIS should develop a brochure giving animal health advice, including advice on Brucellosis, to buyers of cattle for use by agricultural lending institutions as a handout to customers, as well as for their own information.

8. We recommend a training and continuing education program for the FSQS personnel involved in the MCI program.

9. We urge APHIS to increase the level of funding for the Southeast and Southwest AMS/APHIS Regional Information Offices so that they can give the educational support to the Brucellosis Program necessary in these areas.

MOTION ON SWINE BRUCELLOSIS STUDY

It is moved that the Brucellosis Scientific Advisory Committee of USAHA, in conjunction with the Swine Brucellosis Subcommittee of the Brucellosis Committee of USAHA, and in consultation with state and federal regulatory officials, pork producer leaders and marketing interests, conduct a study of the present Swine Brucellosis Eradication Program, as established in the Uniform Methods and Rules, with the aim of modifying the program to solve the problems outlined above; such study to be completed within the next year and a report made at the 1980 meeting of USAHA.

REPORT OF THE SUBCOMMITTEE ON SWINE BRUCELLOSIS

The first meeting of the new subcommittee on swine brucellosis was held July 10, 1979, in Chicago.

Dr. G. H. Frye presented a progress report of the swine brucellosis eradication effort. His report indicated that:

1. Progress is being made toward implementation of the Market Swine Testing Program, although serious problems have been encountered with the new swine skinning procedures.
2. States are encountering problems obtaining adequate data to validate and revalidate their states.

Dr. Neil Becker gave a presentation on brucellosis in the feral swine population in southern Florida. He said the estimated 400,000 to 600,000 feral swine in Florida are not a true wild species. Dr. Becker reported that brucella has been isolated from the Florida feral swine population.

The subcommittee met October 30 and 31 in San Diego, CA.

At the July meeting, it was suggested that greater emphasis be placed on board testing as a means of surveillance. A study made since that time indicated that testing boars alone is not adequate for detecting disease.

A resolution from the Nebraska Department of Agriculture was discussed. This resolution was directed toward the inability of that state to trace 90% of the reactors disclosed at slaughter to the herd of origin. The discussion of this resolution again brought out the problem encountered nationally with program standards. These standards have prevented states from gaining and maintaining recognition for progress despite surveillance evidence that the disease in that state has declined or been eliminated.

In an attempt to correct this situation, the subcommittee made the following request which was approved by the full committee:

The Brucellosis Scientific Advisory Committee of USAHA, in conjunction with the Swine Brucellosis Subcommittee of the Brucellosis Committee of USAHA, and in consultation with state and federal regulatory officials, pork producer leaders and marketing interests, shall conduct a study of the present Swine Brucellosis Eradication Program, as established in the Uniform Methods and Rules, with the aim of modifying the program to solve the problems outlined above; such study to be completed within the next year and a report made at the 1980 meeting of USAHA.

The subcommittee looks forward to working with the Scientific Advisory Committee on this problem during the coming year.
ABSTRACT

Soluble antigens or whole cells of *Brucella abortus* 45/20 were combined with trehalose dimycolate (cord factor, P3) or muramyl dipeptide (MDP) and tested in guinea pigs as immunogens. Purified protein derivative-like preparations of *B. abortus* 45/20 had limited immunogenic properties either alone or when combined with adjuvants. However, emulsions of whole cells with MDP or P3 reduced splenic infections by 95-99% as compared to control animals. Such preparations could be viable alternatives to present Strain 19 or Strain 45/20 vaccines.
PASTEURIELLA HAEMOLYTICA AND RESPIRATORY DISEASE IN CATTLE

G. H. Frank, D.V.M., Ph.D.*

SUMMARY

A review on the relationship of Pasteurella haemolytica to respiratory disease in cattle is presented. From experimental evidence and field observations, it is generally believed that P. haemolytica is commonly carried in undetectable numbers in the nasopharynx of healthy cattle. Under certain stress or disease conditions, the P. haemolytica can replicate rapidly, and can reach the alveoli in aerosolized droplets. The healthy host will clear the bacteria rapidly from the lungs, but the diseased or stressed host may develop pneumonia.

INTRODUCTION

Pasteurella haemolytica is associated with respiratory disease (RD) in cattle, especially with the condition known as "shipping fever." Although it is commonly isolated from the nasal passages of healthy cattle and of cattle with RD and from pneumonic lungs, its role in RD is not well defined. Several review articles on the subject are available.6,9,10,28

NATURAL INFECTIONS

Natural Disease

P. haemolytica is commonly isolated in high numbers from the lungs of cattle that die from acute RD. Often, it is the only bacterium observed on blood agar incubated aerobically. It has not been isolated from the blood during acute RD.7 Although P. haemolytica is isolated from the nasal passages of healthy cattle, it is isolated with greater frequency from those of cattle which have been stressed or are suffering from RD.7,22,24,39,40 Mean colony counts of P. haemolytica from the nasal passages of recently shipped cattle judged to be sick were higher than from those of cattle judged to be well, although mean colony counts of P. multocida were the same in both groups.40

Healthy Carriers

Several studies have focused on the relationship of P. haemolytica with the healthy carrier. No Pasteurella spp. were isolated from 88 lung and bronchial lymph node homogenates from healthy cattle, but bacteria commonly found in soil and in feces were isolated.8 In a long term study on the nasal bacterial flora of 12 healthy calf herds,41 P. haemolytica and P. multocida were found to be widely distributed although isolations

*From the National Animal Disease Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, P.O. Box 70, Ames, IA 50010.
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were sporadic from individual calves. Bacterial flora in the nasal passage varied widely, and at different times different species prevailed over the others. Results suggested recurring colonization by \textit{P. haemolytica} even in the absence of RD over a 7-month period. Similarly, multiple samplings of a healthy sheep flock revealed that any single sampling detected only a small fraction of the sheep which were carrying \textit{P. haemolytica}.4

In calves carrying detectable numbers of \textit{P. haemolytica} in their nasal passages, the bacterium was also isolated from the tracheal air.21 About 50\% of the \textit{P. haemolytica} were in 1–5 micron droplets, which could reach the smallest passages of the lung.21 \textit{P. haemolytica} was also isolated from the oral cavity of calves found negative by nasal swab cultures.21

\textit{Problems in Isolating P. haemolytica}

In healthy calves, \textit{P. haemolytica} probably comprises a very small fraction of the total bacterial population, thus reducing the chances of isolating a \textit{P. haemolytica} colony on blood agar. Selective media are not available. Chances of isolation are less if the sample is incubated in broth before plating, since \textit{P. haemolytica} will be rapidly overgrown.

Since we were involved in field studies which necessitated storing nasal swab samples for a period of some days before they could be processed, we compared two storage methods and determined the death rate of \textit{P. haemolytica} for each. Aliquots of fresh bovine nasal mucus were seeded with \textit{P. haemolytica}. For the first method, blood agar plates were inoculated with 0.05 ml amounts of seeded mucus, then refrigerated. After predetermined time intervals, they were incubated. For the second method, cotton-tipped swabs dipped in the seeded nasal mucus were put in dry tubes and stored in a dry ice chest. After certain time intervals, the swabs were streaked on blood agar which was incubated. The half life of \textit{P. haemolytica} on refrigerated blood agar was approximately 4 days, and of that stored in the dry ice chest was approximately 2 weeks. Before storage, the recovery from a swab streaked on blood agar was only 3 to 4\% of that in a 0.05 ml volume of nasal mucus.

The efficiency of \textit{P. haemolytica} isolation from individual cattle was studied by comparing antemortem and postmortem culturing results.38 Shipped cattle were grouped according to antemortem culture results into high, medium, low, or negative \textit{P. haemolytica} shedders, then 15 areas of the nasal cavity of each animal were quantitatively cultured at postmortem. Many cattle found negative at antemortem yielded even more \textit{P. haemolytica} at postmortem than those in the low or medium shedding groups.38 Results suggested that routine sampling methods are not sensitive enough to detect small numbers of \textit{P. haemolytica} in the nasopharynx.

\textbf{SEROTYPES AND BIOTYPES}

\textit{P. haemolytica} isolated from cattle and sheep have been divided into serotypes and biotypes, thus enabling epizootiologic studies to be con-
ducted. Biberstein et al. divided isolates from cattle and sheep into 12 serotypes plus untypable isolates by an indirect hemagglutination (IHA) procedure. Smith divided the species into biotypes A and T on the basis of colonial morphology and fermentation of arabinose and trehalose. Later, when the 12 serotypes were grouped according to biotype, serotypes 3, 4 and 10 were biotype T and all others were biotype A.

Only a limited number of complete serotyping studies have been done on large numbers of P. haemolytica isolates owing to the time and effort involved in the IHA procedure. Thus far serotype 1 is the predominant serotype isolated from cattle; however, serotype 2 and untypable isolates are frequently isolated. Serotype 1 is most frequently isolated from calves with RD and is the serotype most frequently found in pneumatic lungs. Serotypes 3, 4, 6, 7, 9, and 11 have been infrequently isolated from cattle. In a long term study, it was observed that one serotype predominated in a herd for 6 to 9 months, and other serotypes were isolated infrequently.

Frank and Wessman recently described a rapid plate agglutination (RPA) procedure that yielded essentially the same results as the IHA procedure for serotyping P. haemolytica. The RPA procedure will allow large serotyping studies to be conducted with little effort, resulting in a more complete knowledge of the epizootiology of P. haemolytica in cattle.

EXPERIMENTAL INFECTIONS

P. haemolytica

There have been many attempts to produce a Pasteurella pneumonia in calves by exposing them to P. haemolytica via the respiratory route in the presence of viral agents and stress. Carter was not able to produce RD by exposing calves to pneumatic lung suspensions by various respiratory routes and by injecting the suspension directly through the thoracic wall into the lung. Usually, no clinical signs of RD result from aerosol or intratracheal infection with P. haemolytica cultures. Some have described an immediate febrile reaction which lasts for 1-2 days with no further complications. In our own experience, after exposing calves via aerosol to P. haemolytica grown directly from frozen lung tissue on blood agar, either there has been no reaction or a one day fever response with shedding for 1-2 days or less. There have been reports of producing clinical illness and P. haemolytica shedding for long periods of time, and vary rarely, pneumonia and death.

P. haemolytica and Bovine RD Viruses

Combination exposures with parainfluenza-3 (PI-3) or infectious bovine rhinotracheitis (IBR) virus and P. haemolytica via aerosol have resulted in a range of clinical responses, from none to marked clinical signs of RD. Results are influenced by viral dosage as well as by the exposure method and the time sequence of exposure, but not many combinations of these factors have been investigated.
Calves exposed intratracheally to PI-3 virus, followed by an intratracheal and intranasal exposure to *P. haemolytica* 24 hours later developed fevers beginning on the second day and continuing for about one week. Baldwin et al. reported that combination exposures with PI-3 virus and *P. haemolytica* resulted in more severe clinical signs of RD and more extensive lesions than exposure with either agent alone. Calves exposed to IBR virus intratracheally, then to *P. haemolytica* via aerosol 3 days later developed fevers by the second day which lasted for a week, and had more pronounced signs of RD than that caused by either agent alone. Collier reported producing clinical signs of RD and a febrile reaction by exposing calves to an aerosol of *P. haemolytica* 30 days after they had been exposed to an aerosol of IBR virus. Lobar pneumonia was produced in 11 of 20 calves which had been exposed to IBR virus via aerosol, then to an aerosol of *P. haemolytica* 4 days later. Lungs were examined for lesions 4 days after *P. haemolytica* exposure.

**VACCINATION**

**Problems in Testing**

Several basic problems make testing the prophylactic value of *P. haemolytica* vaccines difficult. *Pasteurella* pneumonia cannot be produced under experimental conditions with reproducible regularity. This is also true of field trials, since one is relying on chance exposure and resulting RD. The logistics of field trials with definite sampling times and times for vaccination and with adequate controls are difficult. Clinical evaluation of the cattle to determine whether individuals have RD after arrival at the feedyard is highly subjective and the laboratory work on collected samples is voluminous.

**Antibody Response**

Studies have been conducted on antibody response to *P. haemolytica* antigens. Calves in normal herds were found to have low antibody titers to several serotypes of *P. haemolytica* by the IHA procedure. Intravenous and subcutaneous injections of live or killed *P. haemolytica* resulted in a nonsignificant serum antibody rise and no nasal washing antibody response. Aerosol exposures with live *P. haemolytica* caused both serum antibody and nasal washing antibody responses, while aerosol exposures with heat-killed *P. haemolytica* resulted in lower serum antibody titers and no nasal washing antibody response. Some calves with nasal washing antibody titers to *P. haemolytica* serotype 1 were found to harbor serotype 1 in their nasal passages.

**Serotypes and Cross Protection**

Nothing has been reported about cross protection afforded by the various serotype in cattle. However, mice vaccinated with a serotype 1 isolate were protected against challenge with a serotype 2 isolate, which
lacked any demonstrable serologic relationship to the immunizing strain. Results indicated that antigens other than somatic and capsular antigens might be involved in protection.

HOST FACTORS

Lung Clearance Studies

Calves exposed to aerosols of *P. haemolytica* cleared 75% of the bacteria from the lungs within 2 hours, 90% within 4 hours and 92% within 8 hours. Pulmonary edema induced by several methods increased lung retention of *P. haemolytica*. The treatment procedure itself had no effect unless edema was actually induced. Aerosol exposure to PI-3 virus 1 day before *P. haemolytica* exposure increased lung retention slightly. In another study, PI-3 virus exposure 3 days before *P. haemolytica* exposure had no effect on lung retention, but with 7 and 11 day intervals between exposures, there was marked lung retention of *P. haemolytica*. Factors which decrease lung retention of *P. haemolytica* are difficult to evaluate, since the normal lung is efficient enough that only very little improvement is possible. Even so, immunization with *P. haemolytica* may have decreased retention.

Alveolar Macrophage Studies

Four hours after aerosol exposure of calves with *P. haemolytica*, there were foci of sublobular atelectasis and an 18% increase in the number of alveolar macrophages. The alveolar macrophages were the main cells to phagocytize the *P. haemolytica*, but some were phagocytized by the polymorphonuclear leukocytes.

Live *P. haemolytica* serotype 1 were cytotoxic to alveolar macrophages in vitro and were phagocytized at a low rate, while heat-killed bacteria were mildly cytotoxic and readily phagocytized. The cytotoxic factor was present in bacteria-free filtrates of culture medium.

Pathologic Studies

Examination of various locations in the nasal cavity of healthy carriers by fluorescent antibody procedures revealed *P. haemolytica* on epithelial cell surfaces and in cellular debris and not in or between cells or within ducts.

Lung lesions were described and graded in 2 to 4-month old calves 18 hours, 3 days, and 7 days after intratrachael inoculation with *P. haemolytica*. Most severe lesions were observed at 3 days and resembled those described for natural cases of pneumonic pasteurellosis.

DISCUSSION

The mechanism of *Pasteurella* pneumonia remains obscure, owing to the difficulty in producing the disease under laboratory conditions. The normal relationship between *P. haemolytica* and the healthy calf seems to be that of a carrier state, during which *P. haemolytica* reaching the lungs
are quickly cleared by the alveolar macrophages. At times, the host situation is such that the *P. haemolytica* population will increase rapidly in the nasopharynx, and more will reach the lungs. The lungs of a healthy calf can clear extremely large numbers of *P. haemolytica* in an inoculum, but when the calf's own *P. haemolytica* population rapidly increases in the nasopharynx, the situation which allowed or caused the increase may be systemic rather than local, and thus affect lung clearance. If the state is prolonged, *Pasteurella* pneumonia may develop. The exact physiological state of susceptibility is unknown, but it can often be caused by such factors as respiratory viral infection and stress.

REFERENCES

**RESPIRATORY DISEASE IN CATTLE**


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

Chairman: G. D. Gurss, DVM, Topeka, Kansas

Co-Chairman: V. A. Seaton, DVM, Ames, Iowa

Arthur A. Anderson, New York; H. A. Arnold, Maryland; R. P. Azelton, Missouri; D. E. Bartlett, Wisc; H. J. Bearden, Miss; W. D. Bolton, Vermont; Dale L. Brinkmeyer, Iowa; L. N. Brown, Texas; E. A. Carbrey, Iowa; George L. Crenshaw, Calif; W. H. Dreher, Wis; F. A. Flipse, Kansas; R. W. Fulton, La; N. Bruce Haynes, NY; Werner Heuschele, Ohio; R. E. Horton, NJ; Jerry D. Houck, SDak; N. W. Kruse, Nebr; George Lambert, Iowa; M. A. Luedke, Colo; A. W. McClurkin, Iowa; R. McClymont, Nebr; Charles A. Mebus, NY; R. Morgan, MD; Robert M. Nervig, Iowa; Carl Olson, Wis; Martin W. Peterson, Ill; M. P. Reeve, Kansas; J. A. Schmitz, Oregon; Ronald Schultz, Ala; Dan E. Suther, Calif; N. R. Swanson, Wyo; John Wilbur, Texas; T. H. Woods, Mo.

The Infectious Diseases of Cattle Committee Meeting was called to order at 1:30 pm on October 29, 1979, in the Forum Room, Town and Country Hotel, San Diego, California. There were 32 members and guests in attendance.

Members of the Committee asked that the following diseases of cattle, Malignant Catarrhal Fever, Bovine Virus Diarrhea, and Foot and Mouth Disease, be discussed at the 1979 annual meeting. The contents of this report support this request.

Dr. John Mare' of the University of Arizona gave a synopsis on MCF. Dr. Mare pointed out that MCF may be one of perhaps several different diseases, and that the disease is caused by at least two and maybe more causative agents. MCF is an infectious disease that produces a mortality in excess of 95 percent. Common clinical signs include a febrile reaction, ocular and nasal discharge, skin lesions, and CNS signs. At times, the lesions are often inconsistent and confusing. In the United States, sheep have been suspected of being carriers of the virus. In Africa, a herpes virus has been shown to be involved when there was spread to cattle from wild animals. The disease can be produced in rabbits, and it has also been shown that cattle-to-cattle transmission can occur. For these reasons, it is believed we are probably dealing with different disease syndromes all under the heading of Malignant Catarrhal Fever.

Dr. Carlos Reggiardo, Texas A&M University, presented a paper on how Bovine Virus Diarrhea virus infection affects immune responses in cattle. His studies showed that BVD virus infects the lymphocytes and that, following this infection, the lymphocytes lose their ability to produce normal immune responses. The immune suppression allows other agents such as IBR virus and Pasteurella hemolytica bacteria to cause extensive respiratory problems. The lack of response by the lymph-
ocytes provides the reasons that treatment used on young, sick calves fails in so many cases.

Dr. John Atwell updated the procedures being considered and executed by APHIS in regard to Foot and Mouth Disease. It was pointed out that previous plans used to eradicate F&M in the United States might need to be modified. The use of quarantines and slaughter has worked well in the past. However, other countries have used vaccination, along with slaughter, and this procedure has been successful.

The Advisory Committee for the Secretary of Agriculture has recommended that a fallback policy be established which would include the use of vaccine in an eradication effort. In order to fulfill this recommendation, APHIS is in the process of purchasing six million doses of F&M antigen. This purchase includes Types A, O, and C. It should be explained that, since there are seven known types of F&M virus, the possibility does exist that the antigen being purchased may not cover a F&M outbreak.

Dr. Van Der Maaten gave the Bovine Leukosis Sub-Committee Report. He reported that the serological testing for bovine leukemia virus infection has resulted in restrictions on the movement of cattle in international trade. Therefore, the sub-committee requested by resolution that the USAHA request APHIS of the USDA to undertake and encourage certain activities that will aid in the exportation of breeding stock relative to bovine leukemia. A motion was made, seconded, and passed that the parent committee accept this request. The resolution has been supplied to the Resolution Committee for their consideration. Some of the activities suggested for APHIS consideration include standardization of testing procedures, evaluation of the prevalence of the disease, and to study the feasibility of developing voluntary official programs for the control and eradication of BLV within herds and provide certification of such herds. Suggestions for consideration in the formulation of programs were outlined in the resolution.

The meeting was adjourned at 3:45 p.m.
LABORATORY PRACTICES INVOLVING THE LEPTOSPIRAL MICROSCOPIC AGGLUTINATION MICROTITER TEST

H. C. Ellinghausen, Jr., PhD
National Animal Disease Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, P.O. Box 70, Ames, IA 50010

The microscopic agglutination (MA) test for the serological diagnosis of leptospirosis using living antigen remains today as the reference test for this disease. Various investigators have concerned themselves with factors which influence this test procedure. Roessler nephelometric turbidity standards and more recent availability of a dry well cuvette holder have aided some laboratory workers to readily standardize antigen density in the MA test. The objectives of this paper are to emphasize: (1) the value of available hyperimmune sera, (2) the limits and advantages of using 2-mercaptoethanol, (3) cross-agglutination of hebdomadis serotypes, (4) the effect of freezing microtiter dilutions (hyperimmune and paired bovine sera), (5) the complexity of the Hebdomadis serogroup, and (6) possible approaches to obtaining comparable results among laboratories when performing the MA test.

MATERIALS AND METHODS

Cultural procedures

The liquid medium to propagate living cultures used as MA antigens has been previously described. This medium with agar at a final concentration of 0.2% was used to grow cultures used for the hyperimmunization of rabbits. The cultures employed in various aspects of MA testing were pathogenic serotypes: copenhageni M-20 (icterohaemorrhagiae), canicola Hond Utrecht IV, ballum S-102, pyrogenes Salinem, djasiman, australis Ballico, pomena DM2H, grippotyphosa Moskva V, bataviae Van Tienen, tarassovi Perepelcin, and 9 members of the Hebdomadis serogroup: hebdomadis, kambale, borincana, szwajizak, hardjo, wolffi, sejroe, balcanica, and saxkoebing, and saprophytic strains: semaranga, Patoc 1, B16, St. Chas #3, Illini 3055, Korman, and Purdue Med.

Three strains of Leptospira hardjo: (a) bovine urine isolate (from Dr. R. M. Nervig) CCNN (U.S. Meat Animal Research Center, Clay Center, Nebraska), (b) hardjoprajitno (WHO Leptospiral Reference Laboratory, Center for Disease Control, Atlanta, Georgia), and (c) New Zealand #12 (N.Z. #12) were used in semisolid growth medium to inject rabbits. The CCNN and N.Z. #12 strains have an infectivity for female weanling hamsters of 2 x 10⁸ cells when inoculated intraperitoneally and may be recovered back from hamsters when posted 14 days later in cultures of urine, kidney, and brain. The hardjoprajitno culture cannot be isolated back from such tissues. Cultures CCNN and N.Z. #12 were periodically
tested for their ability to infect hamsters. It is not known what effect the maintenance of infectivity has upon a culture used to produce hyperimmune sera. The two infectious *hardjo* strains were used as *in vitro* stored cultures of the original isolations.

*Production of hyperimmune sera*

New Zealand white female rabbits (6 to 8 lbs) were bled from the marginal ear vein and their preimmunization sera tested at final 1:10 dilutions in the MA microtiter test against the pathogenic antigens previously mentioned under cultural procedures. All rabbits were injected via the marginal ear vein with 1, 2, and 3 ml of heavy semisolid growth at 0, 7, and 14 days, respectively. Such cultures were grown in bovine albumin polysorbate 80 semisolid medium for 7 days. When the subsurface zone of growth typical of leptospires was readily macroscopically detectable, the growth zone was aseptically aspirated from the culture with needle and syringe. One ml of the parent cultures was inoculated to fresh medium to prepare cultures for the second injection. This procedure was repeated; thus, keeping the cultures in a rapid growth phase. The semisolid growth medium (0.2% agar) cultures used alive have shown no apparent adverse effect upon these animals, with 20 rabbits inoculated with both virulent and avirulent strains.

The pair of CCNN *hardjo* injected rabbits were exsanguinated (bled out) during euthanasia at 28 days after the first 1 ml injection and their sera tested in the MA microtiter test using different dilution schemes to bracket the titers of antisera.

The second pair of rabbits were similarly injected with the *hardjo-projitno* strain and the N.Z. #12 strain. In order to acquire sera for use with the 2-mercaptoethanol (2-me) test, the rabbits were bled at 7 and 14 days. These sera were subsequently tested against 9 serotypes in the *Hebdomadis* serogroup (Table 3); eight other pathogenic serotypes and 7 saprophytic leptospires (Table 4).

*Serological procedures*

Antigens for use in the MA microtiter test were propagated, standardized, and added to microtiter dilutions as previously described. Serial 2-fold dilutions of sera were made with PBS and the final dilutions that are reported include the volume of antigen added. Routine dilutions started at 1:10, however, when more precise bracketing of titers was desired initial dilutions of 1:4 and 1:6 were also used.

Sera were treated with 2-me in order to determine what portion of the agglutinin titer might be attributable to an early antibody response.

In contrast to judging a dilution as positive when it had 50% clearing, a more critical reading procedure was employed. Reactions were rated 4, 3, 2, and 1 + related to 100, 75, 50 and 25% clearing and the titer taken as that showing a + 1 reaction or higher when compared with the antigen control.
In order to determine the effect of freezing on the MA test in its completed state (diluted serum plus antigen) four high titer rabbit sera were reacted with *hardjoprajitno* antigen. The end titer of the reaction was determined after a 2 hour incubation period. This completed test was subsequently frozen at -80 C for 24 hours. Upon thawing and holding at room temperature (25 C) for 30 minutes, the test was reread. These sera were also tested with 2-me. The cooperation of Mrs. Catherine Sulzer, Leptospiral Reference Laboratory, Center for Disease Control, Atlanta, Georgia, and Dr. B. S. Kingscote, Animal Pathology Laboratory, Guelph, Ontario, Canada, in providing the sera is acknowledged.

Using lower titered sera from a previous study, duplicate titrations were made with the negative and positive sera and one set of dilutions stored at -80 C while the duplicate was stored at 6 C for 24 hours. The frozen diluted samples were thawed the next day and after both sets of dilutions had been at room temperature (25 C), the MA test was completed with all the samples after adding freshly prepared live antigen. *Leptospira hardjo* (*hardjoprajitno* strain) was used for the antigen since it produces a very stable suspension of well dispersed cells and rarely, if ever, has agglutinated cell clumps.

Sera from 5 bulls were received from Dr. H. Rubin, Florida Veterinary Diagnostic Laboratory, Kissimmee, Florida. These sera had been judged negative to *pomona* and *grippotyphosa* antigens but reacted to *hardjo* antigen when tested in Florida. Their negativity to *pomona* and *grippotyphosa* was confirmed at the NADC. The sera were tested by us in the MA test with 5 Hebdomadis serotypes: *hardjo*, *wolffi*, *szwajizak*, *sejroe*, and *saxkoebing*. These samples were subsequently treated with 2-me and retested with *hardjo* and *wolffi*.

Agglutinin absorption of 4 bull sera was done with very dense suspensions (6% transmittance at 400 nm) of *hardjo*, *wolffi*, and *szwajizak* that had been stored at -80 C for 12 months.11

The above 5 bull sera originally tested in the Florida laboratory were also tested in our laboratory and in a diagnostic laboratory in Georgia with each laboratory using their own test procedure. The cooperation of Dr. J. Cole, Tifton Veterinary Diagnostic Laboratory, Tifton, Georgia, is acknowledged.

Paired sera from a previous study at NADC were retested in our laboratory and sent to Dr. C. Kirkbride, Veterinary Diagnostic Laboratory, Brookings, South Dakota, and Dr. H. Rubin, Kissimmee, Florida. The South Dakota laboratory using the same culture medium as NADC and *hardjoprajitino* agreed to read the test in the manner in which it is read at NADC (4+, 3+, 2+, 1+); the titer being taken as that showing a 1+ reaction or higher when compared with the antigen control. The Florida laboratory performed the test as far as reading titer as close to NADC as possible and also used their method of judging agglutination.
RESULTS

Hyperimmune sera

a. Titer: In order to more precisely bracket the titer sera, 3 different dilution schemes were used. Using dilutions of serum of 1:5, 1:3, and 1:2 dilution ranges of 10 to 40,960, 6 to 49,152, and 4 to 65,536 result. With one rabbit inoculated with hardjo strain CCNN, its serum was positive at 20,480, 24,576, and 32,768 while negative at 40,960, 49,152, and 65,536. Similar results were obtained with the second rabbit. Since leptospires have few, if any, biochemical characteristics sufficient to establish cultural identity, hyperimmune sera is a mandatory reagent needed for definitive identification of an isolate. In addition, such sera are useful for assessing the antigenicity of strains of serotypes used in serology. Such immune sera should be tested for cross-agglutination reactions with other antigens commonly used in the laboratory. These sera become indispensable for monitoring antigen identity, stability, and sensitivity.

2-me treatment

b. The effect of treating sera with 2-me on the agglutinin titer of rabbits during the course of 3 injections with a virulent and an avirulent strain of hardjo is shown (Table 1). Seven days after the first inoculation both the avirulent and virulent strain injected rabbits had their MA titers to hardjo reduced 99% (2560 to 40). At 14 days postinoculation the titer of the rabbit receiving hardjoprajitno had risen to 81,920 but could be reduced to 20,480 by 2-me treatment. The N.Z. #12 rabbit did have its titer significantly reduced; 14% of the 14 day titer still persisted. At 21 days postinoculation there was little mercaptoethanol sensitive antibody as judged by MA titer in the rabbit inoculated with the avirulent hardjoprajitno strain, while the 2-me sensitive antibody persisted longer in the rabbit receiving the hamster infective strain N.Z. #12. It was not ascertained if this difference was due to the rabbit, strain of organism, or amount of inoculum, since only 2 rabbits were compared.

Cross-agglutination of hardjo rabbit hyperimmune sera against Hebdomadis serotypes, other pathogenic serotypes, and saprophytic strains

c. The antisera harvested at 21 days from rabbits immunized with avirulent and virulent strains of hardjo were reacted in the MA test with 9 serotype antigen strains of the Hebdomadis serogroup (Table 2), 8 other pathogenic serotypes of other serogroups, and 7 strains of saprophytic leptospires (Table 3). Leptospira hardjo antigen reacted to the highest agglutinin titer in both sera. Antigens wolfi, balcanica, and sejroe reacted in high titer to hardjoprajitno antisera (40,960, 20,480, 10,240 and to N.Z. 12 antisera 10,240, 10,240, and 5120). Leptospira saxkoebing, hebdomadis, borincana, szwajiszak, and kambale were much less reactive. When these sera were treated with 2-me some titers remained unchanged (163,840 to hardjo) other
titers were reduced. It is not possible to assess the role of 2-me treatment of hyperimmune sera and its minor agglutinating activity with various heterologous members of the *Hebdomadis* serogroup at this time.

The degree of cross-agglutination of the 2 antisera when reacted with other pathogenic strains of leptospires, not of the *Hebdomadis* serogroup, and saprophytic strains is shown in Table 3. With 12 of the antigens used, the titers were > 93% and > 99% lower for the same antisera. Antigens *pyrogenes* (10,240), *pomona* (1280), and *grippotyphosa* (10,240) did react with the high titer (163,840) *hardjo* antisera. After 2-me treatment all of the titers shown in Table 3 were eliminated.

**Freezing effects**

a. Completed MA test: High titer hyperimmune sera were reacted in the MA test with *L. hardjo* (hardjoprajitno strain) and the completed test frozen at –80 C (Table 4). The agglutinin titers determined before freezing of the MA test were not changed after the tests were thawed and reread. Photographs before and after freezing did not show detectable changes in the morphology of the agglutinated particles when viewed at 100x magnification. Two-mercaptoethanol treatment of these sera did not reduce agglutinin titers suggesting the method of immunization had produced a serum not typical of containing predominately IgM antibodies.

**Frozen dilutions**

b. Dilutions of sequential sera derived from a previous study, having lower titers than hyperimmune sera, after storage at –80 C and 6 C, reacted well in the MA test (Table 5). Negative sera and sera with low titers (320, 640, 1280) did not have their titers or pattern of agglutination changed when the serum dilutions were held for 24 hours at –80 C and 6 C.

**Single sera**

a. Five bull sera gave negative reactions to *pomona* and *grippotyphosa* antigens but when tested with 5 different *Hebdomadis* serotypes all were agglutinated (Table 6). The highest titers were produced by the *hardjo* and *wolffi* antigens followed by *saxkoebing*, *sejroe*, and *szwajizak* antigens.

When the 5 sera were tested with *hardjo* and *wolffi* antigens before and after the sera were treated with 2-me, no significant reduction in titers was obtained. These results indicate that the sera were not taken during the early stages of leptosporal infection when 2-me sensitive antibody is being produced by the animal.

Since only *hardjo* and *szwajizak* serotypes of the *Hebdomadis* serogroup have been reported as isolated from cattle in the United States, four of the bull sera were absorbed with antigens of these 2
serotypes plus *wolffi* because of its close relation to *hardjo* (Table 7). The high density of the 3 antigens caused overabsorption of the agglutinins since the titers were either eliminated or reduced more than 90%. In order to approach speculating the probable infecting serotype of the closely related members of the large membered *Hebdomadis* serogroup, dilute suspensions of antigen should probably be used to absorb the agglutinins.

**Interlaboratory comparability with the MA test**

Five separate bull sera were compared for *hardjo* agglutination at three different laboratories are shown in Table 8. The MA titers obtained were generally comparable. Subsequently, sequential sera from a single experimentally infected steer were tested at the three laboratories (Table 9). Again, results were comparable. While variation was observed between laboratories on individual samples, it seems possible that the MA test can have some degree of standardization. Interpretation still poses the greatest problem.

**DISCUSSION**

The microscopic agglutination test with living cellular antigens remains the reference test for leptospirosis today. The genus *Leptospira* has been subdivided into serogroups; some groups having as few as 3 serotypes, while others such as the *Hebdomadis* group has 38 members. The serogrouping of leptospires can be reasonably accomplished by using hyperimmune sera. Serotyping is a more involved process. In humans, immunization is rarely practiced and serologic diagnosis is probably less confusing. Though paired sera with humans are frequently not available, at least the complexity of repeated exposure and exposure to multiple serotypes present less of an immunological complexity to deal with in humans than in animals.

The diagnosis of leptospirosis is accomplished by isolation of the causative agent, or serological evidence adequate to adjudge that an animal or group of animals has been infected recently. When these judgments have to be made upon field samples, frequently unpaired, the adequacy of the MA test and the manner in which we utilize it is severely tested. The continual need to propagate living antigens, alone, poses a problem to many laboratories.

Assuming the laboratory is able to cope sufficiently with growing its antigens, this is but the first step prior to the use of leptospiral antigens in serological tasks. These living antigens employed in the MA test need to be judged as to identity, sensitivity, and stability. It is this second phase of utilization of leptospiral antigens to which this paper is addressed.

*Leptospira* cannot be readily differentiated by conventional biochemical or physiological methods. The diagnostic laboratory should have as the second part of its MA test system sera from animals or rabbits hyperimmunized against the most common leptospiral serotypes.
LEPTOSPIRAL AGGLUTINATION MICROTITER TEST

that infect cattle (animals). Although absolute monospecific serotype antisera are not available or cannot be made because of limited technology, antisera prepared against specific serotypes can achieve at least a serogrouping of a leptospire. The continual subculture propagation of leptospires in liquid medium should have accompanying it the periodic test of antigens with specific immune sera. This should be done with homologous and heterologous sera as a minimal procedure. Test antigens have been shown to become nonagglutinable to their homologous antisera due to contamination with saprophytic leptospires.12

We are still limited in our ability to differentiate specific serotypes because major and minor antigens are shared by leptospires and cross-agglutination reactions occur with so-called specific antisera. The widespread use of pomona bacterins has possibly influenced the importance that one member of the Hebdomadis serogroup (38 in number), hardjo, has begun to play in cattle disease.13-22 To show the complexity which faces us, a number of serotypes (e.g. sejroe and wolffi) have been used in the past as indicator antigens in the MA test to detect hebdomadis agglutinins. Reporting of titers in cattle as reactions to sejroe or wolffi was a practice in the past23-25 even though isolations of such serotypes have not been made. Our limited study with hardjo hyperimmune serum has shown such sera to agglutinate to extremely high titer when tested homologously while some members of the Hebdomadis group react minimally (Table 2). When tested with other antigens outside of the Hebdomadis serogroup (Table 3) for all practical purposes many antigens were nonreactive. Field isolates of pomona and hardjo can be fairly readily characterized by their reaction with a battery of antisera as reported in our previous studies.9 The presence of the Hebdomadis serogroup in cattle poses a problem of major importance when an attempt is made to employ serology as the major laboratory tool. Multivalent leptospiral vaccines have been developed for use in farm animals. Today products containing five and up to eight different serotypes are in the market. Some of these serotypes have never been isolated from animals in this country and other serotypes are the result of a single isolation. These multivalent products could be of no further protection to our animal populations. A multivalent product probably could produce a lesser immunological response by the host to each of the components and may lead to a nonprotective immunity. This could also complicate the interpretation of serodiagnosis due to the confusing presence of antibodies to unncessary serotypes. The justification to include a large number of serotypes in a bacterin should rest mainly upon the widespread isolation of those serotypes. Leptospiral serologists employing the MA test find it difficult to differentiate between infectious or vaccinal titers. But since the MA test at present represents our reference test for this disease, we must use it to maximum capacity and even employ it in areas unfamiliar to us.

There is some evidence that the MA test, in specific instances, can
have its interpretive value enhanced. In previous studies with appropriate selection of sera at 0, 6, and 13 days all agglutinin activity in field sera and sera of experimentally infected *hardjo* cattle were removed by treatment with 2-me. In this study (Table 6) and, in our study of the *hardjo* and *szwajizak* serology of 4 herds from Florida, 2-me treatment of sera, especially of single samples, was of limited value. During an outbreak of leptospirosis in a herd of cattle, sera might be treated with 2-me in order to possibly detect which cattle were recently infected. Test sera reacting predictably to treatment with 2-me could be a valuable reagent in a diagnostic laboratory as a comparative standard. Our limited study with weekly samples obtained during the hyperimmunization of rabbits, utilizing the orbital plexus technique (personal communication: A. B. Thiermann, NADC, Ames, IA) demonstrates how readily such sera can be evaluated (Table 1). The quantitation of the MA titer of untreated and 2-me specific sera of such interval bleedings lends further support that one can monitor the reliability of the MA test system.

The living antigen MA test for leptospirosis making use of microtiter equipment has brought to the diagnostic laboratory a new dimension for the performance of leptospiral serology. The appearance the agglutination takes on, in some instances, is rounded particle agglunination familiar to many laboratory workers. In other instances, judged by careful assessment of antigen control, the agglutination is a loose yet definite network of leptospires. Training laboratory technicians to recognize these classical patterns is important. Our studies showed that the completed microtiter MA test could be frozen, thawed, and reread when using antisera with little changes in titer. In addition low titer paired sera could be diluted, frozen, thawed, and have living antigen added and the titer was not changed. The appearance of the MA test agglutination should be photographed to record the process which one sees in the microscope when reading the test.

Various aspects of standardizing the agglutinin absorption process with leptospires have been previously reported. Those studies were primarily aimed at documenting that dense absorbing antigens could be simply prepared and subsequently preserved by freezing (−80 C). In these instances success with the agglutinin absorption test was influenced by the serum studied, having for example high titers to *hardjo* (1:12,800) and minor agglutinin titers to *grippotyphosa* and *pomona* of 1:800 and 1:100 respectively. Ninety-nine percent reduction of *hardjo* titer was achieved while the agglutinin activity to *grippotyphosa* and *pomona* remained unchanged. *Wolfii* antigen behaved identically to the *hardjo* absorbed serum. Our routine attempts to clarify the *Hebdomadis* agglutinin activity of bull sera negative to *pomona* and *grippotyphosa* was not successful using dense *hardjo*, *wolfii*, and *szwajizak* absorbing antigens. Agglutinin absorption studies of field sera reacting to members of the *Hebdomadis* serogroup will require extensive and detailed studies because of the closely related antigens of serotypes in this group.
Various aspects of the relative importance of variable factors in the MA test were considered by workers 1-2 30 years ago. Our initial comparison of the MA test among laboratories using the same sera indicated that better agreement of the titers was obtained when the same test procedure was used. When laboratories can agree on (1) the manner of reading agglutination end points, (2) the dilution scheme to use, (3) the antigen strains to use, and (4) the growth medium for preparing the antigen, more comparable results among laboratories can be expected. The variables that are even less obvious appear to be: (1) the age of leptospiral cells used, (2) the stability of antigen and the periodic need to judge this stability using antisera of known experimentally infected and vaccinated animals. Such reference sera would seem a must if the agglutination phenomenon is to be the foundation of leptospiral serology.

The MA test is being more extensively used than previously, but as its use becomes subject to more complex interpretations; alternative techniques to diagnosis can be considered. Renewed attempts to isolate leptospires are still necessary and the use of the diuretic furosemide 26 could aid the matter. The method of direct morphological identification of leptospires in fluids and tissue should be reappraised. Fluorescent staining 27 used in the early sixties needs further attention. The fact that early workers did not achieve absolute serotype specificity should not terminate interest in the technique.

All of the above mentioned techniques—serology, isolation, direct identification—are needed to improve our capabilities of diagnosing the serotypes responsible for—animal, herd, cattle—infections for in so doing we will gain the knowledge required to protect our animals.

SUMMARY

The current use of the microscopic agglutination test for the detection of leptospiral agglutinins related to previous exposure, current exposure, and vaccinal titers requires that a diagnostic laboratory have serological reagents such as sera of specific quality. The utilization of this test requires more quality control in a laboratory, since living antigens are needed to measure leptospiral antibodies. Our findings emphasize that: (1) Rabbit hyperimmune sera harvested at 7, 14, and 21 days has agglutinin profiles sensitive and resistant to 2-me treatment, indicative of early and late immunological response and the appropriate use of such specific sera can serve as a quality control in the diagnostic laboratories' use of 2-me with field samples; (2) The 2-me technique finds usefulness when paired sera are available; (3) The Hebdomadis serogroup, currently 38 in number, requires much needed study; (4) Hyperimmune sera made against cultures of actively growing leptospires whether virulent or avirulent can be simply made and expected to be mostly serotype specific; (5) The MA test in its completed state can be frozen and preserved as a teaching tool; (6) In the absence of an isolation, 3 techniques—cross-agglutination studies, 2-me serum treatment and retest, and agglutinin absorption—can be combined together to further extend
the potential usefulness of the MA test. However, agglutinin absorption long used in isolate identification will require considerable study before it can be routinely used and understood with field sera; (7) The MA has certain aspects which can be standardized.

TABLE 1--EFFECT OF 2-MERCAPTOETHANOL TREATMENT ON AGGLUTININ TITERS IN SERA OF RABBITS INOCULATED* WITH AVIRULENT (HARDJOPRAJITNO) AND VIRULENT HARDJO N.Z. #12

<table>
<thead>
<tr>
<th>Strain</th>
<th>Before 2-me</th>
<th>After 2-me</th>
<th>% titer reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 day P.I.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardjoprajitno</td>
<td>2560</td>
<td>40</td>
<td>98.2</td>
</tr>
<tr>
<td>N.Z. #12</td>
<td>2560</td>
<td>20</td>
<td>99.2</td>
</tr>
<tr>
<td>14 day P.I.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardjoprajitno</td>
<td>81,920</td>
<td>20,480</td>
<td>74.8</td>
</tr>
<tr>
<td>N.Z. #12</td>
<td>20,480</td>
<td>2560</td>
<td>86.0</td>
</tr>
<tr>
<td>21 day P.I.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardjoprajitno</td>
<td>163,840</td>
<td>163,840</td>
<td>0</td>
</tr>
<tr>
<td>N.Z. #12</td>
<td>20,480</td>
<td>5120</td>
<td>75.0</td>
</tr>
</tbody>
</table>

*P.I. = First inoculation of 1 ml was made day zero; 2nd day 7; 3rd day 14. Titers determined 7, 14, and 21 days after day zero.
<table>
<thead>
<tr>
<th>Antigen serotype</th>
<th>Hardjoprajitno avirulent</th>
<th>N.Z. #12 virulent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Titer</td>
<td>After 2-me</td>
</tr>
<tr>
<td>Hardjo</td>
<td>163,840</td>
<td>163,840</td>
</tr>
<tr>
<td>Wolffii</td>
<td>40,960</td>
<td>20,480</td>
</tr>
<tr>
<td>Balcanica</td>
<td>20,480</td>
<td>20,480</td>
</tr>
<tr>
<td>Sejroe</td>
<td>10,240</td>
<td>2560</td>
</tr>
<tr>
<td>Borincana</td>
<td>2560</td>
<td>160</td>
</tr>
<tr>
<td>Hebdomadis</td>
<td>1280</td>
<td>2560</td>
</tr>
<tr>
<td>Saxkoebing</td>
<td>1280</td>
<td>640</td>
</tr>
<tr>
<td>Szwajizak</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>Kambale</td>
<td>40</td>
<td>-</td>
</tr>
</tbody>
</table>

*Serum obtained 21 day P.I.

**Final 0.1 M 2-me, 2 hours, 37 C.

- Negative in the 1:20 final dilution of 2-me treated dilution.
### TABLE 3—PATHOGENIC AND SAPROPHYTIC LEPTOSPIRES AGGLUTINATION TITERS IN SERUM OF RABBITS HYPERIMMUNIZED WITH AVIRULENT AND VIRULENT *L. HARDJO*

<table>
<thead>
<tr>
<th>Antigen serotype</th>
<th><em>Hardjoprajitno</em> avirulent Titer</th>
<th>New Zealand - 12 <em>virulent</em> Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copenhageni</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Canicola</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Ballum</td>
<td>160</td>
<td>10</td>
</tr>
<tr>
<td>Pyrogenes</td>
<td>10,240</td>
<td>-</td>
</tr>
<tr>
<td>Djasamin</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Pomona</td>
<td>1280</td>
<td>-</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>10,240</td>
<td>40</td>
</tr>
<tr>
<td>Tarassovi</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

**Saprophytes**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Semaranga</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patoc 1</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>B-16</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>St. Chas. #3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Illini 3055</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Korman</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>Purdue Med.</td>
<td>160</td>
<td>10</td>
</tr>
</tbody>
</table>
**TABLE 4--EFFECT OF FREEZING AND THAWING ON THE COMPLETED MA TEST USING HARDJO ANTISERA**

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Titer before -80 C</th>
<th>Titer after thawing</th>
<th>After 2-me</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downey</td>
<td>10,240</td>
<td>10,240</td>
<td>10,240</td>
</tr>
<tr>
<td>BV&lt;sub&gt;6&lt;/sub&gt;</td>
<td>10,240</td>
<td>10,240</td>
<td>10,240</td>
</tr>
<tr>
<td>KAP249</td>
<td>10,240</td>
<td>10,240</td>
<td>10,240</td>
</tr>
<tr>
<td>WINT 5</td>
<td>20,480</td>
<td>20,480</td>
<td>20,480</td>
</tr>
</tbody>
</table>

**TABLE 5--COMPARISON OF TITERS OF DILUTED SERA* THAT WERE STORED 24 HOURS**

**AT -80 C AND 6 C**

<table>
<thead>
<tr>
<th>Bleeding Day</th>
<th>Titers of sera stored diluted at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-80 C</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>640 (2+)†</td>
</tr>
<tr>
<td>16</td>
<td>640 (2+)</td>
</tr>
<tr>
<td>18</td>
<td>1280 (1+)</td>
</tr>
<tr>
<td>21</td>
<td>1280 (2+)</td>
</tr>
<tr>
<td>36</td>
<td>1280 (2+)</td>
</tr>
<tr>
<td>43</td>
<td>640 (4+)</td>
</tr>
</tbody>
</table>

* Sera from steer inoculated with *L. hardjo* (N.Z. 12), $4.9 \times 10^9$ cells I.V.; kidney culture positive at 43 days.

** MA test completed after adding live antigen.

†( ) indicates degree of antigen agglutinated as in Materials and Methods, Reference 9.
### TABLE 6--AGGLUTINATION TITERS OF BULL SERA TO FIVE HEBDOMADIS SEROTYPES

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hardjo</th>
<th>Wolffii</th>
<th>Szvajzak</th>
<th>Sejroe</th>
<th>Saxkoebing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10,240</td>
<td>10,240</td>
<td>2560</td>
<td>2560</td>
<td>10,240</td>
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<td>2</td>
<td>1280</td>
<td>1280</td>
<td>160</td>
<td>320</td>
<td>640</td>
</tr>
<tr>
<td>3</td>
<td>1280</td>
<td>1280</td>
<td>160</td>
<td>320</td>
<td>640</td>
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<tr>
<td>4</td>
<td>640</td>
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<td>640</td>
<td>160</td>
<td>640</td>
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<tr>
<td>5</td>
<td>640</td>
<td>640</td>
<td>160</td>
<td>320</td>
<td>640</td>
</tr>
</tbody>
</table>

### TABLE 7--AGGLUTININ ABSORPTION OF BULL SERA WITH CONCENTRATED ANTIGENS

<table>
<thead>
<tr>
<th>Animal</th>
<th>MA titers before and after absorption:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardjo Before</td>
</tr>
<tr>
<td>1</td>
<td>10,240</td>
</tr>
<tr>
<td>2</td>
<td>1280</td>
</tr>
<tr>
<td>3</td>
<td>640</td>
</tr>
<tr>
<td>4</td>
<td>640</td>
</tr>
</tbody>
</table>

- = Negative in the 1:50 final dilution of MA test; serum diluted 1:25 by absorption.
LEPTOSPIRAL AGGLUTINATION MICROTI T E R TEST

TABLE 8—COMPARISON OF MA TITERS OF FIVE INDIVIDUAL BULL SERA TESTED BY THREE LABORATORIES

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hardjo Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Florida Lab</td>
</tr>
<tr>
<td>1</td>
<td>6400</td>
</tr>
<tr>
<td>2</td>
<td>3200</td>
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<tr>
<td>3</td>
<td>800</td>
</tr>
<tr>
<td>4</td>
<td>80G</td>
</tr>
<tr>
<td>5</td>
<td>800</td>
</tr>
</tbody>
</table>

Starting dilution: 1:25

1:25 1:10

TABLE 9—COMPARISON OF MA TITERS OF SEQUENTIAL SERA FROM AN EXPERIMENTALLY HARDJO INFECTED STEER AS DETERMINED BY 3 LABORATORIES

<table>
<thead>
<tr>
<th>Sample</th>
<th>NADC 8-10-77</th>
<th>South Dakota 3-2-79</th>
<th>Florida 2-27-79</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>320</td>
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<td>6400</td>
</tr>
<tr>
<td>3</td>
<td>2560</td>
<td>1280</td>
<td>3200</td>
</tr>
<tr>
<td>4</td>
<td>2560</td>
<td>640</td>
<td>3200</td>
</tr>
<tr>
<td>5</td>
<td>1280</td>
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<td>3200</td>
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<td>6</td>
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<td>9</td>
<td>1280</td>
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<td>1600</td>
</tr>
<tr>
<td>10</td>
<td>640</td>
<td>1280</td>
<td>1600</td>
</tr>
</tbody>
</table>

*Test was performed as close to procedure followed at NADC as to reading.

**Reflects results from the test as had been the practice in the Florida laboratory.
ELLINGHAUSEN

REFERENCES


LEPTOSPIRAL AGGLUTINATION MICROTITER TEST


EVALUATION OF SEROLOGIC REACTIONS IN CATTLE
FOLLOWING VACCINATION WITH MULTIVALENT LEPTOSPIRAL
COMMERCIAL BACTERINS AND COMPARISON OF THE
MICROSCOPIC AGGLUTINATION (MA) ANTIBODY RESPONSE
BY VARIOUS LABORATORIES

D. N. Tripathy, L. E. Hanson and M. E. Mansfield
College of Veterinary Medicine
University of Illinois
Urbana, Illinois 61801

INTRODUCTION

Leptospiral vaccination has proved effective in reducing losses in endemic herds (Hanson et al. 1972). Vaccination not only provides protection from leptospiral disease but also reduces chances of survival of the infecting serovar (serotype) in the immune population. The microscopic agglutination (MA) test has been used extensively in the detection of the immune response both in the naturally infected animals as well as for the evaluation of the vaccination response. As the M.A. response in the natural infection is usually 1:100 or greater, the lack of significant M.A. response in vaccinated animals had been interpreted by many as an inadequacy of the bacterins. However, evaluation with other tests e.g., hamster protection (Huhn, et al. 1971) growth inhibition, (Tripathy, et al. 1974) and challenge of vaccinated animals have demonstrated the adequacy of the leptospiral bacterins for protection. This lack of M.A. response by vaccinated animals, however, has been useful to differentiate the natural infection in which high M.A. titers are detected. However, in recent years improvements in the commercial leptospiral bacterins, due to increased antigen concentrations have stimulated greater M.A. responses in vaccinated animals. The objective of this study was to evaluate the M.A. antibody responses of animals following vaccination with commercial bacterins and conduct a comparison of M.A. test results of some selected sera by different laboratories.

MATERIALS AND METHODS

Animals and Vaccination

Beef steers approximately 1 year of age purchased from the open
market were used in this study. All animals were bled before vaccination. Ten animals were vaccinated subcutaneously with each commercial bacterin.* Two bacterins contained 5 serovars (*icterohemorrhagiae, *canicola, *pomona, *grippotyphosa and *hardjo) and one bacterin contained three serovars (*hardjo, *pomona and *grippotyphosa).

The ten animals of the unvaccinated control group were pastured separately. Animals from each three vaccinated groups were also pastured separately. All vaccinated animals were administered a second dose of the same bacterin one month after primary vaccination. All animals were mixed together at each monthly bleeding and weighing period and maintained as one herd following the five month test period. Animals which received the five serovar bacterins were retained and revaccinated at 10 months after primary vaccination which was followed by another vaccination one month later. All the sera were tested by M.A. test and selected sera were also tested by growth inhibition test.

Comparison of diagnostic test results of different laboratories

Selected sera obtained at three intervals (pre-vaccination, 2 months after vaccination and 5 months after vaccination) from 5 cattle vaccinated with five serovar bacterin and from 5 animals which received 3 serovar antigens were tested by five laboratories in the United States. Our laboratory used the tube method of dilution for the M.A. test while remaining four laboratories employed the microtiter method.

Growth inhibition test

Five sera from cattle vaccinated with a five serovar bacterin obtained at three intervals (prevaccination, 2 months and 5 months after vaccination) were tested for growth-inhibition activity against *hardjo according to the method described earlier (Tripathy et al., 1974). Five vials of media were used for each sample. To 1 ml. of liquid medium 0.05 ml. of serum and 0.05 ml. of *hardjo containing approximately 10⁷ leptospires per ml. were added. The growth was measured nephelometrically after 24 days of incubation. Sera from 5 cattle of the naturally infected group were also tested for the same intervals.

RESULTS

M.A. antibody response

Majority of animals developed detectible M.A. responses to the serovar antigens which were incorporated in the bacterin except with *hardjo in animals vaccinated with the 5 serovar bacterins (table 1). The M.A. response with *hardjo was greater in the cattle vaccinated with the 3 serovar bacterin. As will be seen from table number 1, that while the majority of animals vaccinated with 5 serovar antigen developed low or

*Bacterins were supplied by Philips Roxann Inc., St. Joseph, Missouri, Beecham Laboratories, White Hall, Illinois and Norden Laboratories, Lincoln, Nebraska.
no M.A. titers against *hardjo*, the control animals contracted a natural infection between first and second month of the study and developed high titers against *hardjo* which persisted for one year.

**Comparative M.A. antibody test results of five different laboratories**

The comparative microscopic agglutinin test results of sera from 10 cattle selected from this vaccination study and tested by different laboratories (designated as A, B, C, D, and E) are presented in tables 2, 3 and 4. As indicated in table 2 similar results were obtained by all laboratories only with minor variations. Only one laboratory detected a titer of 1:100 for *icterohemorrhagiae* in sera of cattle No. 49. Similarly, two laboratories detected titers against *hardjo* for animal No. 98 in the prevaccination sera.

The results of the comparative study on sera obtained two months after vaccination are presented in table 3. While some variations in M.A. titers in different laboratories are apparent with different serovars, there is generally a consistency in the data e.g. animal Nos. 18, 19, 26 and 100 were negative for *hardjo* in all five laboratory tests (table 3). Similarly, the results of the animals which had positive response against *hardjo* had only minor variations in titers. Results on sera tested after 5 months of vaccination also had only (table 4) minor variations.

**Growth inhibition test**

Growth inhibiting antibody was detected in sera of both groups of animals at 2 and 5 months. The sera of majority of animals at 2 months caused agglutinated clumps while the sera at 5 months showed some reduction in growth. The differences in growth could be measured nephelometrically (table 5) and were also apparent grossly. The nephelometric reading for each animal is an average of 5 readings.

**DISCUSSION**

Most of the animals vaccinated with leptospiral bacterins developed M.A. antibody titers of incomplete or complete 1:100 and rarely higher against serovars other than *hardjo* with 5 serovar bacterins although 3 serovar bacterin provided higher M.A. response even with *hardjo*. The development of natural infection in control animals provided an interesting direct comparison between the immune response of vaccinated and naturally infected animals. As will be seen from table 1 that the animals receiving multiple vaccination did not develop the high M.A. responses as detected in the sera of naturally infected animals. A greater M.A. response was detected in sera of vaccinated animals after third and fourth vaccination but was still of lower magnitude than detected in naturally infected unvaccinated animals. High M.A. titers were maintained by naturally infected unvaccinated animals for over one year. However, growth inhibiting antibodies were detected in vaccinated and naturally infected cattle sera.

Marshall et al. (1979) also observed low M.A. titers in response to a
bivalent *pomona*, *hardjo* bacterin. Sera of only 2 of the 9 vaccinated and all 10 unvaccinated cattle showed seroconversion to natural *hardjo* challenge by introduction of animals shedding *hardjo*. *Hardjo* was isolated from 6 of 10 unvaccinated but from none of the 9 vaccinated animals.

In this study the vaccinated animals were mixed several times with the naturally infected unvaccinated animals in the first 4 months of the study and were pastured together after 5 months. While naturally infected unvaccinated animals maintained high titers, no similar increase in the titer of vaccinated animals was detected, indicating that the vaccination response interfered with the extension of the natural infection.

Results of the growth inhibition test further indicated the development of an immune response in the absence of appreciable M.A. titer in vaccinated animals. Though both IgM and IgG provide protection, IgM in the early sera causes agglutination while IgG predominates later (Tripathy *et al.* 1975). Nephlometric measurement of growth provided a numerical evaluation of the growth inhibition test.

The comparative study of leptospiral agglutination test techniques of the 5 laboratories provided substantial agreement. This is particularly important in view of the concern which had been expressed at some meetings whether reliance could be placed on the test. Although variations did occur with a few sera, the titer variations were not significant with most sera. In fact, the reproducibility of the comparable test results indicates that proper conduct of either the original or the microtiter M.A. test should provide reliable test results.

**SUMMARY**

Steers approximately one year old, were vaccinated either with 5 serovars or 3 serovar leptospiral bacterins. Immune response was measured by the M.A. test. Higher M.A. titers against *hardjo* were detected in sera of animals vaccinated with 3 serovar bacterin while poor M.A. response against *hardjo* occurred with 5 serovar bacterins. Growth inhibiting antibody was detected in post-vaccination sera of vaccinated animals and the numerical evaluations obtained from nephelometric values provided a more definable procedure. A natural *hardjo* infection which occurred in the control group caused development of much higher M.A. titers than detected in the vaccinated animals which persisted over the one year observation period. This natural infection did not alter the immune (M.A.) response of vaccinated animals although they pastured together for at least 10 months with naturally infected animals indicating protection due to vaccination during the later part.

A comparison of serologic tests conducted by 5 laboratories on 10 cattle sera taken at prevaccination, 2 and 5 month postvaccination intervals indicated substantial agreement between the laboratories. The results also demonstrated that the current techniques can provide reproducible test results.
TABLE 1. MICROSCOPIC AGGLUTINATING (MA) ANTIBODY RESPONSE OF CATTLE VACCINATED WITH FIVE SEROVAR LEPTOSPIRAL-BACTERIUM AND NATURALLY INFECTED CATTLE AGAINST HARDJO

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal Number</th>
<th>0 month*</th>
<th>1 month*</th>
<th>2 months</th>
<th>3 months</th>
<th>4 months</th>
<th>5 months</th>
<th>10 months*</th>
<th>11 months*</th>
<th>12 months</th>
<th>13 months</th>
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<td>1:10</td>
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<tr>
<td>B</td>
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| 45       | 1:100         | 1:100    | 1:100    | 1:100    | 1:100    | 1:100    | 1:100    | 1:50       | 1:100      | 1:500      | 1:100      | 1:100      |
| 46       | N             | N        | NA       | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:1000     | 1:1000     | 1:50       | 1:500      | 1:500      |
| 54       | N             | N        | 1:10,000 | 1:1000   | NA       | NA       | NA       | NA         | NA         | NA         | NA         | NA         |
| 62       | N             | N        | NA       | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:10,000   | 1:500      | 1:1000     | 1:1000     | 1:1000     |
| Unvaccinated |              |          |          |          |          |          |          |            |            |            |            |            |
| 96       | N             | N        | 1:1000   | 1:100    | 1:100    | 1:100    | 1:100    | 1:100      | 1:100      | 1:100      | 1:100      | 1:100      |
| 108      | N             | N        | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:100      | 1:100      | 1:100      | 1:100      | 1:100      |
| 116      | N             | N        | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:100      | 1:100      | 1:100      | 1:100      | 1:100      |
| 116       | N             | N        | N        | N        | N        | N        | N        | 1:500      | 1:10,000   | 1:10,000   | 1:10,000   | 1:10,000   |
| 133      | N             | N        | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:1000   | 1:100      | 1:100      | 1:100      | 1:100      | 1:100      |

NA = Not available for testing. *Vaccination time. Groups A & B vaccinated with 5 serovar (pomona, grippotyphosa, canicola, icterohemorrhagiae and hardjo) bacterin at 0, 1, 10 and 11 months) I = Incomplete reaction.
### TABLE 2. COMPARATIVE DIAGNOSTIC MICROSCOPIC AGGLUTINATION (MA)

**TEST RESULTS OF PRE-VACCINATION SERA AGAINST LEPTOSPIRAL ANTIGENS**

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N = Negative; NT = Not tested
TABLE 3. COMPARATIVE DIAGNOSTIC MICROSCOPIC AGGLUTINATION (MA) TEST RESULTS OF SERA OBTAINED TWO MONTHS AFTER PRIMARY VACCINATIONS AGAINST LEPTOSPIRAL ANTIGENS

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N = Negative; NT = Not tested. The figures in parentheses represent dilutions with incomplete reaction.
TABLE 4. COMPARATIVE DIAGNOSTIC MICROSCOPIC AGGLUTINATION (MA) TEST RESULTS OF SERA OBTAINED FIVE MONTHS AFTER PRIMARY VACCINATION AGAINST LEPTOSPIRAL ANTIGENS

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<th>Animal Number</th>
<th>Autumnalis</th>
<th>Canicola</th>
<th>Grippo-typhosa</th>
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<th>Hardjo</th>
<th>Pomona</th>
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N = Negative; NT = Not tested. The figures in parentheses represent dilutions with incomplete reaction.
TABLE 5. COMPARATIVE GROWTH-INHIBITING (GI) RESPONSE OF SERA FROM VACCINATED AND NATURALLY INFECTED CATTLE AGAINST HARDJO

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<th>5 months</th>
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<td>16.4</td>
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<td>19</td>
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<td>17.1</td>
<td>15.2</td>
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<td>26</td>
<td>18.6</td>
<td>14.6</td>
<td>16.1</td>
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<tr>
<td></td>
<td>100</td>
<td>17.2</td>
<td>16.6</td>
<td>18.9</td>
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<td></td>
<td>105</td>
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<tr>
<td>Naturally infected</td>
<td>32</td>
<td>21.8</td>
<td>8.4</td>
<td>6.0</td>
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<tr>
<td>with hardjo</td>
<td>96</td>
<td>24.2</td>
<td>11.2</td>
<td>8.3</td>
</tr>
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<td></td>
<td>104</td>
<td>19.9</td>
<td>6.7</td>
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<td>18.2</td>
<td>8.1</td>
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<td>133</td>
<td>15.7</td>
<td>7.2</td>
<td>3.9</td>
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*Vaccinated with five serovar bacterin (pomona, grippotyphosa, canicola, icterohemorrhagiae and hardjo).

REFERENCES


LABORATORY DIAGNOSIS OF 
LEPTOSPIROSIS OF DOMESTIC ANIMALS

Prepared by a subcommittee of the 
U.S. Animal Health Association Committee on Leptospirosis

J. R. Cole, Jr., Tifton, Georgia
H. C. Ellinghausen, Ames, Iowa
H. L. Rubin, Kissimmee, Florida

1979

INTRODUCTION

The Leptospirosis Committee of the U.S. Animal Health Association, at its 1977 meeting, expressed concern about the leptospiral serologic tests because of the variety of techniques, and differences in reporting and interpreting test results by animal disease diagnostic laboratories. A subcommittee was formed to develop a leptospirosis laboratory manual to assure uniformity of procedures used in diagnosis of leptospirosis. This manual “The Laboratory Diagnosis of Leptospirosis of Domestic Animals,” will be issued as three separate publications entitled I. Microscopic Agglutination Microtiter Procedure, II. Isolation Procedures, and III. Characterization Procedures. Because of the urgent need for uniformity in serologic testing, it was decided that Part I. would be prepared first and that the other two publications would be prepared and distributed at a later date.

The Committee also emphasized that this manual should be considered as a companion to “Leptospirosis of Domestic Animals,” Agriculture Information Bulletin No. 394.

In preparing this section, the Leptospirosis Committee decided that the microscopic agglutination microtiter test (MAMT) would be the reference test because of its specificity and sensitivity, and its usefulness as a test for determining previous exposure. The MAMT is easily adaptable to all laboratories and, if performed according to protocol, will provide the best uniformity of test results. Because of the rapid and widespread acceptance of the MAMT, only detailed procedures for that test will be specified in this publication.

The conduct of the MAMT consists of four major points: (1) Selection of serovars (serotypes) used; (2) growth and standardization of antigens; (3) performance of the test and (4) interpretation and reporting.

MICROSCOPIC AGGLUTINATION MICROTITER PROCEDURE

Serovars

In the routine testing of animal sera, serovars *pomona*, *hardjo*, *grippotyphosa*, *icterohemorrhagiae* and *canicola*, must be used. Additional serovars, as desired, may also be included.

Because of the serologic cross-reactivity among members of the Hebdomadis serogroup, e.g. *hardjo*, *szwajizak*, *wolffi*, *sejroe*, etc., only
one of these serovars is needed in the routine MAMT. Serovar *hardjo* is the antigen recommended by this committee. At this time, there is no reliable routine way to serologically differentiate infections caused by members of the Hebdomadis serogroup.

Serovars used in the MAMT must be obtained from the WHO Leptospirosis Reference Laboratory, CDC, Atlanta, GA 30333.

**Antigens**

**CAUTION:** Extreme care must be exercised and all safety requirements followed when working with *Leptospira* cultures.

1. The antigens for MAMT are 5-7 day cultures grown in a suitable liquid albumin-polysorbate 80 medium at 29°C. (See Appendix for source of media.)

2. The need for living cultures requires subculturing in liquid medium. Subculturing of the various serovars should be made each time the test is performed, or every 5 to 7 days, by adding 1 ml of culture to 10 ml of medium. Duplicate liquid or semisolid cultures should be available in case the working culture fails to grow or becomes contaminated.

3. After subcultures have been made, transfer sufficient antigen for the test into 13 X 100 mm tubes and stopper tube.

4. Centrifuge at 1500 rpm for 15 minutes to remove debris.

5. Transfer supernate to another 13 X 100 mm tube and adjust the density to one of the following standards: (a) McFarland No. 0.5, (b) Light transmission of 60-75% on a Spectronic 20 or equivalent spectrophotometer set at 400 NM or (c) a Nephelometer set to 25 with either dry well or wet well. These standards are equivalent to a Petroff-Hauser count resulting in the antigen having 200 X 10⁶ cells/ml (100 X 10⁴ cells at test).

**Microtiter Equipment**

1. The following items are needed for the performance of the MAMT.
   a. Standard dust-free* rigid plastic microtiter plates with flat bottom wells.
   b. Disposable 0.025 ml microtiter pipets equipped with a 0.025 ml disposable dropper tip for dispensing serum dilutions and antigens.
   c. 0.025 ml microdiluters for making dilutions. A multimicrodiluter handle is recommended.
   d. If desired, 0.05 ml microtiter pipets and microdiluters may be used in place of the 0.025 ml items.

   (See Appendix for source of microtiter equipment.)

---

*Dust-free plates can be obtained by moving air across the wells of the plate just prior to use.
LEPTOSPIROSIS OF DOMESTIC ANIMALS

2. Microscope

A darkfield microscope equipped with a dry darkfield condenser, 10X eyepieces and a long working distance 10X objective is required to read the reaction in the microtiter well. In the event a 10X long working distance objective is not available, it may be necessary to modify the objective-eyepiece combination. Regardless of objective-eyepiece combination, the final magnification should approach 100X or greater. (See Appendix for source of microscope equipment.)

Procedures

If 5 or more sera are to be tested, it is recommended that they first be screened at a final dilution of 1:50 to eliminate non-reactive (negative) sera and unnecessary titration of negative serovars.

1. Screening Test

a. In a 12 X 75 or 13 X 100 mm tube, prepare a 1:25 serum dilution by adding 0.1 ml of clear, uncontaminated serum to 2.4 ml phosphate buffered saline (PBS). (See Appendix for formulation of PBS.)

b. Add 0.025 ml* (1 drop) of the 1:25 serum dilution to each well of a column (columns on the standard microtiter plates are numbered 1 through 12), e.g. serum 1 in column 1, serum 2 in column 2 and so on, (see example No. 1).

c. Starting at the bottom row (H) of the plate, add 0.025 ml (1 drop) of a different antigen to the wells in each row (rows on the standard microtiter plates are labeled from top to bottom A through H), e.g. pomona in Row H, hardjo in Row G and so on. This procedure allows 12 sera to be screened on a single plate (see Example No. 1).

d. Gently shake plates to mix the contents of each well and cover plates with a plastic lid to exclude debris and prevent drying. Incubate at 29°C for 2 hours ± 30 minutes.

e. After incubation, the microtiter plate is placed on the stage of the darkfield microscope and each well is examined for agglutination and/or clearing of the antigen. The patterns of agglutination vary and some degree of experience and training is required. The textbook picture of a dark black background with varying sized masses of leptospires is quite easy to discern. In other instances, the agglutination is in a lacework pattern and not as compact. Wells in which at least 50% (2+) of the leptospires are agglutinated are to be considered as positive. (See Plate 1.)

f. Sera that are positive against one or more serovars need to be titered only against those serovars to which they are positive at the screening dilution (see Examples 1 and 2).

2. Titrations

a. The 1:25 serum dilution prepared for the screening test is used for the titration.

*All volumes specified should be double if 0.05 ml pipets and diluters are used.
b. Add 0.025 ml* (1 drop) of PBS to each well in the microtiter plate(s) except for the wells in Row H (bottom row).

c. Add 0.05 ml (2 drops) of the 1:25 serum dilution to be titered to the wells in Row H. Use 1 column per sera per serovar. It is more convenient to group sera in the plate according to the serovar being tested (see Example 2).

d. Using the 0.025 ml microdiluters, mix the serum dilutions in Row H by turning the diluters 10-15 times.

e. Transfer the diluters to the next row (G) and mix.

f. Repeat step “e” for the desired number of dilutions.

g. After mixing the last dilution, rinse diluters by twirling in distilled water and blot dry.

h. Repeat the above process for each plate used.

i. Add 0.025 ml of antigen to each well for the serovar involved (see Example 2).

j. Gently shake the plates to mix the contents of each well, cover with a plastic lid and incubate at 29°C for 2 hours ± 30 minutes.

k. Using the darkfield microscope, examine each well for agglutination and/or clearing of the antigen. Report the endpoint as the highest dilution in which at least 50% (2+) of the leptospires are agglutinated (see Example 2).

3. Controls.

The following controls must be included in each series of tests:

a. Antigen Control. 0.025 ml antigen and 0.025 ml PBS. This must be set up for each antigen used.

b. Positive Serum Control. Titrate a known positive reference antiserum with each homologous antigen to assure maximum reactivity of the antigen.

4. Reporting Test Results

The highest dilution with 50% (2+) of the leptospires agglutinating for each serovar is to be reported. An example of a report form is shown in Example 3.

5. Interpretation of Test Results

Since leptospirosis is a herd problem it should be diagnosed on that basis. To obtain meaningful information, samples should be obtained from at least 10 animals or 10% of the herd, whichever is greater.

MA titers of 1:100 or greater against one or more serovars are generally considered significant. Diagnosis of leptospirosis may be justified when most of the seropositive animals have titers of 1:1000 or

*All volumes specified should be double if 0.05 ml pipets and diluters are used.
greater on a single sample, or when paired serum samples show a four-fold rise or fall, or conversion from negative to positive titer. Testing of sera from a single animal should be discouraged unless paired samples are submitted.

Because of serologic cross-reactivity among members of the same serogroup, e.g. Hebdomadis, which includes serovars hardjo, szwajizak, wolffi, sejroe, balcanica, care must be exercised when interpreting test results of serum which react positively to two or more of the antigens within a group.

The leptospiral vaccination history in the herd must be known. This information may help determine whether titers are due to vaccination or natural exposure.

6. Suggested References

APPENDIX

MICROTITER EQUIPMENT

Flat bottom plates, pipets, dropping tips, microdiluters and multi-microdiluter handles may be obtained from:

Linbro Scientific Co.,
Subsidiary of Flow Laboratories
143 Leeder Hill Drive
Hamden, Conn. 06517 or
any Flow Laboratory office.

Cooke Laboratory Products
Division of Dynatech Laboratories, Inc.,
900 Slaters Lane
Alexandria, Va. 22314

MEDIA AND REAGENTS

1. A basic albumin-polysorbate 80 medium and its modifications (Ellinghausen-McCullough, Amer. Jour. Vet. Res. 26:45-51, 1965; Ellinghausen McCullough: Johnson-Harris Modification, J. Bact., 94:27-31, 1967) should be used for the cultivation and maintenance of the serovars used in the MAMT. In the preparation of Leptospira media, glass distilled water should be used.
Caution: Deionized water can be a contaminating source of saprophytic leptospires if only filtration sterilization procedures are employed. Deionized water, if used, must be heat sterilized.

Albumin-polysorbate 80 media or individual solutions are available commercially from Difco Laboratories, P. O. Box 1058A, Detroit, Mich. 48732, Phoenix Laboratories, 1614 N. 74th St., Omaha, Neb. 68114, Reheis Chemical Co., Armour Pharmaceutical Co., Greyhound Tower, Phoenix, Ariz. 85077 and Scientific Protein Laboratories, Inc., P. O. Box 158, Waunakee, Wisc. 53597.

The Difco product (EMJH medium) uses a dehydrated basal to which is added enrichment. The Phoenix, Reheis and S. P. media are supplied as a 5X concentrated liquid. Additionally, because many laboratories encounter problems in filter sterilizing albumin solution, Reheis offers a sterile 30% non-preserved albumin solution for those who elect to make EMJH medium.

Semisolid agar (0.2%) is prepared by adding 2.0 gm of agar per liter (1000 ml) of complete medium (basal + enrichment.)


2. Phosphate buffered saline (PBS)

Any phosphate buffered saline with a final pH of 7.2-7.6 is acceptable. The following two formulas have been used for several years and found to be suitable:

a. 20X Stock Solution
   
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<td>NaCl</td>
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<td>KH₂PO₄</td>
<td>13.6 gm</td>
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<td>Distilled water, to make</td>
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   Working Solution
   
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b. Physiological saline solution (0.85%)

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<td>KH₂PO₄</td>
<td>1.09 gms</td>
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<tr>
<td>Distilled water</td>
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</table>

   Dispense in 200 ml amounts in screw-capped bottles.

   Autoclave at 6.8 kg pressure for 15 minutes. Determine final pH after autoclaving. Final pH should be 7.5.

SPECIAL MICROSCOPIC EQUIPMENT

Eyepiece magnification will depend on magnification of the objective.
LEPTOSPIROSIS OF DOMESTIC ANIMALS

1. American Optical Corporation
   Scientific Instrument Division
   Eggert and Sugar Roads
   Buffalo, N. Y. 14215
   Catalog. No. AO 1019; 10X/0.25
   Note that the old objective, No. AO 1076; 10X/0.25, is very satisfactory.

2. E. Leitz, Inc.
   Rockleigh, N. J. 07647
   Catalog No. 519-438; 10X/0.22. The old long-working-distance objective No. 559-003, 10X is also very satisfactory.

Further information regarding the MA microtiter test may be obtained by contacting the following:

Dr. John Cole
Vet. Diagnostic & Investigational Lab.
College of Vet. Medicine,
P. O. Box 1389
Tifton, GA 31794
(912-386-3340)

Dr. H. C. Ellinghausen
National Animal Disease Center
P. O. Box 70
Ames, Iowa 50010
(515-232-0250)

Dr. H. L. Rubin
Bureau of Diag. Labs.,
Florida Dept. of Agric. & Cons. Serv.
P. O. Box 460
Kissimmee, Fla. 32741
(305-847-3185)
SCREENING SCHEMA
(Example #1)

ANTIGEN

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SAMPLE NO. 1 2 3 4 5 6 7 8 9 10 11 12

+ = 50% or greater agglutination
- = Less than 50% agglutination

SCREENING RESULTS*

Sample No.

1. hardjo, grippotyphosa
2. negative
3. hardjo
4. pomona, hardjo, grippotyphosa
5. negative
6. hardjo
7. hardjo, icterohemorrhagiae
8. hardjo, grippotyphosa
9. pomona, hardjo, grippotyphosa
10. pomona, grippotyphosa
11. pomona, hardjo, grippotyphosa
12. pomona, hardjo, grippotyphosa, icterohemorrhagiae

*See EXAMPLE #2 for titration Schema of above screening positive sera.
TITRATION SCHEMA
(Example #2)

**Hardjo-Hemorrhagiae** Antigen

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**Grippotyphosa** Antigen

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- = 50% or greater agglutination
- = Less than 50% agglutination
LEPTOSPIROSIS MICROSCOPIC AGGLUTINATION TEST REPORT

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Agglutination at a serum dilution of 1:100 is considered suspicious for Leptospirosis. 1:50 equals screening titer.
EXAMPLES OF LEPTOSPIRAL MICROSCOPIC AGGLUTINATION

A = 100% loose agglutination (4+)
B = 100% compact agglutination (4+)
C = 50% compact agglutination (2+)
D = Antigen control (Negative)

Plate #1
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

Chairman: James W. Glosser, Montana
Co-Chairman: S. L. Diesch, Minnesota

Niles H. Bairey, IA; R. Wayne Behan, IL; Alex Canales, FL; C. S. Card, J. J. Cecil, IA; John R. Cole, H. C. Ellinghausen, IA; Lyle E. Hanson, IL; R. Harrington, IA; R. L. Morter, IN; H. Stewart Powell, TN; Guy E. Reynolds, OR; L. A. Rosner, MO; H. L. Rubin, FL; Paul R. Schnurrenberger, AL; Herbert G. Stoenner, MT; James M. Williams, MO.

Current studies concerning serologic responses of cattle tested following vaccination with presently available multivalent leptospiral bacterins indicate diagnostically significant titers can result but persist for periods of only one to three months. Natural infections in unvaccinated contact animals did not induce significant serologic titers in the vaccinated cattle.

A comparative study of leptospiral serologic tests (Microscopic Agglutination and Microtiter) conducted by five selected laboratories on 40 cattle sera indicated substantial agreement between the diagnostic laboratories. Recently a similar comparison of leptospiral serologic tests of experimentally and naturally infected animals was conducted in five independent laboratories and the results demonstrated substantial agreement between the laboratories. These studies demonstrate that current serologic techniques can provide reproducible test results.

The committee approved with amendments, the laboratory subcommittee’s preparation of a laboratory manual entitled “Laboratory Diagnosis of Leptospirosis of Domestic Animals — Microscopic Agglutination Microtiter Techniques.” The preparation of this publication is a reference document in an attempt to assure uniformity of procedures used in the diagnosis of leptospirosis by the various diagnostic laboratories. This section is attached as part of the Committee’s report for adoption and publication in the Proceedings.

The laboratory subcommittee was reappointed to prepare the second section of the manual to be entitled “II Isolation Procedures,” which will be submitted to the committee for its approval at the next meeting.

The committee is concerned about the lack of communication that exists between Federal personnel investigating food animal associated human leptospirosis outbreaks and the State Animal Health regulatory agency. To improve communications, the committee recommends that the Federal Public Health agency involved request the State Animal Health agency to appoint a representative to work with the investigating team.

The committee continues to be concerned about the lack of understanding of some of the USDA-APHIS staff on the importance and significance of the Hebdomadis serogroup infections in United States cattle, particu-
larly *hardjo* infections. The committee plans to meet with APHIS representatives at the next meeting in an attempt to provide more understanding concerning the importance of Hebdomadis infections in cattle.

Concern was expressed that abortion in sheep may be due to infection with serovar *hardjo*. This observation is based on serologic evidence. Attempts at isolating *hardjo* from affected ewes has to date been unsuccessful. Veterinary practitioners and diagnostic laboratories are encouraged to continue with isolation attempts.

Preliminary infectivity studies in pregnant ewes using two different *hardjo* isolates of bovine were attempted by the NVSL. Agglutinating antibodies were produced but clinical or pathological disease did not occur.
EFFECTS OF AFLATOXIN IN CORN ON PRODUCTION AND REPRODUCTION IN DAIRY CATTLE

L. D. Guthrie
Extension Dairy Scientist, University of Georgia, Athens, Georgia

*D. M. Bedell
Extension Veterinarian, University of Georgia, Tifton, Georgia

A Georgia dairy herd that received aflatoxin contaminated corn experienced severe production, reproduction and herd health problems during 1977. Initial assays of samples of corn indicated that the owner had approximately 380 metric tons of contaminated corn. When the source of the problem was discovered, the grain mix (approximately 50% of the dry matter) contained 120 ng/g of aflatoxin (100 B₁ + 15 B₂ + 5 G₁ + G₂). Milk production increased 28% (4.9 kg/cow/day) within three weeks after removing the aflatoxin contaminated corn from the ration. The percent of successful breedings for the 10 months prior to June 1977 averaged 39.1%. The percent of successful breedings for the five months following the exposure to the aflatoxin contaminated corn on 9-17-77 (DHI test 9-30-77 through 3-1-78) averaged 37.4%.

The health problems observed in the herd were: birth of weak, unthrifty and undersized calves, diarrhea, outbreak of acute mastitis, respiratory disorders, erratic milk production, abortions, prolapsed rectum, unexplained deaths, hair loss, depressed appetite and a general unthrifty appearance of the herd. The mature animals may have been exposed earlier to some of the grain with even higher levels of aflatoxin B₁ than reported above.

*Presented to the Environmental Residues Committee, 83rd Annual Meeting United States Animal Health Association, October 28-November 2, 1979, San Diego, California.
EFFECTS OF AFLATOXIN IN CORN AND IN CATTLE

TABLE I

AFLATOXIN LEVELS IN INITIAL SAMPLES 9-13-77

<table>
<thead>
<tr>
<th>Bin</th>
<th>AFLATOXIN (ng/g)</th>
<th>B1</th>
<th>B2</th>
<th>G1</th>
<th>G2</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
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<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>60</td>
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<td>225</td>
<td>N</td>
<td>N</td>
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<td></td>
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<td>23</td>
<td>N</td>
<td>N</td>
<td>1223</td>
<td></td>
</tr>
<tr>
<td>Bin 4</td>
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<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>N</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>Corn Silage 1</td>
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<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Silage 2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td></td>
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<td>Dairy Feed</td>
<td>100</td>
<td>15</td>
<td>5</td>
<td>T</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

N=Negative

TABLE 2

AVERAGE MILK/DAY WEIGHTS (kg) - AFLATOXIN HERD

<table>
<thead>
<tr>
<th>Date</th>
<th>Milk (kg)</th>
<th>Date</th>
<th>Milk (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-76</td>
<td>16.5</td>
<td>6-77</td>
<td>17.0</td>
</tr>
<tr>
<td>8-76</td>
<td>18.2</td>
<td>7-77</td>
<td>15.2</td>
</tr>
<tr>
<td>9-76</td>
<td>18.6</td>
<td>8-77</td>
<td>16.2</td>
</tr>
<tr>
<td>10-76</td>
<td>16.9</td>
<td>9-77</td>
<td>16.5</td>
</tr>
<tr>
<td>11-76</td>
<td>16.8</td>
<td>*10-77</td>
<td>17.4</td>
</tr>
<tr>
<td>12-76</td>
<td>19.8</td>
<td>11-77</td>
<td>19.3</td>
</tr>
<tr>
<td>1-77</td>
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<td>18.9</td>
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<tr>
<td>2-77</td>
<td>23.4</td>
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<td>18.3</td>
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</tr>
<tr>
<td>5-77</td>
<td>20.8</td>
<td>4-78</td>
<td>20.7</td>
</tr>
</tbody>
</table>

*Aflatoxin corn removed 9-16-77 - Test interval 9-30 to 10-27-77
### TABLE 3

**MILK PRODUCTION RESPONSE - AFLATOXIN HERD**

<table>
<thead>
<tr>
<th>Date</th>
<th>Cows</th>
<th>Lbs/Cow/Day</th>
<th>Kg/Cow/Day</th>
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<tr>
<td>8-28</td>
<td>148</td>
<td>41.1</td>
<td>18.7</td>
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<tr>
<td>8-30</td>
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<tr>
<td>9-8</td>
<td>&quot;</td>
<td>41.0</td>
<td>18.6</td>
</tr>
<tr>
<td>9-15</td>
<td>&quot;</td>
<td>39.2*</td>
<td>17.8</td>
</tr>
<tr>
<td>9-16-77</td>
<td>Aflatoxin Corn Removed</td>
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<tr>
<td>9-25</td>
<td>&quot;</td>
<td>43.2</td>
<td>19.6</td>
</tr>
<tr>
<td>10-1</td>
<td>&quot;</td>
<td>46.0</td>
<td>20.9</td>
</tr>
<tr>
<td>10-7</td>
<td>&quot;</td>
<td>49.9</td>
<td>22.7</td>
</tr>
</tbody>
</table>

*Consulting Veterinarian's regular visit

### TABLE 4

**PERCENT SUCCESSFUL BREEDINGS - AFLATOXIN HERD**

<table>
<thead>
<tr>
<th>Test Date</th>
<th>No.</th>
<th>%</th>
<th>Test Date</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
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<tr>
<td>12-76</td>
<td>39</td>
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<td>58</td>
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<tr>
<td>5-77</td>
<td>20</td>
<td>25</td>
<td>2-78</td>
<td>49</td>
<td>43</td>
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</tbody>
</table>

*Aflatoxin corn removed 9-16-77 - Test interval 9-30 to 10-27-77
LABORATORY DIAGNOSIS OF MYCOTOXICOSES OF VETERINARY IMPORTANCE IN THE UNITED STATES

J. L. Richard, PhD; J. R. Thurston, PhD; A. C. Pier, DVM, PhD
The National Animal Disease Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture
P.O. Box 70, Ames, IA 50010.

SUMMARY

Fungal metabolites that produce pathologic responses or other undesirable effects in animals present a real challenge to the veterinarian since the effects vary from acute disease and death to subtle changes in growth, production, disease resistance and immunity. These toxic metabolites (mycotoxins) are frequently encountered by the animal as a contaminant of its food and a toxicosis results following the consumption of an adequate amount of the mycotoxin. However, consideration needs to be given also to skin and respiratory exposure to mycotoxins. The veterinarian must recognize the occurrence of various mycotoxins in feeds and field crops, their effects on animal species, their metabolism and accumulation in certain body tissues and fluids, and their potential for being synergistically or additively involved in animal disease. In addition, the diagnosis of mycotoxicoses requires acquisition of a representative sample of feed involved in the intoxication and an analysis and identification of the responsible mycotoxin(s) in sufficient amounts in the feed sample. This report outlines methods that can be employed for the laboratory diagnosis of mycotoxicoses of veterinary importance in the United States.

Fungal metabolites that produce pathologic responses or other undesirable effects in animals present a real diagnostic challenge to the veterinarian since the effects vary from acute disease and death to subtle changes in growth, production, disease resistance and immunity. These toxic metabolites (mycotoxins) are frequently encountered by the animal as a contaminant of its food and a toxicosis results following the consumption of an adequate amount of the mycotoxin. Skin and respiratory exposure to mycotoxins may also produce disease. The veterinarian must recognize the occurrence of various mycotoxins in feeds and field crops, their effects on animal species, their metabolism and accumulation in certain body tissues and fluids, and their potential for synergistically or additively being involved in animal disease. In addition, the diagnosis of mycotoxicoses requires acquisition of a representative sample of feed involved in the intoxication and an analysis and identification of the responsible mycotoxin(s) in sufficient amounts in the feed sample. Cooperative efforts between the veterinarian and other disciplines are required to meet the challenge of the mycotoxin problem.

No endorsements are implied herein.
The major biological effects and dose responses of mycotoxins in food producing animals have been presented in another paper and are briefly summarized in Table 1. Therefore, the purpose of this presentation is to discuss methods of diagnosis of mycotoxicoses utilizing biological and chemical analysis for specific mycotoxins. The sensitivities of the methodologies discussed are given in Table 2.

**AFLATOXICOSES**

The aflatoxins are a group of chemically related, hepatotoxic compounds produced by certain toxigenic strains of *Aspergillus flavus* and *A. parasiticus*. The major aflatoxins are B₃, B₂, G₁ and G₂; named for their fluorescent color (B = blue, G = green) under ultraviolet light and for their Rf values on thin-layer chromatograms. Another aflatoxin of importance to the veterinarian is M₃ toxin, a metabolite of B₁ aflatoxin which may occur in urine and milk of animals consuming aflatoxin B₁. This toxin has a blue-violet fluorescence under UV light and has a Rf value less than aflatoxin G₂.

Although aflatoxins are produced on a variety of substrates, the commodities of greatest concern, in regard to intoxication in animals, in the United States are corn, cottonseed, and peanuts. These commodities may become contaminated in the field or in storage.

There is considerable variation among animal species to acute and chronic effects of aflatoxin and these variations have been correlated with the metabolic capacities of various animal species and ages within a species.

Reports of aflatoxicosis in farm animals in the United States include primarily swine, cattle and poultry.

Although the literature contains volumes of written material on the various effects of aflatoxins on animals, the major effect is damage to the liver. The lesions are not pathognomonic for aflatoxicosis. Acute aflatoxicosis is typically manifested as hepatic necrosis and widespread hemorrhages while chronic effects are primarily bile duct hyperplasia and portal fibrosis. A well known chronic effect of aflatoxin is the production of tumors in susceptible animals; however, this is of little importance in farm animals as they are not usually retained for periods long enough for this condition to be manifested. However, this aspect of aflatoxicosis may be important to the veterinarian concerned with fish diseases since rainbow trout are quite susceptible to the carcinogenic effects of aflatoxin.

Biological assays for aflatoxins can be used but are of limited value since the chemical tests are more rapid and conclusive. Biological assay consists of feeding suspected aflatoxin containing feed *ad libitum* to day-old white Pekin ducklings for 10 days. At the end of the 10 day feeding period, survivors are killed and the livers of all ducklings are examined histopathologically for necrosis and biliary hyperplasia.
Analysis of aflatoxins by chemical means is utilized by most laboratories and references are given to those various procedures in the following discussion.

The detection of aflatoxins in mixed feeds is more difficult than for grains and procedures have been developed for these commodities. Generally the two recognized procedures involve extraction of the feed with an organic solvent such as methanol or acetone:methylene chloride. The extract is then purified by various chemical techniques and finally by column chromatography. The material from the column can then be subjected to thin-layer chromatography (TLC) for final analysis, confirmation and quantitation.

For grains the method adopted by the Association of Official Analytical Chemists and known as the CB method is preferred. A method of comparable efficiency is the multimycotoxin method as outlined in Cottral. This method can be used for detection of aflatoxins, ochratoxin and zearalenone. Basically, the grain is extracted with chloroform and purified by column chromatography. One of the fractions eluted from the column contains the aflatoxin from the extracted grain sample and the aflatoxins can be analyzed by TLC.

Residues of aflatoxins may occur in certain animal tissues, particularly the liver, and extraction of these tissues may be an aid in the diagnosis of aflatoxicosis. The method of Murthy, et al has been employed successfully in our laboratory in the extraction of aflatoxin from liver tissue. Column fractions obtained from this procedure can be analyzed by TLC. Urine or milk obtained from animals dying or affected by aflatoxin may contain toxin which can be extracted, purified and analyzed by TLC.

Aflatoxin is easily extracted from a 30 ml urine sample by mixing with 45 ml of methanol and partitioning 3 times with 55 ml volumes of chloroform in a separatory funnel. The chloroform extract is saved, concentrated, and analyzed by TLC. For milk, the method of Stubblefield has been used successfully in our laboratory for extracting and purifying the extract by column chromatography. The final column fraction is analyzed by TLC.

The aflatoxins are ammenable to TLC analysis because they are highly fluorescent under long-wave ultraviolet light (365 nm). Portions of the samples obtained by the various procedures discussed above can be applied to thin-layer silica gel plates including appropriate internal and external standards of the specific aflatoxins. The plates can be developed in a variety of solvent systems; however, we have successfully employed chloroform:methanol:formic acid (97:2:1 v/v/v). Aflatoxins may be quantitated on the TLC plates by comparing densitometrically (ultra-violet) sample spots of aflatoxins on developed plates to standard aflatoxin spots of known concentration. Aflatoxins can be confirmed on TLC plates by spraying the plates with 25% aqueous sulfuric acid. Examination of the
plates under long wave UV light shows the aflatoxins have changed to a
brilliant yellow fluorescence. Another excellent method for confirmation
is that described by Pryzybylski.17

All of the samples to be analyzed by TLC from the methods discussed
above may be alternatively subjected to gas chromatography-mass spec-
tral analysis.

TRICHOTHECENE TOXICOSES

The trichothecenes are the largest group of chemically related
mycotoxins produced by several genera of fungi,11 although the genus
Fusarium is predominant. Therefore, an examination of the literature
will reveal the designation of "fusariotoxicosis" to some of these toxic
diseases.

The trichothecenes produce a number of toxic effects on animals such
as epidermal necrosis, vomiting, diarrhea, hemorrhage, edema, hemato-
poietic depression, leucopenia, tachycardia, feed refusal, neural
disturbances16 and changes in immunogenic organs and immunologic
responsiveness.18-21

Two important diseases which have occurred primarily in Russia
known as alimentary toxic aleukia and stachybotryotoxicosis caused by
toxins from Fusarium sporotrichoides (syn = F. tricinctum) and
Stachybotrys atra, respectively, may have been caused by
trichothecenes.22 Trichothecene toxins of importance in the United States
include T-2 toxin, deoxynivalenol and diacetoxyscirpenol since they have
been found to be naturally occurring and associated with disease in
animals. T-2 toxin was found in moldy corn associated with moldy corn
poisoning resulting in death of dairy cattle in Wisconsin23 and in mixed
feed producing hemorrhage and bloody feces of cattle in Nebraska.24
Deoxynivalenol (also called vomitoxin) has been found in corn and mixed
feed associated with feed refusal and bloody feces in swine and vomiting
in dogs in the north central United States.25-26 Diacetoxyscirpenol has
been found in mixed feed associated with a hemorrhagic bowel syndrome
in Minnesota swine.24 Trichothecenes should be suspect when conditions
similar to those described above are observed in animals.

Pathre and Mirocha24 have reviewed a number of biological assay
systems for detection of trichothecenes. However, for nonspecific
screening for these compounds isolated from various commodities and
culture extracts, the dermal necrosis test is frequently employed and has
considerable sensitivity. A successful method11 for this assay is to finely
grind a 100 gm sample of the suspect substrate, add 30 ml of water and
extract three times with 250 ml of ethyl acetate in a Waring blender for
five minutes each extraction. The resultant extract is pooled, filtered,
evaporated to dryness and redissolved in 10 ml of ethyl acetate. Five ul of
this solution can then be applied to the shaved skin on a rat or rabbit.
Using the rabbit, Chung et al17 were able to detect 0.01 ug of T-2 toxin per
test and in the rat 0.04 ug of T-2 per test. The areas to which the extracts
were applied should be examined daily for 5 days; however, positive samples are usually detectable within 48 hr. Repeated daily applications for 3 days may be appropriate in some cases. Positive skin response will be noted as a whitish area becoming erythematous with a white center and eventually necrosis if sufficient quantity of toxin is present. Standard amounts of T-2 toxin should be applied to the shaved skin of the test animal for control purposes.

The trichothecenes possess little or no fluorescent properties and are difficult to detect using thin-layer chromatographic techniques. Anyone employing this technique needs to include a number of standard trichothecenes as internal and external standards. Also, for detection of these compounds on thin-layer plates, the plates must be sprayed with concentrated HSO₄ or with p-anisaldehyde spray reagent and heated at 110°C for 10 minutes. With the latter spray reagent, most trichothecenes are purple or bright yellow under visible light. Frequently, rather crude extracts are applied to the TLC plates (a preferred method as clean-up procedures often result in poor recovery or loss of the toxin) and the trichothecenes are unable to be visualized on TLC plates due to interfering substances in the extract. To overcome this problem, successive development may be used whereby the plate is first developed in petroleum ether:diethyl ether:glacial acetic acid (70:30:2 v/v/v) to move interfering substances up the plate and then rechromatographed in a different solvent such as toluene:ethyl acetate:formic acid (6:3:1 v/v/v) to move trichothecenes away from the origin. Use of this method with appropriate internal and external standards is often successful in determining the identity of some of the trichothecenes. Quantification of trichothecenes on TLC plates is impractical since spray reagents cannot be evenly applied and reactions are not assured of being complete.

Probably the greatest success in analyzing for and quantitating trichothecenes is achieved by use of gas chromatography-mass spectral analysis. The compounds separated from a mixture by gas chromatography can be analyzed and identified by its mass spectrum.

ZEARALENONE TOXICOSES

Zearalenone is an estrogenic metabolite of several species of Fusarium, but F. graminearum (syn = F. roseum) is probably the most frequently involved species. The perfect state of this organism is Gibberella zeae and this name may be used in the literature of zearalenone toxicoses. To anyone unfamiliar with this group of organisms, the literature may be quite confusing since there is current disagreement among mycologists concerning the taxonomy and nomenclature of the Fusaria.

Zearalenone or F-2, when fed to animals (particularly swine), produces estrogenic effects such as mammary hypertrophy, enlarged vulva and prepuce, enlarged uterus, vaginal and rectal prolapse (in severe cases), abortion, infertility, weak piglets, and reduction in litter size. More recently, Shreeve, et al found no evidence of an effect on reproductive
Among the commodities and feeds in which zearalenone has been found are corn, wheat, oats, sorghum, barley, hay, sesame, commercial animal rations and corn silage. The compound has been found in almost any part of the world where these commodities are grown. In the United States, corn is the commodity most frequently involved in intoxications of animals. These intoxications include hyperestrogenism, primarily in swine although cattle have been involved, abortion in cattle and swine, feed refusal, hemorrhage and infertility.

The coexistence of zearalenone in feed samples with deoxynivalenol, T-2 toxin, ochratoxin and aflatoxin has been found and provides the diagnosticians with a diagnostic challenge due to the multiplicity of signs possible in the affected animals.

A method of screening feed for zearalenone has been developed utilizing the effect of zearalenone on the rat uterus. Samples of feed can be fed to female, weanling, white rats for 5 days and weights of excised uteri from the principle rats can be compared to the excised uteri from control rats. A preferred method has been outlined whereby 25 gm of feed is extracted with ethyl acetate, evaporated to dryness, redissolved in acetonitrile, defatted with petroleum ether, the acetonitrile evaporated to a residue, redissolved in a small amount of acetone and applied to the shaved skin of a rat. After 3 days the rats can be killed and the weights of excised uteri compared with those of controls. This method reportedly has a sensitivity of 0.3-0.6 mg zearalenone.

A multimycotoxin analytical method developed by Eppley has been utilized successfully for extraction and clean-up of zearalenone in agricultural commodities and the method has been accepted in official first action by the Association of Official Analytical Chemists and the American Association of Cereal Chemists. The appropriate eluate taken from a column chromatograph in the above procedure can be used for TLC analysis employing appropriate internal and external standards. Zearalenone fluoresces yellow-green under short-wave UV light and has an Rf = 0.5 when chromatographed on a silica gel 60 plate developed in chloroform: methanol:formic acid (97:2:1 v/v/v). Zearalenone can be visualized as an orange-brown spot when the plate is sprayed with the p-anisaldehyde spray reagent and heated at 110°C for 10 min. Since zearalenone is fluorescent under UV light, it can be quantitated densitometrically on TLC plates using an excitation wavelength of 313 nm and the fluorescence can be measured at 443 nm.

Mirocha et al has described the use of gas chromatography for separation of the trimethylsilyl ether derivative of zearalenone and mass spectral analysis utilizing selected ion monitoring for confirmation and quantitation of zearalenone. This technique has a sensitivity to 15 nanograms.
TREMORGEN MYCOTOXICOSES

Tremorgenic mycotoxins have been included in the list of mycotoxins as laboratory curiosities since the discovery of tremors produced in laboratory animals by an extract of *Aspergillus flavus*.\(^4\) Since that time the list has continued to grow and they have been classified into four groups based on chemical similarity.\(^4\) These groups are: the paspaline group, the penitrem group, the tryptoquivaline group, and the fumitremorgen-verruculogen group. The latter two groups have not been associated with naturally occurring disease and will not be discussed further. However, the paspaline group has been implicated in a disease in the United States known as paspalum staggers.\(^4\) The major signs of the disease in cattle are generalized muscle tremors accompanied by hyperexcitability. Frequently, undisturbed cattle will show no sign of disease until they are forced to move. Bermuda grass tremors and rye grass staggers are two additional diseases where affected animals exhibit signs similar to paspalum staggers. Porter, et al\(^4\) have implicated a *Claviceps* sp as the fungus involved in bermuda grass tremors while the etiology of rye grass staggers is unknown, although *Corynebacterium* sp has been implicated.\(^4\) Perhaps as DiMenna\(^4\) has suggested, fungi infecting or infesting the grasses may not be involved in these syndromes, but that fungi growing in the soil and producing toxins may be involved when they and their products are ingested during grazing. Paspalum staggers is associated with the ingestion of grasses of the genus *Paspalum* infected with the ergot fungus *Claviceps paspali*. The infected flowers of the grass are replaced by a hardened mass of fungal tissue called sclerotia which are toxic when experimentally fed to cattle.\(^4\) Three tremorgens, paspaline, paspalinine, and paspalicine have been isolated from *C. paspali* but sufficient toxicity work has not been done with these compounds to demonstrate a causal relationship to paspalum staggers.

The penitrem group of tremorgens are produced by four species of *Penicillium* belonging to the section Asymmetrica, subsection Fasciculata.\(^4\) Fungi with capabilities of producing penitrem A, the most potent of the penitremes, were isolated from feedstuffs associated with toxic syndromes in cattle, horses, and sheep.\(^49,50\) In these cases penitrem A was not found to occur naturally in the feeds. Recently, Richard and Arp\(^51\) have described the natural occurrence of penitrem A in refrigerated moldly cream cheese which had been discarded and caused tremorgenic disease when the cheese was inadvertently ingested by two dogs.\(^52\)

Suspect feeds can be assayed biologically by extracting with chloroform and the extracts given orally or injected intraperitoneally into mice. Within an hour (usually within 20 minutes) mice will develop tremors and if sufficient penitrem is present, mice will develop seizures and eventually die.

Chloroform extracts of suspect feeds may be analyzed by TLC by spotting the extracts on TLC plates utilizing appropriate internal and
external standards. The plates can be developed in chloroform:acetone (93:7 v/v), dried, sprayed with 1% p-dimethylaminobenzaldehyde (in 95% ethanol) and dried again. The blue color of the penitrem A is visualized by placing the plate in HCl vapors for 15 minutes. Color development continues for 24 hours so plates should not be discarded immediately. Quantitation of penitrem A on TLC plates is impractical because spray reagents cannot be applied evenly and reactions are not assured of being complete. However, Hou et al have developed a colorometric test whereby the penitrems can be quantitated in chloroform extracts of feeds.

The penitrems are somewhat unstable in solution; they are chemically very complex compounds (structures undetermined) and have not been amenable to gas chromatographic-mass spectral analysis.

ERGOTISM

Ergotism is probably the oldest recognized mycotoxicosis other than poisonings due to the ingestion of fleshy fungi. Ergot fungi invade the female portion of the parasitized plant and replace the ovaries of individual flowers with a mass of fungal tissue called a sclerotium. The sclerotia are then consumed during foraging by animals or are harvested with grains and then consumed resulting in the disease either as the convulsive form of ergotism or the gangrenous form. The toxins causing the disease may be one or more ergot alkaloids which are contained in the sclerotia. There are two major types of alkaloids from ergot; the lysergic acid derivatives and the clavine alkaloids. Although there are several species of ergot fungi, Claviceps purpurea is the fungus predominately involved in ergotism and it has capabilities of parasitizing a wide range of pasture grasses and cereal crops.

Cattle, swine and sheep appear to be the animal species other than humans most commonly involved in ergotism. Cattle may exhibit lameness progressing to gangrenous necrosis of the feet at the fetlock joint. This disease should not be confused with the syndrome in cattle known as fescue foot, a disease in cattle suspected of being mycotoxic but as yet of uncertain etiology. Balansia spp (species related to the genus Claviceps) have been found growing on fescue and other grasses in toxic pastures. A steroid has been isolated from B. epichloe and from toxic fescue.

Effects usually attributed to ergot are gangrenous necrosis, reduction in weight gains and agalactia. Biological tests, although used in the past, are of little value because chemical tests are much more rapid and conclusive.

Sclerotia of Claviceps purpurea may contain variable amounts of alkaloids; therefore, accurate estimates of toxic alkaloids cannot be made by the concentration of sclerotia in the grains.

The ergot alkaloids can be detected in sclerotia by grinding the latter
to a fine powder and mixing with 0.3 gm sodium bicarbonate with sufficient water to make a crumbly paste. The paste is extracted by shaking for an hour with 100 ml diethyl ether for each of three extractions. The combined extracts are partitioned into 10 ml of 1% tartaric acid. (This step is repeated three times.) The tartaric extracts are collected and brought to a 50 ml volume with additional 1% tartaric acid. Ten ml of this solution is adjusted to pH 8.5 with ammonium hydroxide, extracted three times with an equal portion of chloroform and the chloroform extracts evaporated to dryness. This extract is dissolved in 0.5 ml chloroform and used for spotting TLC plates including known ergot alkaloids for internal and external standards. The plates then can be developed in ethyl acetate:dimethyl-formamide:ethanol (13:1.9:1, v/v/v). Some alkaloids will fluoresce when the plates are examined under UV light (250 nm). For detection of nonfluorescent alkaloids, the plates are sprayed with 1% p-dimethyl-aminobenzaldehyde (in 95% ethanol) and dried. The blue colors of the alkaloids are visualized by placing the plate in HCl vapors for 15 minutes. Color development continues for 24 hours so negative plates should not be discarded immediately. Quantitation of alkaloids on plates is impractical because sprays cannot be applied evenly and reactions are not assured of being complete. An additional qualitative, simple TLC method for ergot alkaloids has been developed by Hassan.

Some of the ergot alkaloids can be quantitated by gas chromatography and confirmation of the alkaloids can be conducted with mass spectrometry.

OCHRATOXICOSES

The ochratoxins are a group of chemically related compounds produced by several species of the two genera *Penicillium* and *Aspergillus*. *Aspergillus ochraceus* and *P. viridicatum* are the most prominent species involved with the latter probably being the most important as it competes better with other fungi under storage conditions. The two major ochratoxins are ochratoxin A and ochratoxin B; however, ochratoxin A is the only one with widespread occurrence as a natural contaminant. Ochratoxin occurs in a variety of cereal grains and has been found in corn and barley in the United States. The major effect of ochratoxin in animals is damage to the kidneys in form of nephropathy manifested as tubular atrophy and interstitial fibrosis. These changes are typified by a condition that has been reported in swine and poultry associated with consumption of grain contaminated with ochratoxin in Denmark. The involvement of citrinin, another nephrotoxic mycotoxin produced by some of the above named fungi, in this syndrome is uncertain as levels of the toxins in involved grains have been below levels which are necessary to produce disease in experimental animals. Although apparently ochratoxicosis is an important disease in Denmark, natural outbreaks of disease in the United States associated with ochratoxin is limited. Only one reference to
outbreaks of ochratoxicosis has been found, and this report still awaits publication confirmation. However, citrinin has been implicated in a toxicosis of feeder cattle in Iowa.

When high doses are consumed and in the syndrome in Denmark called mycotoxic porcine nephropathy, ochratoxin may occur in the urine of the affected animals. This aspect may be used in the diagnosis of the disease.

The lesions produced in animals by ochratoxin are not pathognomonic and chemical tests are quite rapid; thus, biological assay in animals is of little value. However, in animals affected by ochratoxin, the toxin has been demonstrated in kidney tissue by immunofluorescence microscopy.

For diagnosis of ochratoxicosis, analysis for ochratoxin in the urine may be employed by acidifying 50 ml of a urine sample to pH 2 with concentrated HCl and then extracting this mixture in a separatory funnel with two 75 ml volumes of chloroform. The chloroform extract is then evaporated to dryness and redissolved in a small amount of benzene-acetonitrile (98:2 v/v) for TLC analysis.

The multimycotoxin method discussed under aflatoxicoses is suitable for detection of ochratoxins in grains. However, the Association of Official Analytical Chemists (AOAC) has an official first action method for barley which can be used on other grains. This method employs extraction from the commodity followed by a column chromatography clean-up procedure. The appropriate column fractions then can be analyzed for ochratoxin by TLC. An important diagnostic aid for ochratoxicosis is that ochratoxin can occur in the kidneys and blood of affected animals and a method has been developed whereby the toxin can be extracted and identified from whole blood, plasma or tissue. The method employs the extraction of tissue homogenate or blood with chloroform and the transfer of ochratoxin A into a tris (hydroxymethyl) aminomethane-hydrochloride buffer solution. The ochratoxin A is then cleaved, using carboxypeptidase, into ochratoxin and phenylalanine followed by spectrofluorometric analysis and quantitation of ochratoxin A based upon changes in fluorescence intensity at 380 nm of the ochratoxin A containing extract and the cleavage product. Krogh, et al have examined tissues including muscle, kidney, liver and fat for ochratoxin using methods developed by them. Final analysis of ochratoxin is by TLC.

Ochratoxin possesses a yellow-green fluorescence under long wavelength UV light and can be easily visualized and may be quantitated densitometrically on thin-layer chromatograms. For TLC analysis, the samples should be spotted utilizing both internal and external standards. Ochratoxins on a TLC plate will change to a bright blue fluorescence when exposed to ammonia fumes. A further confirmatory test for ochratoxins A and B involves conversion to their ethyl esters and then TLC analysis.

Samples subjected to TLC analysis may also be subjected to gas
chromatography-mass spectral analysis for unequivocal confirmation.

ADDITIONAL MYCOTOXICOSES OF POSSIBLE IMPORTANCE IN
THE UNITED STATES

Rubratoxicosis

The natural existence of this disease is unknown as is the proven natural occurrence of the toxin in commodities. This may be a reflection on our inability to assay appropriate commodities for naturally occurring levels of the toxin. The fungus, *Penicillium rubrum*, which produces rubratoxin has been associated with moldy corn toxicosis in swine, cattle and poultry.78,79 Rubratoxin is hepatotoxic and may cause hemorrhages in affected animal species.

Methods of analysis for rubratoxin in corn have been developed;10 however, the sensitivity is in the range of 50-100 ppm. A radioimmunoassay procedure for rubratoxin has been developed,80 but the technique has not been applied to field or survey investigations. Attempts to analyze rubratoxin by gas chromatography-mass spectrometry have been unsuccessful.

Slaframine Toxicosis

This disease was first reported to occur in cattle and sheep in the United States in 1959.81 The condition was observed to occur in animals ingesting red clover hay and the toxin was subsequently identified as slaframine, a metabolite of *Rhizoctonia leguminicola*, the agent of black patch disease of red clover.82-84 Salivation is the most prominent sign and occurs throughout the intoxication.85 Slaframine is a histamine-like alkaloid capable of producing effects similar to parasympathomimetic drugs and can be partially counteracted by atropine.

*Rhizoctonia leguminicola* should be isolated and identified from the hay given affected animals. Culture material can be fed to guinea pigs to reproduce the excessive salivation. No adequate assay procedures for slaframine from infected red clover have been developed.

Lupinosis

This disease occurs primarily in sheep grazing or feeding on lupines infected with the fungus *Phomopsis leptostromiformis*.87,88 Affected sheep lose their appetite, collapse and often die with hepatic changes manifested as bright yellow, fatty appearing, distorted livers.87 The disease was found to be attributed to toxins produced by *Phomopsis leptostromiformis*.87 The toxins subsequently have been isolated and are known as phomopsins A and B.89 The disease is recognized as a problem especially in Europe, South Africa, and Australia.88 Lupinosis may occur in the United States. It has been suspected in horses in Montana89 and we have examined lupine hay (*Thermopsis montana*) involved in a syndrome similar to lupinosis in sheep in the western United States (Morphet, E. Personal communication, 1978). We failed to find the organism in the submitted samples of hay but the fungus does exist as an important
The only biological assay for these toxins is to feed suspect hay or forage to sheep and observe them for the signs of the disease. The fungus can be isolated and identified from the infected lupine stems and seed pods and feeding trials have been conducted with pure cultures of *Phomopsis leptostromiformis*.

Presently, there are no good chemical assay procedures for the phomopsins and their structure has not as yet been determined.

*Mycotoxic Photosensitivity*

Facial eczema is a mycotoxic photosensitivity disease that occurs primarily in sheep and cattle in New Zealand. A mycotoxin, sporodesmin, is produced by the fungus *Pithomyces chartarum* which grows on certain pasture grasses such as ryegrass. Animals ingesting infected grasses consume a large number of the toxin containing spores. The toxin is hepatotoxic and the result is a failure of the damaged liver to remove phylloerythrin from the blood stream. Phylloerythrin accumulated in the bloodstream and tissues absorbs energy from sunlight resulting in damage to the skin of the animal.

Photosensitivity diseases with pathogenic changes identical to facial eczema have been reported in the United States, but a mycotoxin has not been found as the agent of the diseases. Also of interest is that Budiarso et al were able to induce a phototoxic syndrome in mice fed cultures of a toxic strain of *Penicillium viridicatum*. The possibility exists that a number of fungi may be capable of producing toxins which indirectly cause a photosensitivity in affected animals.

Analyses for sporodesmins have included a bioassay utilizing tissue culture and a chemical titration method. However, Pyle et al recently have developed purification, derivitization and gas chromatographic methods for determining the sporodesmins. The derivative can be unequivocally determined by mass spectral analysis.

**GENERAL DIAGNOSTIC CRITERIA FOR MYCOTOXICOSES**

The diagnosis of a mycotoxicosis is virtually impossible from the clinical picture of affected animals because of: mycotoxin synergism, potentiation or predisposition, the nonpathognomonic nature of the lesions and concurrent signs of other disease in the animals. Unfortunately, the diagnostician may not become concerned with the possibility of a mycotoxicosis until other causes of disease have been eliminated. By this time, the suspect feed is often already consumed and not available for analysis.

When a mycotoxicosis is suspect, a feed sample for analysis should be collected immediately and stored, preferably at \(-70^\circ\text{C}\). The sample must be taken from that lot of feed involved in the intoxication, perhaps necessitating acquisition of the feed from the feed trough. Sampling of a lot of feed to obtain a representative sample is very important as "hot
spots'" may occur in a bin of feed. The occurrence of “hot spots” necessitates taking subsamples throughout the bin with a grain probe to obtain the representative sample. A few kernels of corn highly contaminated with aflatoxin may be sufficient to yield 25-50 ppb of aflatoxin in a 20 pound sample of corn. Sampling procedures for peanuts have been designed and adopted by the National Peanut Council and may be used with modification for other commodities.\textsuperscript{101} The occurrence of “hot spots” makes diagnosis even more difficult as the toxin is diluted in the sampling procedure. Therefore, portions of any obvious moldy areas within a lot or bin of feed should be set aside for a separate analysis.

To decrease the possible heterogeneous distribution of a mycotoxin, the sample of feed collected should be finely ground and mixed thoroughly before a subsample is taken for chemical analysis. A portion of this sample may be used for biological testing, if applicable. When the toxin(s) are determined to be unknown by conducting established laboratory tests, it is often desirable to prove toxicity of the sample before initiating extensive analytical work. To prove the feed to be toxic, it may be necessary to administer the feed to animals of the same species in which the disease occurred. Of course, this may be impossible where the disease resulted from long range feeding on low levels of a toxin.

Samples should not be submitted to the laboratory with the request, “Please examine for mycotoxins” as the laboratory diagnostician has no idea of the kinds of toxins that may be present in the sample. Samples so submitted can become an entire research project and may be entirely neglected. The type of feed, clinical history, and histopathology assist the laboratory diagnostician to establish the type of toxin(s) that may be involved. The suspected type of toxin(s) in the feed sample determine the method(s) of analysis. Most methods employ extraction of sample, cleanup of extract and final analysis with TLC, a test with varying but considerable sensitivity (Table 1). Additional methods such as high pressure liquid chromatography or gas chromatography-mass spectral analysis are often used for confirmatory procedures and may be used in the primary analysis. Multiple toxin analysis procedures should be used where applicable.

Diagnosis of certain mycotoxicoses are aided by analysis of certain tissues and fluids (urine, milk or blood) for toxins or their metabolites.

The toxin(s) found in a feed sample should be of the type and quantity compatible with the condition observed in the affected animals. More than one toxin may be present in a sample and may or may not be involved in the disease syndrome. Also, the disease may be dependent upon the length of consumption of the toxic feed.

Mycological investigations of the feed sample may be helpful in confirming a diagnosis but in itself is not diagnostic. The presence of toxigenic fungi in a feed sample is not proof of the existence of the toxin since appropriate environmental conditions for toxin production may not
have been met. Moldy feed does not insure the presence of a mycotoxin or a mycotoxicosis. Also, particularly in prepared feeds which have undergone heating or some other preparative treatment, the fungus that elaborated the toxin in one of the feed components may no longer be viable.

Safety should be of concern with all of the procedures used in the diagnosis of mycotoxicoses. Precautions should be taken during collection and preparation of samples, extraction, clean-up of extract and final chemical analysis since certain mycotoxins are dermal necrotic, irritants, carcinogens, mutagens and teratogens. Additionally, the solvents and other chemicals used in the procedures may be quite toxic and/or flammable. Precautions should be taken in the use of ultraviolet light in the examination of TLC plates. Protective clothing including safety glasses and disposable gloves should be worn, for handling contaminated materials. Face masks or a respirator should be worn when grain is collected and ground. We know practically nothing of the effects of mycotoxins on animals exposed by the respiratory route. All work with solvents and other procedures should be conducted in a ventilated hood equipped with a filter system.

All purified toxins should be put in solution as soon as possible when working with them since many of them are highly electrostatic in a dry powder form and may be easily aerosolized.

All toxin containing materials including TLC plates for discard should be decontaminated (e.g. hypochlorite is excellent for aflatoxin, ochratoxin, and zearalenone and saturated potassium hydroxide solution is satisfactory for many of the trichothecenes) prior to disposal.
## TABLE 1—Summary of Effects of Some Mycotoxins on Some Animal Species
(From Pier et al, 1979, Reference 1)

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Species</th>
<th>Level</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>Cattle</td>
<td>0.2 ppm - 2-4 wks</td>
<td>Reduced weight gains in calves</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14-46 ppb</td>
<td>Detectable milk residues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 ppm - 59 days</td>
<td>Death in steer</td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>0.26 ppm</td>
<td>Decreased growth rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.62 mg/kg</td>
<td>Single oral LD&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
<td>0.25 ppm</td>
<td>Decreased weight gain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-10 ppm</td>
<td>Decreased egg production, hepatic necrosis and hemorrhage, death.</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>Cattle</td>
<td>0.16 mg/kg - 12 days</td>
<td>Enteritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.64 mg/kg - 20 days</td>
<td>Death with bloody feces, enteritis, abomasal and ruminal ulcers, coagulopathy</td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>0.38 ppm</td>
<td>Hemorrhagic enteritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(diacetoxyscirpenol)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
<td>4 ppm</td>
<td>Oral necrosis, reduced weight gains, neural effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 ppm</td>
<td>Decreased egg production and shell quality</td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>Cattle</td>
<td>1.0 mg/kg - 14 days</td>
<td>Depression, reduced weight gains, nephritis, enteritis in calves</td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>0.2 ppm</td>
<td>Chronic nephropathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ppm</td>
<td>Acute fatal enteritis</td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
<td>0.6 ppm</td>
<td>Acute nephrosis, hepatic change, marrow and lymphoid depression and death in broilers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 ppm</td>
<td>Decreased egg production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4 mg/kg</td>
<td>Single oral LD&lt;sub&gt;50&lt;/sub&gt; in chicks</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Swine</td>
<td>1-5 ppm</td>
<td>Estrogenism</td>
</tr>
</tbody>
</table>
TABLE 2--Laboratory Detection of Selected Mycotoxins

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Substrate</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>Corn</td>
<td>TLC</td>
<td>1-4 ppb</td>
<td>11, 14</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>TLC</td>
<td>0.5-3 ppb</td>
<td>16</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>Corn</td>
<td>TLC</td>
<td>1000 ppb</td>
<td>29, 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01-0.04 µg</td>
<td>27</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>Corn</td>
<td>TLC</td>
<td>100-200 ppb</td>
<td>11, 38, 41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>200-300 µg</td>
<td>33</td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>Corn</td>
<td>TLC</td>
<td>15-20 ppb</td>
<td>11, 14</td>
</tr>
</tbody>
</table>

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REPORT OF THE COMMITTEE ON ENVIRONMENTAL RESIDUES

The Environmental Residues along with the AAVLD Mycotoxin Committee met jointly at the U.S.A.H.A. Conference in San Diego, CA. The following members and guests were present:

*Chairman:* G. T. Edds, Florida


Mycotoxin contaminations of animal feeds produce animal health hazards from growth suppression, reduced milk and egg production along with immunosuppression, with serious economic losses to livestock producers.

Improved differential diagnostic capabilities including new, refined automated analytical procedures for quantitating levels of single or mixed mycotoxins in feeds and tissues, establishment of minimal, acute or chronic toxic levels of the several agents under controlled and field exposure along with residue hazards in animal products, liver, kidney and muscle tissues is recommended.

Broader recognition of the significance of aflatoxin B₁, other mycotoxins, such as zearalenone, T-2 toxin, ochratoxin A and their metabolites, by those in diagnostic laboratories as well as livestock producers, including assay procedures, clinical signs, lesions, pathologic effects and tissue residue levels should be emphasized. Such information was provided in 2 papers presented: "Effects of Aflatoxin in Corn on Production and Reproduction in Cattle" by L. D. Guthrie and D. M. Bedell and "Laboratory Diagnoses of Mycotoxicoses of Veterinary Importance in the U.S." by J. D. Richard, J. R. Thurston and A. C. Pier.

The Committee also reviewed other toxicants present in poisonous plants such as monocrotaline, thiaminase and tremetol affecting animal health with possible tissue residues hazardous to humans. It was recommended that research to develop analytical methods to identify and quantify such toxicants be encouraged.
ADDITIONAL CHARACTERISTICS OF DISEASE CAUSED
BY THE AFRICAN SWINE FEVER VIRUSES
ISOLATED FROM BRAZIL AND THE DOMINICAN REPUBLIC

C. A. Mebus and A. H. Dardiri
From the Plum Island Animal Disease Center,
Agricultural Research
Science and Education Administration,
United States Department of Agriculture
Post Office Box 848, Greenport, New York 11944

Clinical aspects and gross lesion of a highly virulent (Lisbon 60) and 2 low virulent (from Brazil and Dominican Republic) isolates of African swine fever (ASF) virus were compared in a presentation to this association in 1978. In this report, the comparison will be extended by more detailed clinical observations and presentation of histologic lesions caused by the Lisbon 60, and Brazil isolates, and presentation of clinical observations, and gross and histologic lesions caused by the Dominican Republic ASF virus isolate.

MATERIALS AND METHODS

The viruses, type of animals used, inocula, routes of inoculation and procedures for clinical observation,1 swine buffy coat cultures,2 and immunoelectroosmophoresis (IEOP)3 have been reported.

For pathological studies, pigs that died or were moribund were necropsied. Gross lesion were recorded and photographed. The following tissues were collected in 10% phosphate buffered formalin for histologic examination: submandibular, prefemoral, mesenteric, internal iliac, renal, gastrohepatic and bronchial lymph nodes; tonsil; thymus; lung; heart; spleen; kidney; adrenal; urinary bladder; liver; pancreas and brain. Tissues were embedded in paraffin, sectioned at 5 μ and stained with Mayer’s hematoxyline and eosin-phloxine B.

RESULTS

Libson 60 Isolate — Clinical Observations

Three days post-inoculation (DPI), all pigs had fevers ranging from 40°C (104°F) to 41.1°C (106°F). The highest average fever was 42°C (107.6°F) 5 DPI. Four of 8 pigs developed leukopenia. The reduction in total leukocyte counts at 6 DPI was 35% for pig 1L and 43% for pig 3L and at 8 DPI was 27% for pig 4L and 64% for pig 7L. Four of the 8 pigs were dead 7 DPI. Eight DPI, 2 more pigs were dead, and 1 was moribund. The remaining pig had a temperature of 41.7°C (107°F) and was

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African swine fever virus was isolated in swine buffy coat cultures from the blood of all pigs 2 and 4 DPI. No antibody was detected by the IEOP test in serum of any pig before or after inoculation.

The relationships of daily temperatures, total leukocyte counts, and virus isolations from the blood of 2 pigs typical of those inoculated with Lisbon 60 ASF virus isolate are shown in figures 1 and 2.

Brazil Isolate — Clinical Observations

Two to 3 DPI, all pigs had fevers ranging from 40°C (104°F) to 42.2°C (108°F). The highest average fever was 41.7°C (107°F) 6 DPI. Pigs that died (4B, 7B, 8B, 9B) had continuous fevers of 40.6-41.7°C (105-107°F) until shortly before death. The temperatures of 3 surviving pigs (1B, 2B, 5B) returned to normal 20 DPI. Pigs 3B and 6B, which also survived, were febrile 40.2 to 41°C (104.2 to 105.6°F) on days 24 and 30 DPI, respectively. All surviving pigs thereafter had normal daily temperatures through 135 DPI, the end of the observation period.

Total leukocyte counts decreased from an average count of 31,550/mm³ of blood on day 0 to an average count of 12,344/mm³ of blood 6 DPI. The DPI of lowest leukocyte count varied among pigs. After 14 DPI, the total leukocyte counts for all pigs, except pigs 9B and 8B which died 10 and 15 DPI, respectively, returned toward pre-inoculation levels.

African swine fever virus was isolated in swine buffy coat cultures from the blood of all pigs 2 DPI. Pigs that died were viremic throughout the disease course. The duration of detectable viremia in surviving pigs varied from 24 to 38 DPI. Toward the end of viremia, virus was detected only by subculture. Hemadsorption results are shown in table 1.

Antibody to ASF virus was first detected by IEOP in all pigs 8 to 12 DPI except for pig 9B that remained negative until it died 11 DPI. All surviving pigs were still serologically positive 135 DPI when the experiment was ended.

The relationships of daily temperatures, total leukocyte counts, virus isolations and serum antibody from the blood of 2 pigs typical of those inoculated with Brazil ASF virus isolate are shown in figures 3 and 4. One pig died and 1 survived.

Dominican Republic Isolate — Clinical Observations

All 10 pigs ate throughout the illness. No exudate was observed from the eyes or nostrils; pig 3DR had diarrhea. Pig 7DR died 13 DPI and pig 3DR died 22 DPI. Pig 6DR developed severe respiratory distress after being bled from the anterior vena cava 13 DPI and was euthanatized. The surviving pigs remained in good condition and grew.

Two DPI, 3 of 10 pigs had fevers over 40°C (104°F). Four DPI, all but one pig had fevers ranging from 40.8°C (105.4°F) to 41.7°C (107°F). The average highest fever was 41.4°C (106.5°F) 6 DPI. The pigs that died on
13 and 22 DPI were febrile throughout the illness. The temperatures of all pigs surviving returned to the normal range except for pigs 1DR, 8DR, 4DR and 10DR, which had temperatures of 40.4, 40.1, 40.8 and 40.2°C respectively, at 106 and 107 DPI.

Total leukocyte counts decreased from an average count of 19,270/mm³ of blood on day 0 to an average count of 14,470/mm³ of blood 6 DPI.

African swine fever virus was isolated in swine buffy coat cultures from the blood of all pigs 2 DPI. Virus was not isolated from the blood of pig 3DR the day before death. The duration of detectable viremia in surviving pigs varied from 18 to 30 DPI. Hemadsorption results are shown in table 2.

The relationships of daily temperatures, total leukocyte counts, and virus isolations from the blood for 2 pigs typical of those inoculated with Dominican Republic ASF virus isolate are shown in figures 5 and 6.

Lisbon 60 Isolate — Pathology

The gross lesions were similar in all Lisbon 60 pigs. The severity of skin reddening varied; 2 pigs had very reddened skin on the extremities of the ears, limbs and tail and scattered areas of the body. The spleens were 4 or more times normal size, dark reddish black, and friable. Visceral lymph nodes, particularly the gastrohepatic and renal lymph nodes, were enlarged and reddish-black. Peripheral lymph nodes had varying degrees of subcapsular reddening. Several pigs had petechial hemorrhages on the serosal surfaces of the cecum and esophageal area of the stomach, numerous petechial hemorrhages in the renal cortex, extensive hemorrhage around the renal pelvis and scattered petechial hemorrhages in mucosa of the urinary bladder. The wall of the gallbladder in a few pigs was edematous.

Histologic lesions were similar in all the Lisbon 60 pigs. Visceral lymph nodes, particularly the gastrohepatic and renal nodes, had extensive necrosis of reticular cells in the cellular stroma; the necrotic areas were filled with erythrocytes. Peripheral lymph nodes had extensive reticular cell necrosis in the subcapsular and paratrabecular areas, but the amount of hemorrhage was much less into these nodes than into the hepatogastric and renal lymph nodes. The tonsils had numerous necrotic reticular cells in the cellular stroma. The spleen had extensive necrosis of reticular cells in the red pulp, and the area was filled with erythrocytes. Periarterial sheaths had a lymphocyte depletion and necrotic reticular cells. In the lungs, there was an interlobular edema and fluid and a few macrophages in the alveoli. In the liver, scattered Kupffer cells were necrotic, and interlobular septa were widened by connective tissue, appeared edematous and had an inflammatory cell infiltrate and pyknotic nuclei. Scattered hepatic-lobules were congested, and in one pig there were scattered foci of necrotic hepatocytes. One brain section of each of 2 pigs had a vessel with lymphoreticular cell cuffing. Brain sections of pig 7L had marked lymphoreticular cell proliferation around vessels and in the
meninges and choroid plexus.

Brazilian Isolate — Pathology

Pig 9B was found dead 11 DPI. Grossly, the spleen was slightly enlarged and had a normal color and consistency. The kidney had a moderate number of petechial hemorrhages in the cortex. The mucosa of the urinary bladder had scattered petechial hemorrhages. The submandibular, bronchial and mesenteric lymph nodes were a little enlarged and reddened. The gastrohepatic lymph nodes were twice normal size and reddish black.

Histologically, the splenic red pulp was full of erythrocytes, and some of the reticular cells were very large. At the periphery of several periarterial lymphoid sheaths, there were small foci of necrotic cells. The kidneys and urinary bladder had numerous petechial hemorrhages. The submandibular lymph nodes had a few erythrocytes, and the bronchial lymph nodes had numerous erythrocytes in the subcapsular sinus. The gastrohepatic lymph nodes had numerous erythrocytes in the subcapsular and paratrabecular sinuses. One small area of lung had neutrophilic leukocytes in the bronchi, fluid and inflammatory cells and in the alveoli, thrombosed arterials and small areas of necrosis in 2 lobules. The heart had severe myocardial congestion and areas of myocardial and sub-endocardial hemorrhage. One section of myocardium had erythrocytes around large blood vessels and between myocardial fibers and rowing of myocardial nuclei (muscular regeneration).

Pig 8B was found dead 15 DPI. Grossly, the spleen was slightly enlarged and had a normal color and consistency. The gastrohepatic, mesenteric and internal iliac lymph nodes had peripheral reddening. The stomach contained a large blood clot, and there was an ulcer in the esophageal area. The large intestine contained a large amount of soft reddish brown material (blood). This pig apparently died from a hemorrhaging gastric ulcer.

Histologically, the internal iliac, gastrohepatic, bronchial and submandibular lymph nodes had small areas of hemorrhage. One prefemoral lymph node had 2 small necrotic areas. The splenic red pulp appeared to have a decreased number of reticular cells, and an occasional cell had a pyknotic nucleus. The periarterial sheaths were depleted of lymphocytes. Renal sections had petechial hemorrhages and small lymphocytic foci in the cortex. The lung was congested and edematous. The thymus had lost small lymphocytes.

Pig 4B was euthanatized 24 DPI. During the previous 24 hours, its temperature had dropped from 40.6°C (105°F) to 38.9°C (102°F), and the pig was ataxic. Grossly, the spleen, kidneys and gallbladder were normal. The gastrohepatic lymph nodes were enlarged and whitish. The renal, mesenteric and internal iliac lymph nodes had a subcapsular reddening.
Areas of the parietal and visceral pleura were thickened and had a shaggy, reddish appearance (pleuritis). The lower half of the right apical lobe and the tip of the right cardiac lobes of the lungs were reddish purple and consolidated. Scattered lobules in the diaphragmatic lobes were raised and whitish. Scattered in the lungs were firm nodules about 1 cm in diameter. These nodules appeared to have a whitish capsule and caseous necrotic center.

Histologically, there was subcapsular hemorrhage in the renal lymph nodes. The mesenteric lymph nodes had mild peripheral congestion and edema. The bronchial lymph nodes had fairly large foci of neutrophilic leukocytes throughout the section. The gastrohepatic lymph node had hypertrophy and hyperplasia of reticular cells in the sinuses and a lymphoreticular cell infiltrate in the connective tissue trabeculae. Thymus had walled-off necrotic areas. In the lung, there were peribronchial and periarterial lymphocytic hyperplasia, neutrophilic leukocytes in the bronchial lumens, macrophages and, in some lobules, neutrophilic leukocytes in the alveoli. The nodules observed grossly in the lung had a connective tissue capsule lined by granulomatous cells, some of which had bright eosinophilic material in the cytoplasm. The center of the nodule was necrotic granulomatous tissue in which cell outlines were well preserved. The necrotic center of another nodule had a well-preserved alveolar histology. The partial and visceral pleura were thickened, and there was fibrin on the surface. The renal cortex contained a few small lymphoreticular cell foci.

Dominican Republic Isolate — Pathology

Pig 7DR was moribund 13 DPI. Grossly, the peripheral, renal and internal iliac lymph nodes had subcapsular reddening. The gastrohepatic lymph node was very enlarged and dark red, and adjacent tissue was edematous. Both kidneys had numerous, large petechial hemorrhages in the cortex; the right kidney also had severe peripelvic hemorrhage. The gallbladder was distended, the neck of the bladder was edematous and hemorrhagic and there was edema around the bile duct. The serosa over the esophageal area of the stomach contained petechial hemorrhages. There were 2 petechial hemorrhages in the urinary bladder. Areas of lung were atelectatic.

Histologically, there was mild hemorrhage in the subcapsular and paratrabecular areas of mesenteric, internal iliac, prefemoral, submandibular and inguinal lymph nodes. The renal and gastrohepatic lymph nodes had extensive subcapsular and paratrabecular hemorrhage. Adjacent to the hemorrhage in the gastrohepatic lymph nodes were numerous eosinophilic leukocytes. The gross hemorrhage in the kidney was confirmed histologically. Scattered renal tubules contained proteinaceous fluid, and some of these tubules had degenerated and necrotic epithelial cells. The connective tissue around the gallbladder was edematous and hemorrhagic. In the hepatic lobules, Kupffer cells were enlarged, and there were foci of congestion. The portal areas had lym-
phocytic and eosinophilic leukocyte infiltrates, and there were areas of hemorrhage in the interlobular septa. Sections of lung had thickened alveolar septa, macrophages in the alveoli and atelectatic lobules.

Pig 6DR was killed after developing respiratory distress following bleeding 13 DPI. Grossly, there was a large accumulation of blood in the thoracic inlet. The lungs had scattered petechial hemorrhages, and the ventral part of the left cardiac lobe was consolidated. The spleen was normal. The kidney had a few scatter petechial hemorrhages in the cortex. The gastrohepatic lymph node had a pronounced peripheral reddening. The renal lymph nodes had mild peripheral reddening. Other lymph nodes were normal.

Histologically, the lungs had several small hemorrhagic foci and thickened alveolar septa. The gastrohepatic lymph node had an accumulation of fluid in the supcapsular sinus, mild peripheral hemorrhage into the cellular stroma and more pronounced hemorrhage in lymph nodules. The renal, bronchial and mesenteric lymph nodes had a small area of peripheral hemorrhage. The thymic cortex contained a reduced number of lymphocytes.

Pig 3DR died 22 DPI. Grossly the skin was slightly reddened. The conjunctiva was reddened, and there was dried secretion in the medial canthi. The submandibular lymph nodes were slightly enlarged and reddened, and the mesenteric lymph nodes were slightly reddened. The gastrohepatic lymph node was dark red and twice normal size, and the cut surface had peripheral hemorrhage. The left ventricle had paintbrush hemorrhages, and there were subendocardial petechial hemorrhages below the mitral valve. The spleen was normal size. The kidneys had scattered petechial hemorrhages in the cortex.

Histologically, blood vessels in most tissues contained many leukocytes. The hepatic sinusoids were dilated and contained many leukocytes, and the spaces of Disse were very wide. The spleen had extensive hematopoiesis. The bronchial lymph node had mild peripheral hemorrhage and foci of fibrinoid material.

DISCUSSION

The clinical disease in pigs inoculated intranasally and orally with the Lisbon 60, Brazil or Dominican Republic isolate of ASF virus was similar during the first 6 DPI. The pigs were alert and ate in spite of high fevers. After 6 DPI, the clinical differences among the 3 groups were: 1) a short clinical course and a near 100% mortality by 8 DPI in pigs inoculated with the Lisbon 60 isolate but a longer clinical course and lower mortality in pigs inoculated with the Brazilian (4/9) or Dominican Republic (2/10) isolate; 2) a more pronounced leukopenia in pigs inoculated with the Brazilian or Dominican Republic isolate than in pigs inoculated with the Lisbon 60 isolate.

The gross lesions in pigs inoculated with the Lisbon 60 isolates were similar to those described for highly virulent ASF infections4,5,6: enlarged
reddish black, friable spleen and enlarged reddish black visceral lymph nodes. Microscopically, these lesions resulted from extensive necrosis of reticular cells in these tissues and filling of the space with erythrocytes.

Grossly, the lesions in spleens and lymph nodes in the first pig that died after inoculation with either the Brazilian or Dominican Republic isolate were suggestive of ASF but were not the severely enlarged and hemorrhagic lesions associated with the classical or highly virulent ASF. Microscopically, the reticular cell necrosis was much less severe in pigs that died after inoculation with the Brazilian or Dominican Republic ASF viral isolate than in pigs inoculated with the Lisbon 60 viral isolate. Pigs that died after a longer course of illness had gross and microscopic lesions even less suggestive of ASF.

The low mortality and the comparatively less severe gross and microscopic lesions caused by the ASF viruses from Brazil and the Dominican Republic confirmed that these isolates were of low virulence. Low virulence rather than highly virulent ASF virus poses a much greater problem to countries with ASF-infected swine and greater threat to countries free of ASF for several reasons:

1. Because of the low mortality and reduced incidence of classical ASF lesions, the disease may escape detection for some time before it is recognized.

2. The development of antibody during the longer course of infection will likely interfere with direct immunofluorescent antibody and hemadsorption diagnostic tests.

3. The disease may become widespread because clinically recovered animals or the meat products produced from them can be sources of virus.

Laboratory diagnosis of low-virulence ASF infection is complicated by low mortality and development of antibody, so a battery of tests should be used for detection of antigen and antibody. Of all tests for viral detection inoculation of susceptible pigs is one of the most sensitive. Specimens from a suspected case of ASF in a virgin area should be inoculated into susceptible pigs and pigs immunized against hog cholera.

In conclusion, ASF should now be defined as a highly infectious, febrile disease of swine which may occur in any of 3 pathologic forms, depending on the strain (virulence) of the infecting virus. 1) An acute form with nearly 100% mortality caused by a highly virulent virus. This form is characterized by a high fever, short course (7-9 days), marked splenic enlargement and extensive hemorrhage in visceral lymph nodes and kidneys. 2) A subacute or chronic form caused by a less virulent virus. Pigs that die during the early phase of the disease have lesions of an acute disease. Pigs that survive have recurrent febrile periods over a period of weeks or months and then die. The prominent lesions consist of any of the following: enlarged lymph nodes that may contain necrotic areas, pleuritis, necrotic areas in lungs, pericarditis and arthritis. 3) An
MEBUS AND DARDIRI

acute disease with low mortality caused by a low-virulence virus. Clinically, the early stage of this third form is similar to that of the acute form of ASF, but then 60-80% or more of these pigs recover. Pigs that die have a longer disease course (11-22 days), and the lesions consist of a normal or slightly enlarged spleen, mild subcapsular reddening (hemorrhage) in lymph nodes, particularly visceral lymph nodes, and a few petechial hemorrhages in the kidneys.

**TABLE 1**: African swine fever viral titers in blood of pigs inoculated intranasally and orally with the Brazilian (B) isolate

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*DPI = Days post inoculations. ** = Death. N = No virus isolated. S = Virus isolated on subculture.

Number is the logarithm of the highest positive 10-fold dilution of blood. Highest dilution tested was 10⁻⁴.
TABLE 2: African swine fever viral titers in blood in pigs inoculated intranasally and orally with Dominican Republic (DR) isolate

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*DP1 = Days post inoculation. ** = Death.
N= No virus isolated. ND = Not done.
S= Virus isolated on subculture.
Number is the logarithm of the highest positive 10-fold dilution of blood. Highest dilution tested was 10^-9.
Fig. 1: Relationships of daily temperatures, total leukocyte counts (WBC) and virus isolations from the blood of pig 1L, inoculated with Lisbon 60 African swine fever viral isolate. Note the inverse relationship of temperature and total leukocyte counts.

Fig. 2: Relationships of daily temperatures, total leukocyte counts (WBC) and virus isolations from the blood of pig 4L inoculated with Lisbon 60 African swine fever viral isolate. Note the rapid drop in temperature during the last 24 hours of life.
Fig. 3: Relationships of daily temperature, total leukocyte counts (WBC) and virus isolations from the blood of pig 9B inoculated with Brazilian African swine fever viral isolate. Pig died.

Fig. 4: Relationships of daily temperatures, total leukocyte counts (WBC), serum antibody and virus isolations from the blood of pig 6B inoculated with Brazilian African swine fever viral isolate. Pig recovered. Note the inverse relationship of temperature and total leukocyte count.
Fig. 6: Relationships of daily temperatures, total leukocyte counts (WBC) and virus isolations from the blood of pig 7DR inoculated with Dominican Republic African swine fever viral isolate. Pig died.

Fig. 5: Relationships of daily temperatures, total leukocyte counts (WBC) and virus isolations from the blood of pig 9DR inoculated with Dominican Republic African swine fever viral isolate. Pig recovered.
REFERENCES


VISCEROTROPIC VELOGENIC NEWCASTLE DISEASE
California - Florida 1979

D. King, D.V.M.; W. Cowart, D.V.M.
D. Johnson, D.V.M.; G. Slonka, D.V.M.

There have been 16 detected introductions of VVND into this country since the California outbreak in 1971-72. Each has been contained and eradicated before spread to commercial poultry occurred except in El Paso, in February 1974, when 188,000 layers were involved. Surveillance for the disease in these high risk areas must continue at least at the present level.

<table>
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CALIFORNIA 1979

On February 20, 1979, exotic Newcastle disease was confirmed at the National Veterinary Services Laboratories (NVSL), Ames, Iowa, from specimens submitted from a citron crested cockatoo at Stanton, Orange County, California. The virus was originally isolated at the California Department of Agriculture Veterinary Laboratory Services, San Gabriel, California, which was subsequently submitted to NVSL for characterization. The disease was traced to a holding facility used to house birds as they are released from a privately owned import quarantine station, both establishments under the same ownership and management.

After Newcastle disease was found, sales and shipments of birds from the holding facility were traced and evaluated. These and other birds on the premises were sampled by swabbing twice or by both swabbing and sampling at necropsy. Specimens were submitted to an approved diagnostic laboratory for testing. As a result, birds on 10 commercial and 7 privately owned premises in Los Angeles, Orange, San Bernadino, and
Riverside Counties, California were diagnosed positive for exotic Newcastle disease.

Federal and State quarantines were placed on the premises and in some instances the area that surrounded them. Epidemiological tracing and sampling led to positive birds on one commercial and one privately owned premises in Las Vegas, Nevada. Birds on five premises in California and one in Arizona were also depopulated as exposed. All birds on positive and exposed premises were appraised and euthanized and the premises were cleaned and disinfected. In addition to California, movements of birds from infected premises were traced to Arizona, Illinois, Minnesota, Nevada, Oregon, Texas, Utah, and Washington. Except as already indicated, birds evaluated on the receiving premises were negative.

**FLORIDA 1979**

On March 24, 1979, another case of exotic Newcastle disease was confirmed in the holding facility used to house birds after release from a USDA approved privately owned import quarantine station at Miami, Florida. Birds shipped from this holding facility between January 1, 1979, the date the facility was epidemiologically determined to have become infected, and March 20, 1979, the date the State of Florida quarantine was imposed, were traced and evaluated. A total of 109 shipments were made from this infected premises to 20 States and the Commonwealth of Puerto Rico. As a result of these tracings and evaluations, positive cases were disclosed in Illinois, Michigan, North Carolina, Ohio, and Texas. Two hundred and sixty-five shipments were made from the infected premises in North Carolina to 14 States. All receiving flocks were negative for exotic Newcastle disease. Almost one thousand shipments were traced and sampled as a result of movements of birds from the infected lot in Florida.

No commercial poultry were involved in either the California or Florida outbreak. Since there is no known treatment for this disease, the infected caged birds involved were appraised and humanely destroyed and the premises were cleaned and disinfected.

**UNITED STATES — 1979**

The disease initially involved holding facilities under the same management of privately owned bird import quarantine stations. The disease spread from these facilities to caged bird wholesalers, jobbers, pet shops, aviaries and private homes.

The Western and Southeast Regional Emergency Animal Disease Eradication Organizations were activated and headquartered at Santa Ana, California, and Miami Springs, Florida, respectively to combat the outbreak. The total cost to eliminate these outbreaks was approximately 1.8 million dollars.
During fiscal year 1978 domestic surveillance of poultry did not reveal any cases of exotic Newcastle disease in poultry in the continental United States. Increased effectiveness in border and port enforcement and alert surveillance by field personnel aided in the effort. Two infections were detected in caged pet birds which were recently introduced into the United States. One of these infections was in birds entering the United States illegally and detected by United States Customs Service officials and other infection was introduced through the pet birds entered into the United States and undergoing a quarantine period for 30 days at the premises of destination. Prompt detection by Veterinary Services personnel and identification by the NVSL prevented the spread of these outbreaks to poultry and other avian species.

SUMMARY

Since 1971-72, when an extensive task force was required to contain and eradicate the disease introduced in Southern California, exotic Newcastle disease has entered this country at least on 16 occasions. Other than an outbreak in El Paso (February-June) in 1974, when a 188,000 hen layer operation was infected, each introduction has been detected and eliminated before it spread to commercial poultry. The disease is presently very active throughout the world as determined by the increased number of infected lots detected in USDA approved quarantine stations when commercial pet birds are offered for entry.

A vital part of the surveillance system is the effort by Veterinary Services to increase awareness of VVND (or exotic NCD) to the people dealing with exotic pet birds, especially the hazards and risks of buying birds that have been smuggled.

Puerto Rico currently is under Federal quarantine for exotic Newcastle disease. All passenger baggage is inspected on all air flights from Puerto Rico to the United States to remove live poultry and poultry products. In addition, all baggage is inspected at the San Juan airport on flights originating in the Virgin Islands destined for the United States.

On June 4, 1979, a surveillance program to determine the status of Puerto Rico in regard to exotic Newcastle disease began. Surveillance for the presence of VVND has been completed. The data are being prepared and summarized. No evidence of VVND was discovered as a result of analysis of 4,458 blood sera and cloacal swabs which were collected from layers, broilers, backyard birds and game chickens.
CONTAGIOUS EQUINE METRITIS: DEVELOPMENT OF 
ENZYME-LINKED IMMUNOSORBENT ASSAY TO DETECT 
ANTIBODY TO CONTAGIOUS EQUINE METRITIS ORGANISM

S. P. Sahu BVSc, PhD., F. M. Hamdy DVM, PhD., 
and A.H. Dardiri DVM, PhD.

From the Plum Island Animal Disease Center 
Agricultural Research, Science and Education Administration 
United States Department of Agriculture 
Post Office, Box 848, Greenport, New York 11994

SUMMARY

Enzyme-linked immunosorbent assay (ELISA) was adapted to detect 
antibody to Contagious Equine Metritis (CEM) organism. ELISA was 
compared with plate agglutination (PA), tube agglutination (TA), and 
antiglobulin (AG) tests. Antibody titers were higher with the ELISA 
than with other tests. An unknown serum at 1:20 dilution and giving an 
optical density (OD) (at 405 nm) 2.5 times the average OD of controls 
should be considered positive for CEM by ELISA.

INTRODUCTION

Induction of antibodies in mares after infection with Contagious 
Equine Metritis organism (CEMO) has been reported.\textsuperscript{1} Agglutination and 
antiglobulin tests were used to demonstrate the presence of 
agglutinating antibodies in sera of mares infected with CEMO.\textsuperscript{2,3,4} A 
complement-fixation and passive hemagglutination tests were also 
developed and were reported to be useful in detecting carrier state of 
mares.\textsuperscript{5,6} However, reports from the field showed that these tests failed 
to pick up carrier mares.\textsuperscript{7,8} 

This report deals with the development of an enzyme-linked im-
munosorbent assay (ELISA) for detection of antibodies to CEMO and 
compares its efficacy with plate agglutination (PA), tube agglutination 
(TA), and antiglobulin (AG) tests.

MATERIALS AND METHODS

\textit{Bacterial culture and antigens}

A culture of CEMO was obtained,\textsuperscript{9} and bacteria were cultured on 
chocolate plates made from Eugonagar (BBL, Cockeysville, Md.).\textsuperscript{9} The 
CEMO colonies were scraped from plates and suspended in PBS pH 7.2. 
The suspension was cultured for possible aerobic contamination. Mer-
thiolate was added at 1:10,000 concentration to the bacterial suspension 
and it was stored at 4 C. Antigen for the PA test was standardized to 7th 
tube of McFarland nephelometer with PBS pH 7.2 containing 1:10,000
Antigen used for the TA test was heated at 60°C for 1 hour and standardized to 5th tube of McFarland nephelometer. Antigen for the ELISA was first shaken vigorously for 3 to 4 min. and then it was centrifuged at 1000 x g for 15 min. The supernatant fluid was saved. The sediment was suspended in 2 ml of PBS pH 7.2 and sonicated with a semimicro tip at 40 watt for 90 sec. at 30 sec. pulse. During sonication, the tube with bacterial suspension was kept in ice-alcohol mixture to keep temperature of the bacterial suspension cold. The sonicated material was centrifuged at 1400 x g for 15 min. and the supernatant fluid was mixed with previously saved supernatant and labeled as "soluble antigen." The protein content of the soluble antigen was determined. About 25 to 30 mg of protein was chromatographed on Sephadex G-100 Column (15 mm x 1½ mm). One ml fractions were collected and optical density (OD) of each fraction was determined at 280 nm. Fractions showing high optical density at 280 nm were pooled and frozen at -60°C to be used as antigen for the ELISA.

Source of Negative, Hyperimmune, and Convalescent Sera

Sera collected before inoculation of pony mares were used as negative sera. Hyperimmune sera were prepared by immunizing pony mares by intravenous or subcutaneous injection of killed CEMO whole bacterial suspension in PBS pH 7.2 (60-65 mg of bacterial protein) as described for bacterial antigen. A 2nd vaccination was given 15 days after the 1st. Sera collected 10 to 15 days after the 2nd vaccination were used as hyperimmune sera. Sera collected at various times after intrauterine inoculation of pony mares with live cultures of CEMO were designated as convalescent sera.

ELISA

The procedure was essentially the same as previously described. Crystalline alkaline phosphatase precipitated with 3.2 M of ammonium sulfate (Type VII, Sigma Chemical Co., St. Louis, MO) was conjugated with rabbit antiequine IgG globulin (Miles Biochemicals, Elkhart, IN) at a concentration of 2 mg of antibody and 5 mg of alkaline phosphatase enzyme. The globulin-alkaline phosphatase mixture was stirred gently at room temperature (24°C) for 1 hour followed by dialysis in PBS pH 7.2 until (NH₄)₂ SO₄ dialyzed out. Glutaraldehyde was added to the enzyme-globulin mixture to a final concentration of 0.2% and allowed to mix for 2 hours at room temperature; 4 to 5 changes of dialysis 36 to 38 hours in PBS pH 7.2 at 4°C followed. Glutaraldehyde-enzyme globulin mixture was finally dialyzed 12 to 18 hours in Tris-HCl pH 8.0 The alkaline phosphatase conjugated rabbit antiequine IgG globulin was diluted in Tris-HCl buffer supplemented with bovine serum albumin at a final concentration of 1% and was stored at 4°C.

Reagents and Buffers for ELISA

Carbonate-bicarbonate (Na₂CO₃·H₂O-1·59 g, NaHCO₃-2·93 g, sodium
azide 0.2 g, distilled water 1000 ml) buffer pH 9.6 was used to dilute antigen for coating microELISA U-plates. The PBS (0.01M) containing 0.05% Tween-20, 1% bovine albumin (Fraction V) and sodium azide 0.2 g/L (PBS:T20:BA) pH 7.4 was used for washing microtiter plates as well as for diluting of sera and conjugate. Diethanolamine buffer (Fisher Scientific, Fairlawn, NJ) containing 97 ml diethanolamine, 100 μg of MgCl₂·6 H₂O, and 800 ml of deionized distilled water pH 9.8 was used to dissolve phosphatase substrate (Sigma Chemical Co., St. Louis, MO).

Coating of soluble antigen to microtiter plates

MicroELISA U-plates (Dynatech Lab Inc., Alexandria, VA) with 96 wells were used. Antigen was diluted in 0.02 M of carbonate-bicarbonate buffer pH 9.6 containing 200 μg/ml of sodium azide. A 0.1-ml sample was put in the inner 60 wells of each plates. The outer wells received a similar volume of buffer. The plates were incubated at 4 C for 18 to 20 hours. The plates may be kept for several weeks at 4 C until use. Before use, all wells were washed 3 times with 0.25 ml of PBS:T20:BA buffer.

Test

The procedure for ELISA has been described. Multichannel pipettes (Flow Lab. Inc., McLean, VA) were used to dispense the reagents. In brief, microELISA U-plates coated with partially purified CEMO antigen was washed 3 times with PBS:T20:BA buffer. The plates were inverted and tapped gently several times against a thick cotton towel to remove traces of buffer. The sera were diluted in PBS:T20:BA buffer 1:10 to 1:20,480, and 0.1 ml of each dilution of the serum was dispensed in wells. Each sample was run in triplicate. The plates were covered with a plastic lid and incubated at 37 C for 30 min. At the end of the incubation period, the wells were washed 3 times with 0.25 ml of PBS:T20:BA buffer. The buffer was aspirated from the wells and the excess buffer was removed by striking plates gently several times on a thick cotton towel with wells facing down, and 0.1 ml of 1:750 dilution of rabbit-antiequine IgG conjugated with alkaline phosphatase was added to each well. This dilution was predetermined. It was covered with a plastic lid and incubated at 37 C for 1 hour. The plates were washed again 3 times with PBS:T20:BS buffer. Phosphatase-substrate, p-nitrophenyl phosphate disodium ("Sigma 104", Sigma Chemical Co., St. Louis, MO), in a concentration of 1 μg/ml was made in 10% diethanolamine buffer pH 9.8 containing MgCl₂, and 0.15 ml substrate was added to all wells. The plates were incubated at room temperature for 30 minutes. At the end of the incubation period, 0.15 ml of 3M NaOH was added to each well to stop the reaction. Samples from each of 3 wells were pooled, and OD was determined at 405 nm in a spectrophotometer (Gilford, Model 250). The following controls were used in the experiment with antigen: Control buffer - PBS:T20:BS, 3 known CEM negative horse sera and 1 hyperimmune serum.

The spectrophotometer (Gilford 250) was set to zero reading of control buffer, and then OD was determined on all samples. The following for-
mula was used to determine a positive reaction at 1:20 dilution:

\[
\text{OD of Unknown} \quad \frac{\text{average of OD of negative sera}}{2.5}
\]

**Plate Agglutination Test**

The PA test was used to measure the presence of antibody in the mare's sera. The sera were diluted two fold in physiological saline pH 7.2 starting from 1:2 to 1:2048. A 0.03 ml aliquot of each dilution was mixed with 0.03 ml of antigen on a glass plate and rotated constantly in a circular motion for 9 min. at room temperature before it was read for agglutination reaction. The degree of agglutination observed was read as follows: -, +, ++, +++ or ++++ representing no reaction, 25%; 50%; 75% and 100% agglutination respectively. The highest dilution of serum giving 50% agglutination reaction or better was considered the titer of the serum.

**Tube agglutination test**

Heated antigen (60 C for one hour) standardized to 5th tube of McFarland nephelometer and PBS buffer pH 7.2 containing 0.5% phenol was used. The sera were diluted two fold in PBS-phenol buffer pH 7.2. The 1 ml antigen-antiserum mixture (equal volume) was incubated for 1 hour in 37 C waterbath and then for 18 hours in 37 C incubator.

**Antiglobulin Test**

After the agglutination test was completed, the tubes were centrifuged at 1400 g for 20 minutes and pellet was resuspended in 3 ml of PBS pH 7.2. This suspension was centrifuged again. The whole process was repeated 3 times. The pellet was suspended in 0.2 ml of 0.5% phenol saline, and 0.2 ml of a 1:150 dilution of rabbit anti-horse gamma globulin was added. The mixture was incubated for 1 hour in waterbath and for 18 hours in 37 C incubator.

**RESULTS**

**Standardization of alkaline phosphatase conjugated antiequine IgG and CEMO antigen for ELISA**

When optical density of the sera from noninfected and immunized pony mares were compared, a 1:750 dilution of alkaline phosphatase conjugated antiequine IgG and 11 µg of protein of CEMO per well were found to be best suited for the experiments (Fig. 1). This concentration of antigen and this conjugate were used throughout the experiment.

**Comparison of ELISA, plate agglutination, tube agglutination and antiglobulin tests**

1. Sera from noninfected pony mares. No agglutination reaction was observed when sera were mixed with CEMO antigen; however, antiglobulin titer on these sera varied from < 1:10 to 1:20 (Table 1). At 1:10 dilution, the OD varied from 0.189 to 0.086 but at 1:20, other than 2 sera (Nos. 2 and 3), all sera had a reading under 0.087.
2. Sera from hyperimmunized pony mares. The PA titers were 1:20 to 1:640, the TA titers were 1:80 to 1:1280, the AG titers were 1:80 to 1:2560, and the titers for ELISA were varied from 1:160 to 1:5120 (Table 2).

3. Sera from infected pony mares. The PA and the TA titers varied from negative to 1:20, the AG titers varied from < 1:10 to 1:80, and ELISA titers varied from 1:10 to > 1:80 (Table 3).

DISCUSSION

Standardization of any immunological test is extremely necessary so that results may be uniform. At 1:10 dilution, 10 out of 12 preinfected sera had OD at 405 nm which varied from 0.093 to 0.189; but at 1:20 dilution other than sera Nos. 2 and 3, sera OD decreased considerably (Table 1). Also, the difference in OD value of preinfected sera, especially Nos. 2 to 7 and in that sera from infected mares (Nos. 13 and 14) at 1:10 dilution is less than 2. But at 1:20 dilution, other than sera Nos. 2 and 3, the difference is greater than 2.5. Therefore, the ELISA on all noninfected and convalescent sera were run at 1:20 dilution. Even at 1:20 dilution, OD reading in sera Nos. 2 and 3 was much higher than other preinfected sera (Table 1). It appeared very necessary to include 3 or more control uninfected sera in the test. Therefore, an unknown serum was considered positive if OD of unknown sera divided by average OD of 3 or more uninfected sera was equal to or greater than 2.5. This value (≥ 2.5) helped in rapid screening of unknown sera. A titer was run on each serum that gave a value of ≥ 2.5 at 1:20 dilution. AG titer of 1:20 or less was considered normal, as has been considered by other workers.2

A comparison of immunological tests for hyperimmune sera showed the ELISA test to be more sensitive than PA, TA and AG tests (Tables 2 and 3). A 2-to-64-fold higher titer was obtained by ELISA than PA and TG tests for hyperimmune sera (Table 2). The ELISA test detected 13 of 15 sera has positive titers (1:20 and higher) for CEMO and 8 of them had titer greater than 1:80 (Table 3). By the AG test, only 6 had titers of 1:80; and by the PA test, 12 samples had titers of 1:20 or less. The fact that 4 of the samples that were negative by the PA, the TA and the AG tests had higher titer by ELISA indicates its sensitivity. In addition, ELISA required only about 4 hours to obtain a result, the TA required 24 hours, and the AG test required 48 hours.
Figure 1 - Standardization of concentrated of alkaline phosphatase conjugated rabbit antiequine IgG (conjugate) and Contagious Equine Metritis Antigen.
TABLE 1 - Comparison of Contagious Equine Metritis negative, immune and convalescent sera in the ELISA and antiglobulin test

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TABLE 2 - A comparison of plate agglutination (PA), tube agglutination (TA), antiglobulin (AG) tests and ELISA on sera from Contagious Equine Metritis organism immunized pony mares.

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<td>6</td>
<td>20</td>
<td>80</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td>7</td>
<td>640</td>
<td>640</td>
<td>2560</td>
<td>1280</td>
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<td>8</td>
<td>20</td>
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<td>640</td>
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<td>9</td>
<td>80</td>
<td>160</td>
<td>160</td>
<td>2560</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>160</td>
<td>160</td>
<td>2560</td>
</tr>
</tbody>
</table>

*Titer = Optical Density (OD) of dilution of hyperimmune sera at 405 nm > 2.5
Average OD of 3 negative control sera at 1:20 dilution
TABLE 3 - A comparison of plate agglutination (PA), tube agglutination (TA), antiglobulin (AG) tests and ELISA on sera from experimentally Contagious Equine Metritis organism infected pony mares.

<table>
<thead>
<tr>
<th>Sera Number</th>
<th>OD at 405 nm</th>
<th>TITER</th>
<th>ELISA*</th>
<th>TITER</th>
<th>TITER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:10</td>
<td>1:20</td>
<td>1:40</td>
<td>1:80</td>
<td>PA test</td>
</tr>
<tr>
<td>1</td>
<td>0.231</td>
<td>0.148</td>
<td>0.078</td>
<td>0.056</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>0.231</td>
<td>0.126</td>
<td>0.088</td>
<td>0.055</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>0.232</td>
<td>0.188</td>
<td>0.064</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>0.254</td>
<td>0.207</td>
<td>0.109</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>0.215</td>
<td>0.192</td>
<td>&gt;80</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>0.223</td>
<td>0.147</td>
<td>0.098</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>0.338</td>
<td>0.270</td>
<td>0.200</td>
<td>&gt;80</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>0.309</td>
<td>0.265</td>
<td>0.192</td>
<td>&gt;80</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>0.421</td>
<td>0.321</td>
<td>0.218</td>
<td>&gt;80</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>0.317</td>
<td>0.206</td>
<td>0.163</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.282</td>
<td>&gt;80</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>0.418</td>
<td>0.321</td>
<td>0.218</td>
<td>&gt;80</td>
</tr>
<tr>
<td>13</td>
<td>ND</td>
<td>0.329</td>
<td>0.210</td>
<td>0.172</td>
<td>80</td>
</tr>
<tr>
<td>14</td>
<td>ND</td>
<td>0.374</td>
<td>0.321</td>
<td>0.296</td>
<td>&gt;80</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>0.257</td>
<td>0.211</td>
<td>0.202</td>
<td>&gt;80</td>
</tr>
</tbody>
</table>

* Average optical density (OD) at 405 nm of all 3 negative sera at 1:20 dilution were: 0.068

† Highest serum dilution giving an OD of >= 0.0170 which is >= 2.5 x average of control serums.

- = negative; U = undiluted; ND = not done.
ACKNOWLEDGMENT

The authors acknowledge Daniel A. Zavaski for technical assistance.

REFERENCES


IMMUNE RESPONSE OF STEERS, GOATS AND SHEEP TO INACTIVATED RIFT VALLEY FEVER VACCINE


Plum Island Animal Disease Center, Science and Education Administration, United States Department of Agriculture, P.O. Box 848, Greenport, NY 11994

and

*United States Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701

SUMMARY

Three groups of 10 steers, 5 groups of 4 goats, and 5 groups of 10 sheep each were inoculated with five-fold dilutions of a formalin-inactivated Rift Valley Fever (RVF) vaccine prepared in tissue culture for human use. The serological response of the steers and goats were assayed for the 30-day trial period. The 50 sheep were reinoculated with undilute vaccine, their serological response was assayed and their immunogenic response evaluated by challenge inoculation with virulent RFV virus. Virus neutralization titers as determined by the plaque reduction tests were generally low in the steers and sheep and much higher in the goats. The revaccinated sheep challenged with virulent RFV virus developed no thermal response, detectable viremia or clinical illness. Under the conditions of this controlled experiment, the inactivated vaccine produced solid immunity in 100% of the sheep.

INTRODUCTION

Rift Valley fever (RVF) occurs naturally only in Africa and is endemic in the Rift Valley in Kenya and South Africa. It is an arthropod-borne virus disease that causes epizootics among sheep, goats, and cattle; and man is also highly susceptible. The most severe epidemic of RFV so far experienced in southern Africa was in 1975. In October and November of 1977, an extensive epidemic occurred for the first time in Egypt along the Nile Delta; thousands of people were affected and 60-120 deaths with hemorrhage and jaundice were reported.

Vaccines have been developed for prevention of RVF in domestic animals with varying immunogenic properties. The attenuated vaccines cause abortion when used in pregnant animals. Live neurotropic virus vaccines have been used in veterinary medicine but have produced untoward reactions in animals; they would be very dangerous to use in humans. Inactivated vaccines that are safe and immunogenic have been

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very effective in protecting many laboratory workers from infection of RVF.\(^5\) An inactivated vaccine produced for the United States Army Medical Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland, for human use has not been tested in domestic animals. This paper discusses the immunogenic response of steers, goats and sheep inoculated with the USAMRIID vaccine; it also describes the immune response of revaccinated sheep after challenge with a RFV virus isolate from Egypt.

MATERIALS AND METHODS

Cell Culture — African green monkey kidney cell (Vero) monolayers prepared in 16-mm, 24-well, tissue culture plates.\(^a\)

Growth Medium — Minimum essential medium (Eagle) containing Earle's salts, L-glutamine and non-essential amino acids without sodium bicarbonate (F-15) plus 10\% fetal bovine serum\(^b\) inactivated at 56\(\degree\) C for 30 minutes.

Agar Overlay — Combination of equal volumes of 2X concentrated F-15 medium and 1.2\% agarose,\(^c\) plus 8\% heat inactivated fetal bovine serum and 0.1\% HEPES buffer.\(^d\)

Counterstain — Agar overlay containing 1:9000 neutral red.

Diluent (used for serums and RFV virus) — F-15 media with added 2\% inactivated fetal bovine serum and 0.1\% HEPES buffer.

Neutralization Tests — Antibody titers to RFV virus were determined by the plaque reduction neutralization (PRN) method.\(^e\) Sera were serially diluted four-fold with microdiluters in 96-well microtiter plates.\(^e\) Equal volumes of previously titrated RFV virus containing 80-100 plaque-forming units (pfu) per 50 \(\mu\)l were added. After incubation at room temperature for 60 minutes, 50 \(\mu\)l of the mixture was inoculated into each of 2 wells of the tissue culture plate containing 2 to 4 day-old monolayers. After 60 minute's adsorption, 0.5 ml of agar overlay per well was added. At 72 hours after overlay, 0.5 ml of counterstain was added to each well, and plaques were counted 24 hours later. An 80\% reduction of plaques was used as the index for virus neutralization titers.

Vaccine — The vaccine was prepared from the 180th mouse passage of Entebbe strain of RFV virus grown in monkey kidney tissue culture and was inactivated with formalin. The vaccine was obtained from the USAMRIID and had been developed for immunizing humans. The dosage recommended for humans is 1 ml of undilute vaccine, the initial vaccination is followed by another vaccination 10 and 28 days later. For each group, 1 ml amounts of different dilutions of vaccine were inoculated into

\(^a\)Costar, Cambridge, MA
\(^b\)Grand Island Biological Company, Grand Island, NY
\(^c\)Bio-Rad Laboratories, Rockville Center, NY
\(^d\)Calbiochem-Behring Corp., LaJolla, CA
\(^e\)Dynatech Laboratories Inc., Alexandria, VA
each animal: steers received undilute vaccine or 1:5 or 1:25 dilutions goats and sheep received undilute vaccine or 1:5, 1:25, 1:125 or 1:625 dilutions. The sheep were each revaccinated with 1 ml of undilute vaccine 90 days after the first inoculation.

Virus for Plaque Reduction and Challenge Studies — The RFV virus was obtained from the USAMRIID as the second fetal rhesus lung cell culture passage of Zagazig 501 strain originally isolated from a human case of fatal hemorrhagic fever in Egypt in 1977. The virus dose used to challenge immunity of sheep 75 days following the second vaccination contained 1 x 10⁶ pfu/ml. Stock virus was prepared from second Vero culture passage virus. It was stored in 1 ml aliquots at −70°C and assayed by plaque count. A recently thawed ampule was used for each neutralization test.

Animals — Steers, goats and sheep were separated into groups, and each was inoculated with the previously indicated doses of vaccine.

Thirty (30) Steers — Three groups each of 10 grade Herfords approximately 18 months old.

Twenty (20) Goats — Five groups each of 4 mixed-breed approximately 6-12 months old.

Sixty (60) Sheep — Five groups each of 10 mixed-breed approximately 6 months old. Ten sheep were used as non-vaccinated controls.

Mice — Albino mice, Plum Island colony of the Rockefeller-H strain 6-9 days old, were used to evaluate viremia in serum of sheep challenged with virulent RVF virus. Each of 8 mice per litter received 0.03 ml intraperitoneally.

Sampling — Serum samples from steers and goats were assayed for neutralizing antibodies 14 and 30 days post vaccination (DPV). Serum samples from sheep were assayed more frequently for neutralizing antibodies after both the first and second vaccination. After challenge of the sheep with virulent RFV virus, serum samples taken daily for 10 days were assayed for viremia by mouse inoculation.

RESULTS

Response of Vaccinated Steers

The PRN titers were less than 1:40 in all but one steer within the 30 day RFV vaccine trial period (Table 1). Antibody was detectable in 50% or more of the steers inoculated with undilute vaccine or a 1:5 dilution; but only 3 of 10 steers inoculated with the 1:25 dilution developed neutralizing antibodies.

Response of Vaccinated Goats

Only 3 vaccinated goats failed to elicit a neutralization antibody response within the 30 day RVF vaccine trial period; the most concentrated vaccine doses produced the highest PRN titers (Table 2).
Response of Vaccinated Sheep

Inoculation of sheep with undiluted RVF vaccine or 1:5 dilutions elicited maximum neutralization titers of 1:40 in some sheep within 14 days; higher dilutions of vaccine produced neutralization titers of 1:10 in only a few sheep (Figure 1). With undilute vaccine, the antibody titer increased slightly between 30 and 90 DPV; but with the dilutions of vaccine, the antibody titer was nearly undetectable in most sheep by 90 DPV.

After revaccination of all sheep with undilute RVF vaccine, some PRN antibody titers increased 640-fold. At the time of revaccination, 80% of the vaccinated sheep had undetectable antibody levels and within 20 days, 94% developed PRN antibody titers of at least 1:40; a few titers were as high as 1:2560.

All vaccinated sheep resisted challenge with virulent RFV virus and developed and increased (PRN) antibody titer. The challenge RVF virus inoculated into non-vaccinated sheep produced viremia, clinical signs of disease and death.

DISCUSSION

This study was undertaken to evaluate the immunogenic effect in steers, goats and sheep of a vaccine prepared for human use. This vaccine was prepared from monkey kidney cell culture infected with pantropic Rift Valley Fever (RVF) virus and inactivated with formalin.

The effect of the vaccine in these domestic animals was measured by the capacity of their serums to neutralize virus, a method that has been used extensively as a measure of immunity in RVF. A single inoculation of different doses of vaccine elicited only a 1:10 virus neutralization titer in slightly more than 50% of the steers within 30 days, but elicited a 1:10 to 1:160 virus neutralization titer in 85% of the goats within that time. Low neutralization titers may not reflect the degree of protective immunity, and factors other than neutralizing antibodies may play a role in resistance against RVF virus infection; however, the inoculated steers and goats were not challenged with live RVF virus.

At the time of the second inoculation of the sheep with undilute RVF vaccine, 80% had neutralizing antibody titers of less than 1:10. Within 20 days, 95% developed upwards of a 640-fold increase in antibody titer. Apparently, a booster vaccination after the slightest sensitization from an initial inoculation elicits an amnestic response. The vaccinated sheep resisted challenge with virulent virus as indicated by the lack of thermal response or clinical signs of disease, and viremia studies as assayed by intraperitoneal inoculation of suckling mice were also negative.

Reports indicate that live RVF vaccines in general produce a higher neutralizing antibody titer and instill more prolonged immunity than inactivated vaccines. However, the inactivated products are safer in that they do not produce such complications as abortions and viremia.
The vaccine tested here is an experimental human vaccine and exists only in limited quantities. It represents the only known stock of RVF vaccine for human use. Because of the safeguards employed in human vaccine quality control and the containment facilities necessary for working with RVFV in this country the vaccine is also quite expensive. However, the product has maintained its potency for over ten years when stabilized with human serum albumin and stored in the lyophilized state at -20° C. Thus, a formalinized vaccine for veterinary use could well be developed in a less expensive cell culture system such as baby hamster kidney cells and stockpiled for emergency use in the event RVF is introduced into the livestock of the USA. This study demonstrates the principle that this type of vaccine is immunogenic as measured by the PRN response and by actual challenge with RVF virus. Furthermore, we have shown that animals having PRN titers as low as 1:10 are fully protected.

**TABLE 1:** Plaque reduction neutralization titers from 3 groups of 10 steers each inoculated with different dilutions of inactivated Rift Valley fever vaccine.

<table>
<thead>
<tr>
<th>Vaccine Dilution</th>
<th>Days Post Inoculation</th>
<th>Plaque Reduction Neutralization Titers*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;10**</td>
</tr>
<tr>
<td>Undilute</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Group 2</td>
<td>1:5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Group 3</td>
<td>1:25</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7</td>
</tr>
</tbody>
</table>

* Number of steers reacting

** Reciprocol of serum dilution reducing plaque-forming unit by 80%
TABLE 2: Plaque reduction neutralization titers from 5 groups of 4 goats each inoculated with different dilutions of inactivated Rift Valley fever vaccine.

<table>
<thead>
<tr>
<th>Vaccine Dilution</th>
<th>Days Post Inoculation</th>
<th>Plaque Reduction Neutralization Titers*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;10** 10** 40** 160**</td>
</tr>
<tr>
<td>Undilute</td>
<td>15</td>
<td>0  2  2  0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0  0  2  2</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:5</td>
<td>15</td>
<td>0  4  0  0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0  2  0  2</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:25</td>
<td>15</td>
<td>0  1  2  1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1  1  1  1</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:125</td>
<td>15</td>
<td>1  2  1  0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2  2  0  0</td>
</tr>
<tr>
<td>Group 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:625</td>
<td>15</td>
<td>1  3  0  0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0  2  2  0</td>
</tr>
</tbody>
</table>

* Number of goats reacting.

** Reciprocal of serum dilution reducing plaque-forming unit by 80%.
RESULTS OF INOCULATION OF 5 GROUPS OF 10 SHEEP EACH WITH DIFFERENT DILUTIONS OF INACTIVATED RIFT VALLEY FEVER VACCINE. THE MEAN, MAXIMUM VIRUS NEUTRALIZATION TITERS BEFORE AND AFTER REVACCINATION AND CHALLENGE ARE SHOWN.
ACKNOWLEDGMENT

The authors would like to thank Messrs. P. Mikiciuk, D. Perkins and R. Trower for their technical assistance.

REFERENCES


FOOT-AND-MOUTH DISEASE — A WORLD PROBLEM

J. J. Callis, D.V.M.
Plum Island Animal Disease Center, Agricultural Research,
Science and Education Administration
United States Department of Agriculture
Greenport, New York 11944

DESCRIPTION OF THE DISEASE

Foot-and-mouth disease (FMD) is also known as fievre aphteous (French), Maul-und Klauenseuche (German), fiebre aftosa (Spanish), and afta epizootica (Italian). Foot-and-mouth disease is acute and highly communicable and affects almost exclusively cloven-footed animals, domesticated and wild. Animals susceptible to the disease include cattle, swine, sheep, goats, wild pigs, wild ruminants, hedgehogs, armadillos, rats, nutria, grizzly bears, elephants, and buffalos. Experimental infection has been reported in young dogs, cats, rabbits, and chinchilla. There are very few scientifically authenticated cases of FMD infection in man.

The disease is characterized by vesicles or erosions in the mucosa of the mouth and by a sharp rise in body temperature. Vesicles may also be found on the coronary band and on the skin between and above the hoofs of swine and other species of cloven hoofed animals. Vesicles may be found on the teats of nursing sows and are frequently found on the snout and back of the rim of the snout, and may sometimes extend into the nares of swine. Lesions are found in cattle on the teats, udder, muzzle, between the claws, and in the buccal cavity. The vesicles rupture soon after they appear and leave raw hemorrhagic eroded surfaces with ragged fragments of necrotic epithelium attached.

When secondary infections do not occur, the lesions tend to heal rapidly. The incubation period after natural exposure varies from 48 hours to a few days, depending upon the particular strain of virus and the nature and extent of exposure to infection. Signs of infection include increase in body temperature, lassitude, anorexia, and lameness when involvement of the feet is evident. The severity of lameness will depend upon the extent of foot involvement. Abortion, mastitis, and chronic deformities of the feet are common. Sometimes the hoofs will slough. Mortality seldom exceeds 5%, however, in outbreaks in young animals the heart may be involved with an overall mortality as high as 50%. In cattle, dribbling of foamy saliva is usually profuse.¹

HISTORY OF THE DISEASE

The first written account of FMD was a description of an outbreak in Italy in 1514. Foot-and-mouth disease occurs at least intermittently in most major livestock producing areas of the world (Fig. 1). Exceptions are: North America, Central America, Australia, and New Zealand. It last
Foot-and-mouth disease occurred in Australia in 1872 and in North America in 1954 and has never occurred in Central America, north of the Panama-Colombia border. Ireland, Norway, Greenland, Iceland, Japan, and most of the smaller islands of Oceania and the Caribbean are free of FMD. England is free of the disease for years at a time. The largest and most costly epizootic in England occurred in late 1967 and early 1968. This outbreak involved more than 2,300 premises and resulted in the destruction of 430,000 livestock before the disease was brought under control. The United States has experienced 9 outbreaks of FMD, the first in 1870 and the latest in 1929. The disease was eradicated, in each instance, by slaughter of the infected and exposed animals and disinfection of the premises. Foot-and-mouth disease occurred in Canada in 1952, and this outbreak was eradicated within a few weeks. The disease occurred in Mexico in 1946, and that country was not declared free of the disease until 1954, after nearly one million animals had been slaughtered and approximately 60 million animals had been vaccinated. Although the mortality rate is not often high, FMD is a major concern to disease control veterinarians in most countries, because of its rapid spread, the resulting loss of milk, meat, and other animal products, expensive eradication and vaccination programs, and restrictions on international trade.

DESCRIPTION OF THE VIRUS

Foot-and-mouth disease virus is a picornavirus of the genus apthoviridae. There are seven immunological types of virus: A, O, C, Southern African Territories (SAT) 1, 2, 3, and Asia 1. Within the 7 viral types, at least 65 subtypes have been diagnosed by the complement-fixation test conducted by the World Reference Laboratory for FMD in England and by the Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil. Types A, O, and C virus are found in most areas where FMD occurs (Europe and South America). Types SAT 1, 2, and 3 virus were found only in Africa until 1962 when SAT 1 was found in the Middle East. Type Asia 1 virus has been found in various parts of Asia and has been identified in the Middle East. Typing of FMD virus is determined by the complement-fixation test and sometimes the viruses are given synonyms. At times, however, the virus type and subtype may be given the name of the place where the virus was isolated, such as O, Brugge.

The protein coat of FMD virus contains 4 major polypeptides (VP$_1$ through VP$_4$) and two minor polypeptides (P$_{40}$ and P$_{52}$). The P$_{40}$ and P$_{52}$ polypeptides are also found in small 12 S particles produced by disruption of the purified virus at pH 6.5 or by heating at 56°C. The virus has a molecular weight of about 6.9 million Daltons, of which about 31% is ribonucleic acid (RNA). The RNA of FMD virus may survive boiling under certain conditions. The intact virion is more susceptible to heat as a result of rapid denaturization of the protein capsid above 43°C. When protected by organic materials, FMD viral preparations may retain limited infectivity after they are heated at 85°C for as long as 4 hours. The FMD virus is more labile to pH changes than are most of the small
viruses containing RNA. Strong bases, such as sodium hydroxide, and the organic acids such as acetic acid 2% solutions, are commonly used as disinfectants. When highly purified virus is used, the virus is inactivated at specific isoelectric points that are governed by both the ionic strength and pH.\(^2\)

When FMD virus is present in milk or milk products, the virus has an unusual ability to survive otherwise adverse temperatures and pH changes. For example, some viral particles in milk will survive heating to 72\(^\circ\) C for 15 seconds and acidulation to pH 4.6. Some properties of the virus which make FMD a difficult disease to control are the multiple types and subtypes of the virus, ability of the virus to mutate, multiple species susceptibility, and the relative ability of the virus to withstand extreme environmental conditions.\(^1\)

GEOGRAPHIC DISTRIBUTION OF FMD VIRUS TYPES

Foot-and-mouth disease virus types A, O, and C have the widest distribution throughout the world. Type A virus exists in Europe, the Near East, Central Asia, the Far East, Africa, and South America. Type O virus continues to infect many countries in Europe, the Near East, Southern Asia, a part of Africa, and South America. Type C virus causes only sporadic outbreaks in Europe and also exists in the USSR, Southern Asia, the Far East, Africa, and South America. Asia 1 virus exists only on the African continent and was last diagnosed in Turkey in 1965. Type SAT 2 virus was identified in 1978 in Mozambique, Botswana, the Republic of South Africa, South-west Africa, and Rhodesia. Type SAT 3 virus has been recognized in Rhodesia since 1974.\(^8\)

FMD INCIDENCE IN EUROPE IN RECENT YEARS

Although FMD continues to occur in Europe, the disease is at a low incidence in many European countries (Figs. 2, 3, 4). It last occurred in Finland in 1960, in Norway in 1952, in Sweden in 1966, in Ireland in 1941, and in the Federal Republic of Germany FMD was last diagnosed in April 1978 and in Austria in 1975. The disease last occurred on the Isle of Jersey in 1974, in the Azores in Madeira and in Portugal in 1971, in Luxembourg in 1964, and in Denmark in 1970. The last case of FMD occurred in Belgium in 1976, and in Holland the last case was reported in January 1977. The disease has never occurred in Iceland, and has not been reported in the United Kingdom since 1968.

Foot-and-mouth disease caused by type O virus occurred in France in March 1979 and caused that government to destroy 514 pigs, 1,619 cattle, 17 sheep, and 4 goats.

In Switzerland in March 1978 there was an outbreak of type C virus in a herd of cattle which had been partially vaccinated using a trivalent vaccine. The German Democratic Republic also reported an outbreak in cattle in 1978 caused by type C virus, and in the European part of the USSR type C virus caused an outbreak in January 1979.
There were no outbreaks of FMD reported in Spain in 1978, but there have been several outbreaks in 1979 due to types A and O. Albania has been free since 1960, Cyprus since 1964, and the Isle of Malta since 1975. Italy reported 18 outbreaks in 1977, 43 outbreaks in 1978, and through February of 1978, 4 outbreaks had been reported. Yugoslavia had been free of the disease since 1974, but reported an outbreak in November 1978 caused by type A virus. (This outbreak reportedly occurred in cattle from Hungary in transit through Yugoslavia.) Greece reported no outbreaks in 1978 or thus far in 1979. The Thrace region of Turkey which has been free since 1973, was affected with type O, virus in 1977; in 1978 there were 31 outbreaks, and January through August 1979 there had been several hundred outbreaks in the Thrace region and the remainder of Turkey.34

The incidence of FMD in Western Europe has been reduced by the strict annual application of well produced and tested trivalent types A, O, and C FMD vaccines. When outbreaks occur, infected and exposed animals are slaughtered, the carcasses are destroyed, and the premises are disinfected. All cattle are vaccinated annually and young animals are vaccinated twice during their first year of life. Sheep and goats are sometimes vaccinated in outbreak areas. In some countries, especially Spain, large numbers of swine are vaccinated with oil-adjuvanted vaccines, which have been shown to be superior for that species. The principal sources of virus responsible for the few outbreaks that occur each year in Western Europe include imported meat and improperly inactivated vaccines.

FMD INCIDENCE IN ASIA IN RECENT YEARS

Reports of incidence of the disease in some countries of Asia are difficult to obtain; therefore, this section will not be as complete as information given for Europe and South America.

Israel, which was free of FMD in 1977, reported an outbreak in 1978. In Kuwait, 454 cases were reported in 1977; 1,710 cases in 1978; and the disease continues to be reported in 1979. The disease was not reported in Bahrain in 1978. In Iraq it continues to be enzootic, with 154 outbreaks in 1978. The disease has occurred there in 1979, but the final tally is not yet available. In Saudi Arabia, 967 outbreaks were reported in 1977, and this epizootic continued into 1978. In Oman, Yemen, and United Arab Emirates, types A or O virus caused outbreaks in 1977, 1978, and 1979. Iran experienced 58 outbreaks in 1977 and 37 in 1978. Jordan reported 18 outbreaks in 1978 caused by types A and O virus. Syria reported type O outbreaks in 1977, 1978, and 1979. Foot-and-mouth disease was not reported in Afghanistan in 1978. In the Asian part of the USSR, types O, and A2 virus caused outbreaks. The disease was reported in 1978 and 1979 in Pakistan, India, Nepal, Sri Lanka, Bangladesh, and Burma caused by types A, O, C, and Asia 1 virus. Thailand reported outbreaks in 1978 due to types A, O, and Asia 1 virus. Malaysia, Laos, and Indonesia (Isle of Java) reported FMD in 1978 due to type O virus. Korea, Japan, the
Island of Taiwan, Sarawak, Timor, and New Guinea remain free of FMD. Hong Kong reported outbreaks in 1978 and 1979 due to type O virus. Type C FMD was reported in the Philippines in 1978.3,4

RECENT INCIDENCE IN AFRICA

In the north, Morocco which had been free since 1965 was affected in 1977 with type A virus, causing 1,611 outbreaks. This epizootic continued into 1978. Algeria reported 23 outbreaks in 1977 due to type A virus and no outbreaks in 1978. Type A virus caused FMD in Libya in 1979. Egypt reported type O virus in 1977, 1978, and 1979.

In the Western and Central sections of Africa, Gambia and Sierra Leone did not report FMD in 1978, and Liberia has been free since 1975. On the Ivory Coast, 2 outbreaks were reported in 1977, with no outbreaks reported since then. Ghana reported outbreaks of SAT 1, 2, and 3 in 1974 and 1975, but no further reported outbreaks since. In Togo, FMD appeared first in 1973 and was again reported there in 1975. In Benin, a highly contagious form of FMD was reported in 1973, 1974, and 1975. Type SAT 1 was identified in Niger in 1976, 1978, and 1979. In Nigeria, virus types A and SAT 1 were identified in 1976 through 1979. Chad reported outbreaks of FMD in 1975, 1976, and 1977. Upper Volta experienced an outbreak in 1976 and another in 1978. Type A virus caused FMD in the Cameroons in 1978 and 1979. Type SAT 2 virus reportedly caused outbreaks in Mauritania in 1975 and 1976. Senegal also reported outbreaks due to type SAT 2 in 1975, 1976, and 1979. No FMD was reported in Bagon on the Congo in 1977 or 1978. Zaire reported 1 outbreak in 1977.


In Kenya, types A, O, C, SAT 1, and SAT 2 caused outbreaks in 1976, 1977, and 1978. In 1976, type SAT 1 virus was reported in Somalia, and in 1977 type O and in 1978 type A. In Uganda, outbreaks were reportedly caused by types A, O, SAT 1, and SAT 2 in 1976 and 1978. Zambia was free of FMD for 5 years, but the disease reappeared in 1975 caused by type SAT 2, thought to have been introduced by wild buffalos. Type SAT 3 caused FMD in Malawi in 1976 and no further outbreaks since. In Tanzania types A, O, SAT 1, and SAT 2 viruses were responsible for outbreaks in 1975, 1976, 1977, and 1978. Type SAT 3 reappeared in 1975 for the first time since 1955. Types SAT 1, 2, and 3 were reported in 1978. Mozambique reported types SAT 1 and 2 in 1977 and 1979. Botswana, free since 1968, experienced outbreaks in 1977 and 1978 due to SAT 1 and 2. No outbreaks have been reported in Swaziland since 1970 nor in Angola since 1974. In the Republic of South Africa, free of FMD since 1975, SAT 2 virus was reported in 1977, 1978, and 1979. Type SAT 2 virus was also identified in South-West Africa in 1978.

RECENT FMD INCIDENCE IN THE AMERICAS

In the United States, type O, FMD virus escaped from a laboratory
building at the Plum Island Animal Disease Center in September 1978, and infected cattle in a normal cattle holding area. There was no evidence the virus escaped to the mainland.

South America

Types A and O FMD virus are responsible for enzootic disease in Colombia, Venezuela, Ecuador, and Peru, and in 1978 caused 208, 48, 46, and 9 outbreaks, respectively. In Brazil, 6,061 outbreaks were reported in 1978 caused by types A, O, and C virus. Bolivia reported 5 outbreaks of type O virus and 4 outbreaks of type A virus in 1978. Paraguay experienced 8 outbreaks of type O virus and 2 outbreaks of type C virus; type A virus was not identified for the first time since 1968. The incidence of the disease in Chile has dropped in recent years. In 1978 there were 2 outbreaks in cattle imported for slaughter. It is possible that Chile may be declared free of FMD in the not too distant future. There were 28 outbreaks in Uruguay in 1978 and, like Argentina, these were caused by types A, O, and C virus. In Argentina there were 816 outbreaks in 1978 (Fig. 5).

During 1978 it is estimated that approximately 800 million doses of FMD vaccine were produced worldwide. More than half of that was used in South America where cattle in many countries are routinely vaccinated three times a year. In Argentina and Uruguay, large numbers of sheep are also vaccinated.

SUBTYPES OF THE VIRUS USED IN VACCINES

Despite progress made in eradicating the disease in a number of countries, the chances for any country in the world to be invaded by FMD virus have not lessened. The disease is overt or latent in Europe, Asia, Africa, and South America. For this reason, those countries that are free must be on the alert and prepare themselves for introduction of FMD virus. Even those countries that are infected and practice vaccination on an annual basis are just as much at risk to the exotic types of subtypes of the virus as are those countries that are free from all types. For this reason, consideration should be given to international collaboration in maintaining reserves of concentrated and inactivated antigens, from which vaccines can be quickly prepared and applied in the event of an outbreak that cannot be controlled by slaughter alone. At present, to be fully prepared, this bank of antigen and/or vaccine should contain at least the following subtypes of the virus: A₅, A₂₅, A₃₄, O₁, C₂, SAT 1, 2, 3, and Asia 1. Viruses responsible for new epizootics, such as currently being experienced in Brazil, known locally as A-Bage and A-Venceslau, must be compared with vaccine strains already in use or in storage to assure that they afford adequate protection against the new strain.

Although the incidence of the disease in many parts of the world has been brought under control through vaccination, application of sanitary measures, and slaughter, FMD is still surely one of the major deterrents to livestock production in vast areas of the world. Those countries that
are free of FMD place restrictions on importations of animals and animal products from countries that are infected. In addition, the infected countries oftentimes go to the added expense of processing meat products to render the product free from infective virus and thus acceptable to FMD-free countries. Foot-and-mouth disease does not respect national boundaries; therefore, international cooperation is required for effective control.

Fig. 1: Worldwide distribution of Foot-and-Mouth disease.

Fig. 2: Foot-and-mouth disease outbreaks in Europe, 1960-1978.
Fig. 3: Foot-and-mouth disease outbreaks in Europe, 1977.

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of Outbreaks</th>
<th>Type of Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>Fed. Rep. of Germany</td>
<td>3</td>
<td>A, C</td>
</tr>
<tr>
<td>Italy</td>
<td>18</td>
<td>A, O, C</td>
</tr>
<tr>
<td>Spain</td>
<td>26</td>
<td>C</td>
</tr>
<tr>
<td>German Dem. Rep.</td>
<td>1</td>
<td>O</td>
</tr>
<tr>
<td>Greece</td>
<td>3</td>
<td>A</td>
</tr>
<tr>
<td>Turkey</td>
<td>735</td>
<td>A22, O</td>
</tr>
<tr>
<td>USSR</td>
<td>101</td>
<td>A22, O</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>888</strong></td>
<td></td>
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</table>

Fig. 4: Foot-and-mouth disease outbreaks in Europe, 1978.

<table>
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<tr>
<th>Country</th>
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<th>Type of Virus</th>
</tr>
</thead>
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<td>C</td>
</tr>
<tr>
<td>Fed. Rep. of Germany</td>
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<td>C</td>
</tr>
<tr>
<td>Italy</td>
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<td>C, A</td>
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<td>Switzerland</td>
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<tr>
<td>German Dem. Rep.</td>
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<td>C</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>1</td>
<td>A</td>
</tr>
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<td>Malta</td>
<td>14</td>
<td>A</td>
</tr>
<tr>
<td>Turkey</td>
<td>830</td>
<td>A22, O</td>
</tr>
<tr>
<td>USSR</td>
<td>32</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>908</strong></td>
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Fig. 5. Foot-and-mouth disease incidence in South America, 1978.

<table>
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<tr>
<th>Country</th>
<th>No. of Outbreaks</th>
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</thead>
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<tr>
<td>Colombia</td>
<td>208</td>
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<tr>
<td>Venezuela</td>
<td>48</td>
<td>A, O</td>
</tr>
<tr>
<td>Ecuador</td>
<td>46</td>
<td>A, O</td>
</tr>
<tr>
<td>Peru</td>
<td>9</td>
<td>A</td>
</tr>
<tr>
<td>Brazil</td>
<td>6,061</td>
<td>A, O, C</td>
</tr>
<tr>
<td>Paraguay</td>
<td>10</td>
<td>O, C</td>
</tr>
<tr>
<td>Chile</td>
<td>2</td>
<td>--</td>
</tr>
<tr>
<td>Uruguay</td>
<td>28</td>
<td>A, O, C</td>
</tr>
<tr>
<td>Argentina</td>
<td>816</td>
<td>A, O, C</td>
</tr>
<tr>
<td>TOTAL</td>
<td>7,237</td>
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</tbody>
</table>

ACKNOWLEDGMENTS

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REFERENCES


REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

Chairman: Thomas G. Murnane, D.C.
Co-Chairman: Gilbert S. Trevino, Texas


The Committee on Foreign Animal Diseases met on October 30 and 31, 1979, during the annual meeting of the USAHA, held at the Town and Country Hotel, San Diego, California. Thirteen of the Committee Members and approximately 60 guests attended the meetings.

During 1979, African Swine Fever (ASF) continues to occur in the Western Hemisphere; Contagious Equine Metritis and Swine Vesicular Disease continue to occur in Western Europe, and a potential new Swine "Vesicular Virus" has occurred in Tasmania, Australia. These diseases were either unknown a few years ago or have invaded major new territory (ASF) in recent years and are illustrative of the serious threat of foreign animal diseases to our meat-producing industries. Continued vigilance alone will not protect this country for long, and the downward trend of support for foreign animal disease research, domestic and international, is of great concern to this Committee.

INTERNATIONAL OPERATIONS

Vesicular Disease Diagnostic Laboratory

Construction of the Vesicular Diseases Diagnostic Laboratory in Panama has been delayed, but dollars are now available and construction should begin by January 1, 1980. This will be a regional laboratory supporting Central America and Panama in vesicular disease surveillance. The laboratory is being constructed by and will be operated by the Panama-U.S. Foot and Mouth Disease Commission.

Pan American Highway

The U.S. Government continues to withhold funds for the construction of the remaining segment of the Pan American Highway until Colombia can provide assurance that an adequate FMD Control Program is operational and that the disease will no longer pose a threat for transmission northward during construction and upon completion of the highway. The initial lack of Colombian political support in the first four and one-half years of the highway construction was reflected in the lack of commitment to carry out effectively the FMD Program on which further U.S. involvement in highway construction is predicated. The
present administration in Colombia, however, appears to be fully com-
m\nnitted to construction and completion of their portion of the highway, and has agreed to contribute substantial funds to the program.

US/MEXICO SCREWWORM PROGRAM

The major objective of the screwworm (SW) eradication program is to eradicate the parasite from the United States and Mexico down to the Isthmus of Tehuantepec, and there establish a barrier to prevent rein-festation of free areas to the north. Departments of Agriculture from both countries participate in the endeavor along with livestock industries and local government officials.

A new strain of flies was developed by the USDA Science and Education Administration for introduction into the sterile fly rearing facilities during the latter part of 1978. The new strain was developed from collections of native screwworms from Arizona and Veracruz, Mexico, and was identified as the Aricruz strain. The Mission facility began production of this new strain in January, 1979, and in Tuxtla Gutierrez, Chiapas, in March, 1979. The Aricruz strain has apparently performed well in the field.

The Screwworm Adult Suppression System (SWASS) is an attractant-bait-toxicant system designed for aerial distribution in areas of wild SW fly activity to reduce the wild fly population down to low levels where sterile flies will be most effective. SWASS was used in 1979 as an aid to the sterile fly technique in widespread application.

Baja California received first priority for eradication when the effort in Mexico was initiated. The last confirmed case in the peninsula was collected on June 30, 1979, and it appears eradication of self-sustaining populations has been achieved. A quarantine line to control livestock movements between the Mexican mainland and the peninsula has been established.

Releases of sterile flies during 1979 were concentrated in south-western United States and northern areas of Mexico. After a difficult beginning, with heavy infestations in many locations, control of the native populations of wild flies was achieved and record low numbers of cases resulted.

The 77 screwworm cases confirmed between April and September of 1979 is lower than any previous year for the same period in the southwest United States. Moving the eradication effort southward will depend on continuing levels of funding, adequate to overcome the increased costs of labor and materials in Mexico and the United States. Improvements in program techniques, and development of new systems are also vital to the success of the campaign. Extensive research is now underway to determine the possibilities of genetic differences in SW flies.

Status of Global Animal Disease

Foreign animal diseases continue to pose a serious threat to livestock
health and economy in the Western Hemisphere. The less virulent form of African Swine Fever in Brazil and the Dominican Republic is causing severe difficulties in diagnosis and eradication. An unidentified “Vesicular Disease” in swine, in a remote area of Tasmania, Australia, continued spread of Swine Vesicular Disease, and decreasing support for rinderpest control in Africa is discouraging to the international effort to control foreign animal diseases.

FMD continued to occur in South America, Asia, and portions of Africa. Sporadic outbreaks occur in Western Europe, resulting probably from importations of contaminated meat products. These outbreaks are quickly eradicated by quarantine and test and slaughter control procedures. The SAT strains no longer occur except in South Africa. Two new A isolates from Brazil may be new strains, but typing is not yet complete.

A new import station on Fleming Key, Florida, which will allow the import of cattle from FMD-free herds within FMD infected countries, should be operable by January 1, 1980.

Rinderpest

This disease continues to occur in portions of Africa and Asia. The vaccination campaign appears to be lagging in West Africa and to be losing its momentum throughout the continent. There is increasing risk of resurgence of the disease in Africa where cautious optimism for its ultimate control formerly existed.

African Swine Fever

In the Western Hemisphere only Brazil, the Dominican Republic, and Haiti are known to be infected, and epidemiological evidence indicates all three countries became infected in 1978. The lack of spread into new countries during 1979 is encouraging.

Laboratory diagnostic procedures were not as effective for identifying the ASF virus isolated in Brazil and the Dominican Republic as for the virus from Africa and the Iberian Peninsula. The hemadsorption inhibition test is considered the most accurate procedure for detecting and identifying the ASF virus, but many isolated from the Western Hemisphere do not hemadsorb on first passage in buffy cells; therefore, the direct fluorescent antibody procedure has become increasingly important.

Research data from PIADC and Europe indicate the ELISA test has the potential for being the most sensitive and accurate test for ASF antibodies. The need for tests to detect antibodies has increased since the mortality rate for ASF in the Western Hemisphere is lower than last reported from Africa and Europe.

Swine Vesicular Disease

This entity continues to occur sporadically in Western Europe. The disease has occurred for the first time in Greece. Surveillance is continuing.
Swine "Vesicular Disease" in Tasmania

An outbreak of a "vesicular disease" in swine has occurred in Northern Tasmania. The outbreak has symptoms identical to those of Vesicular Exanthema of Swine. The disease appears mild in character, and affected swine appear active. Feedlot cattle in close contact showed no signs of infection, but were destroyed as a precautionary action. The disease has been reproduced in the laboratory and initial laboratory tests are negative for FMD and vesicular stomatitis. It would appear this may be a new swine virus capable of producing vesicular lesions.

TRAINING

Since the last USAHA meeting and as a result of a resolution passed at the 1978 meeting, one additional training program on differential diagnosis of African swine fever and hog cholera was held for diagnosticians in the U.S. Approximately 20 diagnosticians attended this program. This was the third such program which was held at the Plum Island Animal Disease Center for representatives of laboratories in the U.S. At the 1978 USAHA meeting, there was also a resolution that PIADC should hold an additional training program on recognition and diagnosis of foreign animal diseases for veterinarians who work at zoological gardens. Such a program was held in July of 1979 for veterinarians from 16 zoos.

Two training programs were held in 1979 for veterinarians from the Animal and Plant Health Inspection Service and from State and Universities. These two programs were attended by approximately 20 veterinarians each. This brings the number of veterinarians trained in the recognition of foreign animal disease to 247.

RESEARCH ON VESICULAR DISEASE

The vesicular diseases group of viral origin consists of foot-and-mouth disease (FMD), swine vesicular disease (SVD), vesicular exanthema of swine (VES) and vesicular stomatitis (VS). The following report is confirmed to a review of reports selected on the basis of relevance to a particular disease. The review does not purport to include all of the literature.

Foot-and-Mouth Disease Virus (FMDV)

A water-oil emulsion, trivalent FMDV vaccine with a shelf life of more than a year when stored at 4°C induced a rapid immunity in pigs which lasted to the end of the fattening period. Formalin inactivated aluminum hydroxide adjuvanted vaccine to which diethylaminoethyl dextran (DEAE-D) was added, induced higher levels of neutralizing antibody in pigs and provided better protection against contact challenge than vaccine not containing DEAE. In a study of FMD vaccination in pigs, the workers demonstrated that protection is often influenced by the method used to challenge immunity. Scientists in the USSR reported an effective immunization of sheep against FMD with aluminum hydroxide formalin-treated, lapinized type O virus. Cattle vaccinated simultaneously with a
combination FMD and vesicular stomatitis live virus vaccine developed much lower antibody levels against each virus than if each vaccine was administered alone.\textsuperscript{5}

In a recent study in England, scientists have reported that virus infective antibody (VIA) heretofore thought to be induced primarily as a result of FMDV replication, may also result after as few as 2 vaccinations with formalin inactivated FMD vaccines. Repeated vaccination resulted in more positive VIA reactions. This new observation indicates that the full range of conditions under which VIA will develop needs further study.\textsuperscript{23}

The European Commission for Control of FMD is interested in determining the availability on short notice of vaccines against types OAC FMD vaccine in Europe for use in countries which are free of FMD.\textsuperscript{10} FAO has also expressed interest in initiating a feasibility study to establish a strategic vaccine reserve (with particular reference to exotic viruses) and to determine the degree of interest among member countries of the Commission in such a reserve.

In a study in Holland FMD vaccinated cattle which were intranasally challenged, FMDV multiplied to a limited extent in the pharyngeal area but virus was not detected in milk samples taken 1 - 19 days post challenge.\textsuperscript{19} In two studies FMDV was detected in milk, blood, and muscle 24 - 32 hours before lesions appeared.\textsuperscript{9,26} Casein prepared from raw skim milk and pasteurized skim milk (72°C for 15 seconds) collected from cows infected with FMDV was shown to still contain FMDV when inoculated into cattle. The same samples were negative when tested in cell cultures. In a study of experimentally infected cows, FMDV was demonstrated in semen when the semen was tested for infections in cell cultures, however, when gilts were inseminated with this semen, they failed to develop FMD.\textsuperscript{20}

In a study of FMD in guinea pigs, Knudsen demonstrated that recovered animals developed cell mediated and humoral immunity.\textsuperscript{14}

In a study of disinfectants against FMDV, sodium hydroxide and sodium and potassium hypochlorites were shown to be the most active disinfectants tested against FMDV.\textsuperscript{4}

Antibody to FMDV was detected in buffalo, eland, cattle, sheep, and goats on a ranch in Southeastern Kenya, but not in oryx and camels.\textsuperscript{21}

Antibody to FMDV was detected by the indirect complement fixation (CF) tests 4 - 7 days after inoculation and for 232 days after infection; antibody titers were lower than neutralizing antibody titers, but were type specific.\textsuperscript{27,28}

The enzyme labeled immunosorbent assay (ELISA) is 50 - 100 times more sensitive than the CF test for specific detection of FMDV.\textsuperscript{7} The test is also useful for detecting 140 S virus (whole virus) in the presence of 12 S material.\textsuperscript{1}
Swine Vesicular Disease Virus (SVDV)

A thorough pathogenesis study of SVD in pigs by clinical observation, immunoflorescence, histopathology, and virus neutralization was accomplished. Immunoflorescence studies showed SVD virus had strong affinity for the epithelia of the tongue, snout, coronary band, and lips, the myocardium and lymphoid elements of the tohsil and brain stem. Cox-sackie B5 and SVDV both produce CNS lesions in exposed pigs, however, only SVD produced clinical symptoms. Pigs exposed to Coxsackie B5 virus had high neutralizing antibodies to SVDV and Coxsackie B5 viruses.

Swine vesicular disease virus did not survive heating to 60°C in MORTADELLE sausage, but did survive the aging process in three types of salami sausage. SVDV was inactivated when the virus was dried at high relative humidity, but the infectivity persisted when dried at a low relative humidity.

Polyacrylamide gel electrophoresis examination showed a variation of structural polypeptides of SVDV isolates from the United Kingdom and Hong Kong strains of virus. This observation is in accordance with competition radioimmunoassays and antibody blocking tests.

By assaying several thousand swine serums for neutralizing and precipitin antibodies, it was determined there was no evidence of widespread undetected SVD in England, Scotland, and Wales.

Vesicular Exanthema of Swine Virus (VESV)

San Miguel Sea Lion Virus (SMSV)

The physical and biochemical studies have been established for VESV as the prototype of the calicivirus group.

By immunoelectron microscopic comparisons VESV and SMSV showed common antigenicility, however, there was only a slight comparison with feline calici-virus. Broad cross-reactivity was observed between serotypes of VESV and SMSV by radioimmune precipitation using staphlococal protein A as IgC adsorbent.

Serum neutralizing antibody to SMSV was detected from gray foxes resident on Santa Cruz Island, California, but not arctic foxes from Alaska. Virus was isolated from the blood and rectal swabs of minks fed ground meat from seals showing skin lesions or pig meat contaminated with SMSV, but the minks developed no vesicular lesions.

Vesicular Stomatitis Virus (VSV)

All research reported this year is on molecular biology which has little relevance to this committee.
REFERENCES


The contagious equine metritis (CEM) situation in the United States can be divided into three parts as follows:

1. Outbreaks of CEM and the aftermath of these episodes.
2. Surveys for CEM of recently imported animals.
3. Considerations regarding the importation of horses and other equidae into the United States.

OUTBREAKS OF CEM AND THE AFTERMATH OF THESE EPISODES

In Central Kentucky the population at risk for CEM consisted of 6,000 resident mares and 1,500 transient mares and a population of stallions to serve these 7,500 mares applying a ratio of 1 stallion to 35-40 mares.

1978 — Kentucky

1. In early March, CEM was confirmed and was found to have spread in the breeding sheds of two major Thoroughbred breeding farms in central Kentucky, where a total of 58 stallions were standing at stud.

2. In the breeding sheds at the two farms 412 mares were considered exposed to CEM. This was ascertained during a two-week interfarm breeding moratorium imposed on all central Kentucky Thoroughbred breeding farms.

3. Using epidemiological investigations, bacteriological cultures, and serological testing, these 412 mares were classified into high, medium and low-risk groups.

4. A Kentucky State quarantine was placed on all exposed and affected animals and a CEM code of practice was formulated.

5. A Federal quarantine was placed on Kentucky to prevent the movement of exposed and affected equidae from that State.

6. During the breeding moratorium, all affected and exposed stallions were scrubbed (according to the Kentucky CEM Code of Practice) and breeding was resumed. The final tally of mares pregnant in 1978 exceeded that of 1977.

1979 — Kentucky

1. The 1978 risk mare groups were released from State quarantine by passing negative: sets of bacteriologic cultures and negative complement-fixation test results.

2. Since CEM was new, a cautious approach to quarantine release was used. All medium and high-risk mares, while being bred under specified conditions were retained in Kentucky. Low-risk mares
were released from quarantine (State and Federal) after negative sets of bacteriologic cultures and negative serological findings were submitted.

3. No new cases of CEM were found in the central Kentucky Thoroughbred population during the 1979 breeding season.

4. Recently, the Federal quarantine was changed from being statewide to described premises.

1978-1979 — Missouri

1. This outbreak involved a population of 11 horses in three herds in central Missouri, predominately Trakehner horses.

2. In spring of 1978, four mares showed clinical signs compatible with CEM (using natural breeding).

3. In 1979 CEM clinical signs recurred in the primary herd with a subsequent positive bacteriologic culture being found to confirm a diagnosis of CEM in four mares among ten covered. The method of breeding in 1979 was artificial insemination using unextended semen and no antibiotics in the AI deposited semen.

4. Epidemiological studies to include tracing have been completed and circumstantially one Trakehner stallion seems to be the source of CEM here.

5. State and Federal quarantines were placed on the three horse farms and the Trakehner stallion has been scrubbed up and we anticipate resumption of breeding soon.

SURVEYS FOR CEM AMONG RECENTLY IMPORTED ANIMALS

1. Beginning in December 1977, recently imported horses from the following countries have been surveyed for CEM: United Kingdom, Republic of Ireland, France, Federal Republic of West Germany, Belgium, and Australia. The survey consists of a cooperative State and Federal breeding history scrutiny and bacteriologic culturing and where indicated quarantine.

2. Surveying such risk animals is a difficult way to "play the game," and we believe a better way to deal with this problem is to categorize foreign countries as CEM dirty or clean on a worldwide basis.

CONSIDERATIONS REGARDING THE IMPORTATION OF HORSES AND OTHER EQUIDAE INTO THE UNITED STATES

1. In September 1977 general prohibitions on horse importations into the United States made.

2. Judgments have been made to allow importation of certain classes of horses such as weanlings, yearlings, and competition horses where solid evidence could be furnished by foreign governments that CEM exposure has not taken place.
3. Pressures continue to mount to resume international commerce in certain classes of horses.

4. Periodic reviews are being made in this area of CEM. The U.S. Animal Health Association (USAHA) has been involved in these reviews as has the American Association of Equine Practitioners (AAEP); also, the horse industry has been included in several review meetings. The most immediate CEM horse industry wide review meeting is scheduled for November 8, 1979, South Agriculture Building, SW, Room 4306, Washington, D.C., at 1:30 p.m., and if you have not been priorly notified by the American Horse Council or through reading the Federal Register, you are at this time invited to attend if interested in the overall United States CEM problem.

SPECIAL CONSIDERATIONS

1. Stallions are only contaminated not truly infected.

2. Mares are difficult to classify regarding CEM due to an inapparent carrier state and shortage of knowledge on treatments.

3. Clitoral sinusectomy needs special investigation as a possible key to freeing mares of CEM.

4. A research thrust needs to be carried out to continue to unravel the "secrets" of CEM.

SUMMARY

1. CEM is a new disease of horses and other equidae, first sorted out as a separate entity in May 1977.

2. International commerce in horses has spread CEM among eight nations in recent years.

3. Two segments of the United States horse industry took decisive action to contain CEM.

4. In each outbreak, State animal health officials moved with dispatch to control CEM and were reinforced by Federal actions. The net result I believe minimized State embargoes due to CEM.

5. CEM is a highly transmissible disease, but it has been shown in the United States that effective measures can stop its spread.

6. Research thrust should be carried out to make better "tools" available to eradicate CEM.

7. It behooves us all; that is, horse owners, horse councils, and regulatory bodies, to complete the job of CEM eradication in the United States and to preclude its re-entry through our borders.
REPORT OF THE COMMITTEE
ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. C. L. Campbell, Tallahassee, Florida

Dr. W. L. Anderson, Dallas, Texas; Mr. Ronald Blackwell, Amarillo, Texas; Dr. Jesus Castaneda Garcia, San Juan de Los Morros, Venezuela; Dr. George C. Cilley, Concord, N.H.; Dr. Leroy Coggins, Ithaca, N.Y.; Dr. C. S. Duncan, Selkirk, N.Y.; Dr. R. K. Farrell, Pullman, Wash.; Dr. John B. Healy, Sacramento, CA; Mr. E. H. Honnen, Englewood, CO.; Dr. Floyd Jones, Austin, TX.; Dr. M. J. Kemen, Ithaca, N. Y.; Gen. Wayne O. Kester, Golden, CO.; Dr. John Kimsey, Atlanta, GA.; Dr. Wayne Kirkham, West Lafayette, Ind.; Dr. Ralph C. Knowles, Hyattsville, MD.; Dr. T. S. Maddox, Frankfort, Ky.; Dr. Donald Mossbarger, Bloomingburg, Ohio; Mr. Michael Nolan, Washington, D.C.; Dr. S. R. Nusbaum, Trenton, N. J.; Dr. D. D. Philson, Hyattsville, Md.; Mr. Wilson Powell, Tallahassee, FL.; Dr. Victor Schroeder, Mexico, D.F., Mexico; Mr. John Smiley, Augusta, Me.; Dr. James Smith, Lexington, KY.; Dr. M. B. Teigland, Miami, FL.; Dr. Charles D. Vail, Littleton, CO.; Dr. Thomas E. Walton, Jr., Lakewood, CO.; Dr. Elina White, Weimar, TX.; Dr. Sam Winkelmann, Austin, TX.

The Committee on Infectious Diseases of Horses convened on October 29, 1979, with an excellent representation of membership in attendance.

The initial topic requiring much Committee deliberation was that of the regulations proposed by the U. S. Department of Agriculture for the importation of stallions into the United States from countries affected with Contagious Equine Metritis. This proposal as published in the August 3 Federal Register had heretofore allowed receipt of comments by October 2; however, the Department has currently extended this period for an additional 45 days. The Committee appreciates this consideration to afford opportunity for input from the U.S. Animal Health Association and other interested organizations and persons intimately affected by the ultimate decision. Since this proposal involves the importation of animals from affected countries, the Committee on Import-Export, feeling that it could benefit by the discussions, met with us and provided sage advice in developing our recommendations. Their action on the matter will be presented in the report of the Committee on Friday morning.

As the discussion on the matter ensued, it developed that while most members recognized that the procedures developed for treating stallions at the point of debarkation were adequate if properly performed, there was concern that the efficacy of such treatment procedures were entirely dependent upon the integrity of the person performing the procedures at the point of debarkation, since USDA seems reluctant to implement any treatment procedures on such animals after arrival at the import station. The Committee was advised of regulations of the State of Kentucky...
which provide for additional treatment of stallions imported from any country outside the continental United States, its territories and possessions. The Kentucky regulation includes any stallion for breeding which had at any time been present in such country for any purpose other than racing. It is the recommendation of the Committee that in the event the proposed amendment is adopted by USDA that requirements for post import cleansing and treatment procedures of stallions imported from any country outside the continental United States, its territories or possessions, such as those that have been adopted by Kentucky, be considered for adoption by the various states as precautionary post entry measures. These requirements can now be found in the Kentucky Code of Practice for handling Contagious Equine Metritis.

The Committee further proposes that in the event the proposed Amendment is adopted, USDA should assist the states in providing diagnostic assistance and personnel to follow through with post import treatment procedures, if requested.

*[After considerable discussion by Committee members and others present, it was determined that the Committee should recommend adoption of the proposed Amendment to Title 9, Part 92, on the importation of stallions from CEM affected countries, as published in the August 3 Federal Register.]*

The Committee received a report on work which has been done toward improvement of the equine infectious anemia agar gel immunodiffusion test and endorses the changes recommended in the official protocol for conducting the test in approved laboratories as soon as practicable. A copy of the report is attached to this Committee Report.

The Committee discussed a proposed rule amendment by the Deputy Administrator of APHIS which would discontinue the equine piroplasmosis test on horses being imported into this country. Previous objections to this proposal have been voiced but it is apparent that the proposal is still receiving consideration. The Committee wishes to iterate its strong urging that this import test requirement be continued.

A proposed change in USDA procedures in the importation and quarantine of Mexican horses consigned to slaughter was brought to the attention of the Committee. It was felt that the controls over these imported animals before going to final destination was not adequate and the Committee opposes any changes in the rules relating to the identification and final disposition of these animals.

The Committee discussed the EIA test requirements for Canadian-U.S. import-export horse traffic and a proposal that test interval be changed from 30 days to 90 days. The American Horse Council and the American

* *(The Executive Committee of the USAHA did not concur in this recommendation, and adopted that of the Committee on Import-Export recommending rejection of the proposed Federal regulation).*
Association of Equine Practitioners have recommended the test interval be increased to 6 months. In view of the high level of EIA control in Canada and the economic burden of frequent testing of the classes of horses traveling between the two countries, the Committee recommends that the test interval be increased to 6 months.

In last year’s report, this Committee voiced objection to a proposal to permit entry of horses into the United States at any port designated by U.S. Customs as an international port or airport where a quarantine facility has been provided by the importer. The objection was based on information that no procedures were included to restrict such importations to horses for competitive events on an infrequent basis. The Committee recognizes the need for adequate import facilities for horses in competitive events and a subcommittee will be appointed to study this problem and make recommendations to this Committee next year for setting up standards to permit horses in competition to be imported without jeopardizing the health of our domestic equine population.

The Committee was privileged to receive an update from Dr. Victor Schroeder, Chief of Equine Health from Mexico, on the Venezuelan Equine Encephalitis vaccination and surveillance program in that country. We wish to commend Dr. Schroeder and the government of Mexico in these efforts which serve materially as a deterrent to the introduction of this disease into the United States. Pertinent data concerning morbidity, mortality and vaccinations during the past few years in Mexico is presented in the following chart.

Committee member Floyd Jones provided the Committee with pictorial information on a horse disease survey on VEE conducted by him and two of his counterparts from El Salvador and Honduras.

IMPROVEMENT OF THE EQUINE INFECTIOUS ANEMIA AGAR GEL IMMUNODIFFUSION TEST

J. E. Pearson and J. W. Black

INTRODUCTION

The agar gel immunodiffusion (AGID) test is widely recognized as a dependable method for the serological diagnosis of equine infectious anemia (EIA). The technique described by Coggins and Norcross1 was accepted with only minor changes as the official protocol2 for conducting the test.

This report will describe 3 modifications of the EIA AGID procedure and the results of an evaluation of these techniques as compared to the standard technique.

From the National Veterinary Services Laboratory, Animal and Plant Health Inspection Service, P. O. Box 844, Ames, IA 50010 (Pearson) and C. E. Kord Animal Disease Laboratory, Nashville, TN 37204 (Black).
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** Jornada de vacunación

*** Programa nacional de vacunación: 5.7 millones

1/ Total reportes de vacunación en México: 1.2 millones.

2/ Información parcial hasta mayo. La programación es de 1.2 millones.

3/ Estimados

- Borunda y colaboradores, 1973
MATERIALS AND METHODS

Agar Gel Immunodiffusion Techniques

The AGID technique as described by the Committee on Equine Infectious Anemia of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) was used as the standard for this comparison. The method as described in the AAVLD procedure was followed except that the size and location of the wells cut in the agar were changed. Therefore, all the techniques were compared using the same 7-well pattern with one center well and 6 wells in a circle around it. Antigen was placed in the center well and positive control serum was placed in wells on each side of the sample to be tested. Commercially available antigens and positive control serums were used.

Seven mm diameter wells 3 mm apart were stipulated by the AAVLD approved technique. A comparison was made between this standard technique and the following: 1) a pseudorabies (PR) slide technique described by Gutekunst in which 4.4 mm wells 3.0 mm apart were used. 2) a method described by Kanitz for bluetongue (BT) using a commercially available cutter and template with 5.0 mm wells 3.0 mm apart, 3) a method developed by Black using 5.3 mm wells 2.4 mm apart. The template used by Black is shown in Figure 1. The thickness of the agar used in the PR technique was 2.0 mm while with all of the other techniques the agar was 2.8 mm thick. Using the method described by Black, 7 of the patterns were used in 100 mm X 15 mm Petri dishes or four patterns were used in commercially available 45 mm X 95 mm trays. The standard technique has 4 patterns on a 100 mm Petri dish. Sixteen ml of agar was used in the 100 mm Petri dishes and 13 ml in the trays. The standard technique has 4 patterns on a 100 mm Petri dish. Sixteen ml of agar was used in the 100 mm Petri dishes and 13 ml in the trays.

Comparison of Techniques

The test findings of all the techniques were compared against those of the standard technique. The evaluation was based on the capability to detect samples ranging from weak to strong positive reactions, the quality of the precipitin line formed, and the length of time required before the precipitin reaction was complete.

The initial comparison was performed at the National Veterinary Services Laboratories (NVSL) using all 4 techniques on a 30-sample proficiency check test. The check test contained samples that produced the following reactions: very weak positive (5 samples), weak positive (8 samples), positive (6 samples), and negative (11 samples). The techniques were also used to test 50 field samples of which 20% were positive.

The method described by Black was subsequently selected for an indepth parallel evaluation against the standard technique. They were

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a Pitman Moore, Inc., Washington Crossing, N. J.
b Miles Laboratory, Inc., Elkhart, IN. 46514
used at NVSL to test the above proficiency test 5 different times and to
test 1,000 field samples of which at least 20% were positive. The
proficiency test samples were coded and were tested by 3 people, all of
whom had been trained to perform the EIA AGID test. The results were
interpreted by 5 EIA competent workers. Black's method was evaluated
against the standard method by the following laboratories: Cornell
University (Dr. Leroy Coggins), Ithaca, NY; Iowa State University (Dr.
Howard Hill), Ames, IA; Louisiana State University (Dr. Charles Issel),
Baton Rouge, LA; C. E. Kord Animal Disease Laboratory (Mr. John
Black), Nashville, TN; and Pitman Moore, Inc., Washington Crossing, N.J.

Each laboratory used both techniques on the above described
proficiency test and at least 25 field samples. C. E. Kord Laboratory
tested 39,946 field samples in duplicate; approximately 450 of these
samples were positive. Sixteen students in 2 classes of a course given at
NVSL to teach the EIA AGID technique evaluated Black's technique as
compared to the standard technique. The students tested the above
proficiency test plus 20 more samples that were very weak to weak
positive.

RESULTS

On the initial comparison of all the methods it was determined that
the PR technique was unsatisfactory. The very weak positive and many
of the weak positive samples were not consistently detected by this
technique.

The BT technique was marginal in that some of the very weak positive
samples were not always detected. However, all the weak positive
samples were detected. The reaction was slower with the BT technique
as it usually took 48 hours before the reaction was complete.

The in-depth comparison at NVSL determined that the accuracy and
the quality of the reaction with Black's technique equaled or surpassed
the standard technique. The reaction with Black's technique was usually
complete at 24 to 36 hours, which is 24 to 36 hours sooner than with the
standard technique. The wells of Black's technique required only 0.06 ml
of reagents as compared to 0.1 ml for the standard technique, and 0.05 ml
for the BT technique.

The results of the student evaluation of the standard as compared to
Black's technique was that the two were equal in quality and accuracy.
The other laboratories that evaluated Black's technique unanimously
agreed with the above results and recommended the adoption of the
technique as the standard method.

DISCUSSION

The conclusion of the people participating in this evaluation was that
the 5.3 mm template produced equal or better results than the standard 7
mm template. The advantage of the smaller wells is that the reaction is
faster and less reagents are used.
SUMMARY

Three different well sizes and arrangements were compared to the standard method for performing the EIA AGID test. The accuracy of a technique which used 5.3 mm wells 2.4 mm apart equaled the standard method that is now approved. The wells of the new method hold only 0.06 ml of antigen or serum as compared to 0.10 ml for the standard method. The test using the smaller wells can be read 24 to 36 hours earlier than the standard method. The test was evaluated in 6 laboratories with unanimous agreement that the new technique was better than the present one.

Metal template which produces a 7-well pattern with 5.3 mm diameter wells 2.4 mm apart.
REFERENCES


ISOLATION UNITS FOR QUARANTINE
OF PERSONALLY OWNED PET BIRDS

Keith Hand,* D.V.M., E. C. Sharman,** D.V.M.

Following the introduction of velogenic viscerotropic Newcastle disease virus (VVND) into Southern California in late 1972, the United States Department of Agriculture (USDA) placed a ban on bird importations. In 1974 USDA promulgated regulations to prevent the introduction of this virus into the United States. Privately owned quarantine stations were approved for the importation of large commercial lots of birds. Personally owned pet birds were allowed entry provided they were accompanied by the owner, and at the port of entry the owner signs and furnishes to the Deputy Administrator, Veterinary Services, a notarized declaration under oath or affirmation a statement signed by the owner and witnessed by a Department inspector stating that the bird or birds have been in his possession for a minimum of 90 days preceding importation and that during such time such birds have not been in contact with poultry or other birds and an agreement on a form obtainable from a Federal inspector at the port of entry stating (1) that the birds will be maintained in confinement in his personal possession separate and apart from all poultry and other birds for a minimum period of 30 days following importation at a place approved by the Deputy Administrator and will be made available for health inspection and testing by the Department inspectors upon request until released at the end of such period by such an inspector.

Since the beginning of the program, difficulties in enforcing the personally owned pet bird import regulations have been experienced. Usually, the owners' residences were the designated quarantine point in the United States. In some cases, when an inspector attempted to locate the recently imported birds, they would find a vacant lot at the address given by the owner or a fictitious address that could not be located. In one case, an imported personally owned pet bird was advertised for sale in the local newspaper immediately following importation. Much manpower was lost trying to locate and inspect imported personally owned pet birds.

In early 1978, a committee was appointed to review the entire bird import program and make recommendations for improvement. One recommendation was that an isolation cage be developed and all personally owned pet birds be quarantined and tested in a manner similar to the commercial lots of birds. The committee discussed at some length the requirements and design of an isolation unit.

A model isolation cage for trials was constructed. The model unit was

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* Regional Poultry Epidemiologist, USDA, APHIS, VS.
** Senior Staff Veterinarian, Import/Export, USDA, APHIS, VS.
constructed of plexiglass with filters for intake and exhaust air. A small fan installed in the neck of the exhaust plenum pulled the air through the filters and $2\frac{1}{4}' \times 2\frac{3}{4}' \times 2\frac{3}{4}'$ cage.

A closed system for introducing food and water was devised to prevent the necessity of opening the cage during quarantine. The closed systems consist of plastic containers filled with feed or water with a plastic tube attached to the mouth of the container and passing through the top of the unit to the feed or water container inside the isolation unit.

A stainless steel removable tray $2''$ deep covered with $\frac{3}{4}$-inch mesh wire was constructed to fit into the bottom of the unit to catch all refuse. The tray can be removed following 30-day use for easy cleaning.

Trials were conducted with the model unit using adult chickens and pet birds. The birds remained obviously healthy during this 30-day test period, however, a trial using larger numbers of birds and cages as well as biological agents was needed to prove the feasibility of quarantining birds for 30 days in such an isolation unit.

MATERIALS AND METHODS

Specific pathogen-free five-week-old chickens were placed in twenty-five isolation units (two per unit) equipped with biological filters on both the intake and exhaust sides comparable to the model cage described. In addition, four units were used as controls with three chickens per unit, except they were without filters. Ten filtered air-cage units were located in one building along with two unfiltered control units using negative pressure. These units pulled air from inside the building and exhausted the air through a common six-inch square metal duct to the outside of the building.

Ten units with filters were located in another building. Air intake was outside the building, and after the air passed through the cages, it was exhausted inside the building. Five additional units with air intake from inside the building plus two other nonfiltered units were in the same room with the positive pressure units. The buildings were separated by a distance in excess of one-half mile.

All units were equipped with $2\frac{1}{2}$-gallon plastic jugs that served as feed storage containers. These jugs were attached to the feed spout and sealed using a silicone rubber sealant as were all connections relating to the duct work. Filters were held in place by furnace-type duct tape. The chickens were watered by a common plastic tubing with cut-off valves for each unit.

All of the chickens were bled and swabbed (tracheal and cloacal) prior to placement in the units. After placement, every other unit was sealed. The remaining chickens were then inoculated by interocular and in-

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*a USDA Contract Number 3294-54-8-2, dated March 24, 1979
*b Owens Corning fiberglass filtration media-type FM004
ternasal routes using a B1-type La Sota strain Newcastle virus (NVD) in combination with Massachusetts and Connecticut type infectious bronchitis virus (IBV) vaccine. A drop of the vaccine was placed in a vial of Brain Heart Infusion (BHI) broth prior to and immediately after the chickens were inoculated to check the viability of the vaccine.

The twelve chickens were placed in the four units without filters before any birds were inoculated. Since there was some possibility for exposure during inoculation, swabs (T & C) were collected from this group at post-inoculation day five of the field trial.

The field trial lasted 31 days. Blood for serum was collected from the inoculated chickens. All chickens were euthanatized and tissues (lung, trachea, spleen, colon, and brain) as well as blood were collected from uninoculated chickens.

All of the samples collected at day zero of the field trial were negative for antibodies and virus. Newcastle virus was isolated from the two vials containing a drop of vaccine, however, the infectious bronchitis virus was not recovered. This could have been due to an overwhelming amount of Newcastle virus in the same vial.

No virus was isolated from any of the tissues from non-inoculated chickens including those units without filters and none seroconverted.

All inoculated chickens except one developed antibodies to both Newcastle and infectious bronchitis virus. Results are illustrated in Table I.

RESULTS

Both the positive and negative pressurized cages apparently contained NVD and IBV, since all inoculated chickens developed antibodies except one failed to develop IBV antibodies, and since none of the uninoculated chickens in the same buildings developed antibodies to either virus.
**SEROLOGY**

**TABLE I.**

Building with Negative Pressure Units - Inoculated Chickens

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*\( \log_{10} \) of protection in embryonated chicken eggs.
Pet bird isolation cage 2 ft. x 2 ft. x 2 ft. Constructed of 1/4-inch plexiglass and stainless steel. Air filters 20 in. x 20 inches for both intake and exhaust.
INSIDIOUS METHODS BY WHICH FOREIGN ANIMAL DISEASE MAKES AN ENTRY INTO THE UNITED STATES

J. S. Walker and J. J. Callis,
U.S. Department of Agriculture
Greenport, New York 11944

Constant vigilance and the efforts of many different agencies, organizations and people have helped U.S. livestock remain free of many foreign diseases. However, it is increasingly evident that this task is more and more difficult, and the future does not look bright. Diseases, both human and animal, are on the move around the world, and the rate at which they can move is extremely rapid. For example, parvovirus infection of dogs recently appeared almost simultaneously on five continents. The rapid spread of this disease was apparently associated with several international dog shows. A second canine disease recently imported into the United States is Babesia gibsoni, a disease of domestic and wild canines of Africa and Asia. The index case was a standard poodle living in Ridgefield, Connecticut. Since this dog had never been out of the United States, the infection must represent a secondary case of an imported disease. This can be taken as presumptive evidence that we now have a new disease of dogs in the United States.

Contagious equine metritis (CEM) is another new disease that has involved three continents and at least eight countries in the past two years. It has actually been introduced into the United States twice. Massive educational and control programs were deployed to control this disease. That this is a venereal disease associated with thoroughbred horses helped in the rapid identification and treatment of CEM. Years ago it would have taken 10 or 15 years for such new diseases to spread so extensively, if they spread at all. However, airplane travel has changed the entire picture and will continue to do so in the future. For example, in the thoroughbred business, it is not uncommon for a mare bred in Kentucky this spring to drop her foal in England next spring, where she is bred and flown to Ireland; after dropping her foal, she may be bred and brought back to Kentucky for the third breeding season. The point is that animals are moving around the world with increasing frequency.

All kinds of exotic animal species are in great demand for exhibition, for research, or for pets. The story of exotic Newcastle disease and pet birds is a familiar one in the United States. Just when everything appears under control and all the holes have been plugged, new ways are found to import potentially infected birds; this has happened twice within just the last few months. Two other incidences within the last year are less well known. One involved the importation of snakes from Ghana.

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Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.
FOREIGN ANIMAL DISEASE

into New York. Within a few days, nine out of eleven employees who worked in or around the snake building of the pet shop became ill. Investigation revealed that these people had Q fever. This was the first outbreak of Q fever on Long Island in recent memory. Epidemiological investigation revealed that the snakes, particularly *Python regius*, were heavily infested with ticks upon arrival and the ticks had been picked off the snakes by hand. The ticks were of the aponomma genus, involving three species. It has recently been reported that several poikilotherms can act as reservoirs of Q fever. What is unsettling is that no controls whatever govern the importation of poikilotherms. Little, if anything concrete, is known about their involvement in the spread of economically important animal diseases. This is but one example whereby a zoonotic disease was imported into the United States via an exotic species and the airplane. In this instance the consequences were not as severe as they might have been. All the employees eventually recovered from their Q fever infections.

A second incident attracted more attention, and before it was over, involved four Departments of the U.S. Government — USDA, HEW, DOD and Interior — plus the WHO Arbovirus Laboratory at Yale. The story starts in the Sudan in Africa where USAID had a project involving rodent control. USAID asked the Department of Interior to do a bait study for them. Rather than spend the money and time to go to the Sudan to do the study, the Dept. of Interior had some of the Nile rats (*Arvicunthus niloticus*) trapped and sent them to their laboratory in Denver. The rats started dying soon after arrival and one of the animal handlers got sick. The local physician contacted CDC, which contacted Plum Island because the rats were from an area south of Khartoum in the Sudan where Rift Valley fever is occurring. Work with these specimens at the PIADC was not begun because some highly lethal viruses, such as Ebola and Lassa fever, which occur in the Sudan may be transmitted by these rats. Indeed further investigation revealed that *Arvicunthus niloticus* rats can carry many viruses (Table 1). Thus, all work involving isolation was done at CDC and the U.S. Army Med. R&D Command Laboratory, USAMRIID at Frederick, Maryland. Serological studies were done at the WHO Arbovirus Laboratory at Yale. Although work at USAMRIID is not completed, some rats were serologically positive for Rift Valley fever, although no virus was isolated from the submitted specimens. The animal holding facilities at the Denver laboratory were not adequately screened and an insect vector might have escaped. Apparently we were lucky again. Work is continuing because serological studies at three different laboratories have revealed that several viruses may have been erroneously classified. The end result will probably be a major change in the classification of several arboviruses.

Thus, importation of exotic species continues to present real and constant dangers. The importation of major zoo species is under tight control and poses a minimum hazard. Many exotic species are not con-
trolled, or their importation is under only minimal control. Table 1 graphically presents examples of some of these hazards.

Another area that is overlooked by many regulatory veterinarians is the possible importation of a zoonotic disease via humans. We constantly worry about luggage and what might be smuggled in, but tend to forget about the person carrying the luggage. Table 2 presents a list of zoonotic diseases of economic importance that could be imported into the United States any day by a person returning from overseas, particularly from the remote areas of Africa and Asia. We tend to dismiss this concern until we realize that diseases such as malaria are imported into the United States at the rate of approximately 500 cases a year. Likewise, Cholera, a disease transmitted by other than arthropods was recently imported into Louisiana where secondary cases occurred around a lake. It has since been brought under control. It is extremely likely that at least several of the diseases listed in Table 2 are imported into the United States every year. Why have these diseases not appeared in livestock or caused an epidemic in the human population? The answer is that a "hit" has not occurred in the right place at the right time. In our opinion, the probability of such a "hit" is high and, as traffic between the remote areas of the world and the United States increases, the time until such a hit occurs is getting shorter. Recall that dengue fever, a disease of southeast Asia, has been imported not once, but several times during the 1970's into the Caribbean Islands where it has caused epidemics. Examples are all around us; all we have to do is look.

Although many other examples can be given of the insidious ways that exotic diseases find their way into the United States, suffice it to say that as we increase our foreign travel and increase the importation of just about everything imaginable, the risk goes up. As veterinarians, we tend to think only in terms of the classic exotic animal diseases, such as FMD, ASF or rinderpest. In today's world we must constantly be on the lookout for any outbreak that doesn't fit the picture of our domestic diseases.
TABLE 1

Seroconversion in Arvicanthus Rats, Cattle, Sheep and Goats to African Viral Diseases*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rift Valley Fever</td>
<td>Bwamba</td>
</tr>
<tr>
<td>Wesselsbron</td>
<td>Nyando</td>
</tr>
<tr>
<td>O'Nyong Nyong</td>
<td>Spondweni</td>
</tr>
<tr>
<td>Sindbis</td>
<td>Zika</td>
</tr>
<tr>
<td>West Nile</td>
<td>Germiston</td>
</tr>
<tr>
<td>Ntaya</td>
<td>Pongola</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Foreign Zoonotic Diseases of Economic Importance to the Livestock Industry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fowl Plaque</td>
</tr>
<tr>
<td>Glanders</td>
</tr>
<tr>
<td>Japanese Encephalitis</td>
</tr>
<tr>
<td>Louping-Ill of Sheep</td>
</tr>
<tr>
<td>Melioidosis</td>
</tr>
<tr>
<td>Rift Valley Fever</td>
</tr>
</tbody>
</table>
FOREIGN ANIMAL DISEASE

REFERENCES


5. Personal Communication, Dr. M. Mayer, Deputy Director, Division of Disease Control and Environmental Health, Suffolk County, NY.

REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: Glenn B. Rea, OR
Co-Chairman: Clint Booth, TX

Ron Caffey, DC; R. L. Evinger, OR; John H. Gray, TX; Frank Harding, IL; Royce Henderson, TX; C.E. Herrick, ME; Bob Mathis, AZ; E. G. Ongert, MD; R. H. Rumler, VT; E. C. Sharman, MD; Donald H. Spangler, WA; H. M. Steinmetz, DC; J. E. Thomas, NV; J. S. Walker, NY; B. D. Ward, MN

The meeting of the Import-Export Committee was called to order at the scheduled time and place, with thirteen (13) of the seventeen (17) members present.

The first order of business was a quick review of last year's report including the appended resolutions.

Dr. E. C. Sharman was then asked to review the past year's activity of the Import-Export Staff. After making a few general statements, he introduced several staff members who provided the committee with pertinent details.

On the Canadian Border in FY 1979, a total of 449,481 animals were permitted entry and 6006 were rejected. Of this total, a breakdown by species shows as the following:*  

<table>
<thead>
<tr>
<th>Species</th>
<th>Entered</th>
<th>Rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>335,620</td>
<td>5,621</td>
</tr>
<tr>
<td>Swine</td>
<td>137,215</td>
<td>303</td>
</tr>
<tr>
<td>Sheep</td>
<td>9,961</td>
<td>29</td>
</tr>
<tr>
<td>Horses</td>
<td>15,201</td>
<td>53</td>
</tr>
<tr>
<td>Others</td>
<td>1,484</td>
<td>6,006</td>
</tr>
</tbody>
</table>

For the Mexican border, the summary shows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Entered</th>
<th>Rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>636,659</td>
<td>64,755</td>
</tr>
<tr>
<td>Horses</td>
<td>1,429</td>
<td>1,433</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>638,088</td>
<td>66,188</td>
</tr>
</tbody>
</table>

*Figures include estimate for fourth quarter.

For the air and ocean ports:

<table>
<thead>
<tr>
<th>Species</th>
<th>Entered</th>
<th>Rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>249</td>
<td>0</td>
</tr>
<tr>
<td>Swine</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sheep</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Horses</td>
<td>1,224</td>
<td>5</td>
</tr>
<tr>
<td>Goats</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,623</td>
<td>7</td>
</tr>
</tbody>
</table>

Swine are prohibited entry from Mexico because of hog cholera, while sheep and goats are not permitted entry because of scrapie.
The reason for the rejections was asked. The majority of rejections were caused by improper forms on presented animals and not meeting quarantine requirements.

Export Animals — All livestock offered for export were inspected for proper health certification, proper identification, and evidence of disease. The following table summarizes the overseas export of livestock during the past two years.

<table>
<thead>
<tr>
<th>Livestock Exported</th>
<th>Canada</th>
<th>Mexico</th>
<th>Overseas</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>41,452</td>
<td>25,135</td>
<td>54,418</td>
<td>121,005</td>
</tr>
<tr>
<td>Horses</td>
<td>39,745</td>
<td>240</td>
<td>2,947</td>
<td>42,932</td>
</tr>
<tr>
<td>Sheep</td>
<td>102,695</td>
<td>81,445</td>
<td>1,898</td>
<td>186,038</td>
</tr>
<tr>
<td>Swine</td>
<td>184</td>
<td>2,392</td>
<td>7,844</td>
<td>10,420</td>
</tr>
<tr>
<td>TOTAL ANIMALS</td>
<td>184,076</td>
<td>109,212</td>
<td>67,107</td>
<td>360,395</td>
</tr>
</tbody>
</table>

| Poultry            | 9,791,333| 568,687| 20,310,141| 30,670,161|
| Hatching Eggs      | 8,964,541| 39,807 | 10,048,141| 19,052,489|

There were two resolutions regarding export animal inspection last year.

1. Modification of Canada’s bluetongue test requirements.

2. Development and recognition of laboratories fully qualified to perform testing of livestock to qualify for export from the United States.

A proposal was presented to Canada by Dr. Chaloux in July 1979 requesting that consideration be given to 21 Northern States for eligibility to enter Canada based upon one bluetongue test. Canada is considering this proposal but wishes to know if animals from other states entering these states could be tested negative to two tests prior to entry. This could be controlled by import permits being issued by the 21 above-mentioned states.

The development of laboratories on a regional basis for export testing of animals was considered. It is against Veterinary Services policy to have regional laboratories.

EEC countries are considering banning entry of U. S. cattle because of bluetongue. Dr. Chaloux is conferring with EEC officials.

A proposed rulemaking was reported which would delete the presently required test for equine piroplasmosis. It has not been published in final form, though there was strong public response to do so. The reason for the delay was that the drug Immidocarb produced by Burroughs Wellcome was not available in the U. S. in sufficient quantities for private use. The implementation of this proposal by the Staff is presently dependent upon approval, by the F.D.A., of the production of Immidocarb in Great Britain. This may take approximately another eight to nine months.
REPORT OF THE COMMITTEE

After a lively and occasionally heated debate on the subject, the Committee feeling that the pending proposal would be detrimental to the National interest, voted seven (7) to two (2) with three (3) abstaining (the Chair not voting) to endorse the stand of the Committee on Infectious Disease of Horses, which rejected the proposed rulemaking.

Another proposed rulemaking discussed was that of the importation of stallions into the U. S. from countries affected with Contagious Equine Metritis. That portion of the report of the Committee on Infectious Diseases of Horses was read. As discussion on the proposal developed, consideration to the Kentucky Code of Practice was given. No one denied the efficacy of the Kentucky approach, but several illustrations of movement of livestock by "white washing" or "laundering," were heard. These methods of evading approved procedures caused alarm. Testimony to difficulty in recovering the CEM organism from the stallion is parallel to that of recovering vibriosis from the bull. This increased the concern already expressed. The possibility of one or more infected animals causing the development of a foci of infection in a part of the U. S. not alert to equine diseases in general let alone CEM greatly influenced the committee. The disease then could be chanelled back into the heavily populated horse areas without any reference to foreign importation.

The difficulty and sometimes failure to treat fractious stallions, by experts, raised a question as to the results of such treatment in the hands of the inexpert. Kentucky, because of its horse population and its equally numerous expert equine practitioners, can provide adequate protection from this disease if the origin of its imports are known. What would happen to the Kentucky horse industry if an infected mare was imported to Kentucky from some state not having that expertise and through which some infected foreign stallion had been "laundered?"

After a lengthy indepth and sometimes heated debate, the committee voted 9 to 0 (the chair not voting) to oppose the proposed rule change (there were three obstinations) and to submit a resolution to this effect.

Several other proposed rulemakings and changes were presented. These had to do with importation of poultry, swine, cattle and horses from Mexico and Canada. The committee found no objections.

A problem in granting import permits for sheep from Australia was discussed. A new strain of bluetongue and several other anthropod bovine viruses, such as Akabane, Aino, and Peaton, were found to exist in that country. Finally, an acceptable protocol was established whereby importation into the U. S. could be allowed. This protocol was explained to the committee.

In connection with the opening of the H.S.T. Import Quarantine Station at Fleming Key, problems involved in approving the requested five hundred and eighty-five (585) animals were explained. As a result of these problems, only three hundred and fifty (350) head are expected to be eligible for the first lottery.
Organisms and Vectors

Two hundred and twenty-six organisms and vectors were permitted interstate movement and four hundred and forty-six (446) were authorized importation. Two hundred and seventeen (217) permits were issued for movement of animal and poultry blood fractions, serums, and tissue specimens, either for research or diagnostic purposes. Three hundred and thirty-eight (338) bacterial and viral cultures (inactivated organisms, toxins, and antitoxins) for identification and research, and thirty-four (34) permits for biological material in the vector class, which included ticks, flies, and other insects, and internal parasites, both live and preserved. The USDA Lab at Ames, Iowa, received sixty-three (63) permits and Plum Island received sixty-one (61). Sixteen (16) requests for permits were denied.

The committee heard that more research was needed with regard to milk products and by-products. This need dealt with time and temperature requirements. Similar work is needed in certain other products.

Dr. Ron Caffey, Asst. to the Deputy Administrator for Plant Protection and Quarantine Programs, reported on the past year's activity. Statistics from his report follow:

**Plant Protection and Quarantine Programs**

Port Inspection Activities — FY 1979*

**Garbage Disposal**

2,986,630 pounds of garbage removed from vessels and 10,362,891 pounds of garbage removed from aircraft was disposed of in an approved manner.

**Passenger Baggage Inspection**

783 lots — 4,154 pounds meats and animal products were confiscated from ships' passenger baggage

65,656 lots — 186,416 pounds meats and animal products were confiscated from airplane passenger baggage

27,498 lots — 75,381.25 pounds meats and animal products were confiscated from land/border passenger baggage

1,315 pairs of shoes were cleaned and disinfected at U. S. ports of entry.

**Post Office/Mail Inspection**

4,225 lots — 13,431 pounds of meats and animal products were removed from international mails.

*FY '79 ended September 30, 1979, therefore, the figures reported above may be adjusted slightly following review of monthly reports for September.
Commercial Importation of Animal Products

25,282 lots — 1,101,094,376 pounds were inspected and 457,058,002 pounds restricted entry passed for entry, 644,036,374 pounds unrestricted entry.

The committee was told of the confiscation of contraband product which included Spanish sausage. Although ship stores and garbage are under surveillance, crew members can apparently bring contraband items such as sausage and other items of food without interference. Proposals for surveillance of this activity have to be presented.

Dr. Sharman next presented a paper concerning the importation of birds, showing the results of experimentation and research with closed environment cages for the purpose of enhancing bird quarantine. This paper is being presented for separate publication in the proceedings book.

The paper just presented by Dr. J.S. Walker was reviewed. The committee was alarmed at some of this information, and a resolution pertaining to efforts to prevent or at least more adequately supervise such imports has been presented to the Resolutions Committee for separate action.

The recent report of the Comptroller General of the U. S. Study — GG-79-84 — was discussed. This report requested that the involved agencies, i.e., Customs, Immigration, and Agriculture, revise their inspection procedures to the end that passenger processing at ports of entry be speeded up. This report stated that passenger retention time could be reduced without risk. By using the very figures projected by the authors of the report, it was determined that one-tenth of one percent of the two hundred and ninety-four (294) million passengers left two hundred and ninety-four thousand arriving in the U.S. with material in violation of U.S. Import laws and regulations. Further, that the two hundred and sixty-five thousand nine hundred and forty-one (265,941) pounds of restricted product confiscated could easily provide entry of any one of a variety of exotic diseases, which USDA officials have estimated might possibly cost the general public up to twelve (12) billion dollars to control and eradicate along with untold damage and loss of international markets and the ensuing damage to the U. S. balance of trade. The “Green Door-Red Door” method whereby a traveler decides whether he has anything to declare without any interrogation, such as some European Countries employ and as suggested in the Comptroller's report, is just not acceptable. Pre-investigation at foreign embarkation points is acceptable both to Immigration and Agriculture but not to Customs. A resolution concerning these recommendations has been forwarded to the Resolution Committee.

The subject of private quarantine stations for horses imported for competitive events was discussed, but no action taken.
A resolution was presented by The Holstein Association, which requested that Veterinary Services and the several university and/or state animal health diagnostic testing laboratories to develop complete facilities for the purpose of providing tests and diagnostic results to enhance and simplify the exportation of livestock to foreign countries.

The committee heard from representatives of the Import-Export Staff and the Director of the National Veterinary Services Laboratory to the effect that: (1) Veterinary Services is not in position to designate or to insist that states or universities provide "Full Service" laboratory facilities; (2) The NVSL will and does provide training and reagents to state and university laboratories; (3) Presently all such requests have been met; (4) that if and when such services are needed, requests should be made to existing facilities in the exporters' local area; (5) Finally — in effect the proposed resolution would require all such laboratories to take training, stock reagents, and conduct check tests for many diseases of species that are never exported from a given area. Such a system would be wasteful and counter-productive.

With this information, the committee decided not to present the proposed resolution.

There being no further business, the meeting was adjourned.
Pesticides are defined in Dorland's Illustrated Medical Dictionary as poisons used to destroy pests of any sort. Their regulation by our federal bureaucracy is nearly as all encompassing as the definition. Prior to 1972, most pesticide regulation was under the auspices of the USDA. However, with the advent of the Environmental Protection Agency, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) has been administered by that agency.

Almost immediately after one begins to think about pesticides, a question arises about the difference between a pesticide and a drug, and whether or not a particular pesticide should be considered a drug rather than a pesticide. EPA and FDA apparently thought of this same question and, late in 1971, attempted to define the responsibilities for pesticides and appropriately divide them between the two agencies. Incidentally, although the agreement was apparently reached in December of 1971, it was not published in the Federal Register until the 6th of September 1973.

Under the terms of that agreement, pesticides were divided into five classes: products considered to be human drugs; products considered to be both pesticides and human drugs; products considered to be animal drugs; and products considered to be pesticides. In general, FDA is responsible for regulation of those products that are human or animal drugs. The products considered to be both human drugs and pesticides are the responsibility of EPA so long as the primary use of the product is on inanimate objects and the human label claim is secondary.

The real confusion begins to begin when the specifics of which product is an animal drug and which product is a pesticide are considered. There are about five categories used to establish that a product is an animal drug; however, several of those categories have exceptions to complicate the issue. Usually, if a product is used parenterally, orally, or in wounds or body openings, is absorbed through the skin or is applied topically for its systemic action, then it is considered to be an animal drug. Exceptions include products given orally but acting in the manure after leaving the body, and products for treatment of screw worms. Except for the exceptions these products are under the jurisdiction of FDA.

Most products that are both animal drugs and pesticides come under FDA's jurisdiction. Oral or parenteral products for treatment of horse bots, cattle grubs, fleas, lice, demodectic mange, ear mites, and ticks if the product is labeled for use against ear mites, and aquatic treatments for parasites and diseases of fish are primarily regulated by FDA. Conceivably, a product that is effective against the spinose ear tick and
used in the ear would be regulated by FDA. Yet, the same identical product could be labeled for use against the brown dog tick and be regulated by EPA. There is another exception that applies in this area and that is, if a product is considered not to be a new animal drug, then it is not subjected to FDA requirements and comes under EPA jurisdiction.

The fifth group of products under this FDA-EPA agreement is one for which EPA has primary responsibility. Included are the pesticides for topical administration for control of cattle grubs, screwworms, wool maggots, horn flies, face flies, mange mites, ticks, sheep keds, and fleas. Also included are treatments administered orally that control horn flies or face flies in cattle manure, aquatic treatments that are not used for control of fish parasites, sanitizers for aquarium equipment, and sanitizers for inanimate objects or water treatments not aimed at disease control.

The cork on this can of worms has to be the last paragraph of the agreement that advises manufacturers, who aren't sure which agency would be in charge of regulating a particular product, to get in touch with either EPA or FDA.

This EPA-FDA agreement is presently being revised. One would hope that the revised version would clarify matters, but as complex as the issues are, it's more likely that the next agreement will be more complicated.

The next big link in the bureaucratic chain of pesticide regulations is the division by EPA of pesticides into two categories: restricted use pesticides (RUPs) and registered pesticides. EPA has developed four toxicity categories for pesticides and all those falling in Category I and some of those that fall in Category II are classified as restricted use pesticides. A few years ago, farmers and ranchers in my practice area in Wyoming were all advised to become certified applicators. I can recall that several of our clients attended schools sponsored by the local weed and pest control district and completed the requirements to become certified applicators. The reasoning behind such training was to prepare farmers and ranchers to be able to acquire and use the pesticides they had been using all the time in their agricultural endeavors. The "word" was out then that all pesticides would be restricted and unless you were a certified applicator, you'd have to hire someone everytime you wanted to spray your crops or livestock. The applicator schools were of 3 days' duration and were well attended.

Unfortunately — or perhaps fortunately — the restricted use category for pesticides is not very large at present and EPA appears to be very cautious in its approach to placing pesticides in that category. I believe the first list of pesticides in the restricted use category was published about two years ago. There have been one or two updates to the restricted use pesticide list and thus far only one or two of those products would have any application in the practice of veterinary medicine. I am aware of only two products that might be used by prac-
ticing veterinarians that are in the restricted use category—they are strichnine and fenthion. Fenthion is classified as a restricted use pesticide in formulations greater than 70% or for aquatic, nondomestic ornamental, and bird control uses. However, products containing fenthion that are likely to be used by veterinarians are subject to New Animal Drug Application approval by FDA and I'll mention this specifically in a few minutes.

Registered pesticides comprise the bulk of pesticides that may be used in the practice of veterinary medicine. According to EPA regulations, registered pesticides must be used only according to label directions and only for the purposes specified in the label. The Federal Insecticide, Fungicide, and Rodenticide Act, as it was amended in 1978, allows veterinarians who are not in the business of being certified applicators to use restricted use pesticides in the course of their normal practice, but FIFRA does not exempt veterinarians from the other requirements of the Act. This was vividly illustrated a few years ago in a midwestern state, when a veterinarian who repackaged and dispensed a topical pesticide was cited and fined by EPA. He had, by the act of changing the container of the pesticide, become a “producer” and had not registered this “product” with EPA. This created a problem of magnificent proportions for practicing veterinarians and for the profession as a whole.

AVMA has been involved in the pesticide issue since at least 1974 when the Council on Biologic and Therapeutic Agents urged that FIFRA be amended to exempt practicing veterinarians from the requirements for certified applicators. Shortly after that, the Council began to talk about the need for regulations that would allow veterinarians to use and prescribe pesticides in accordance with good medical judgment in a similar fashion to FDA's policy that a veterinarian may use any drug he may legally obtain with the understanding that the veterinarian assumes complete responsibility for use of the drug for non-label uses. AVMA's Washington Office staff has worked as closely as possible with EPA and the Council on Biologic and Therapeutic Agents has had an interested and active consultant from EPA. He is a veterinarian, Dr. Roland Gessert, and has done a good job of keeping us informed and has worked hard to convince his colleagues at EPA that veterinarians should be provided with enough leeway under EPA regulations to allow the practice of good veterinary medicine to continue.

The first encouraging sign, of course, was the 1978 amendment to FIFRA exempting the veterinarian from certified applicator requirements so long as he used restricted pesticides in the normal course of practice and was not in the business of being a pesticide applicator for hire. The next and most hopeful sign that EPA has begun to recognize that veterinarians do have a bit of expertise in the use of pesticides is the proposed policy enforcement statement that describes the responsibilities of veterinarians who use RUPs and/or repackage registered pesticides. EPA anticipated publishing the statement sometime last spring and we've been assured several times in the last few months that it would
PESTICIDE REGULATIONS

appear in the Federal Register in a couple of weeks. Looking back at the EPA-FDA agreement which took 21 months to publish, I'd say we may have another year before we actually see the statement in print.

AVMA's Council on Biologic and Therapeutic Agents has had a couple of opportunities to review drafts of the statement and a summary of the proposal's language was published in the June 1, 1979, JAVMA. Roughly, the policy allows a veterinarian or his supervised employees to use restricted use pesticides within his practice and allows a veterinarian to dispense RUPs to certified applicators. Additionally, the policy allows the practicing veterinarian to repackage or mix registered pesticides and to dispense them on a case-by-case basis. Unfortunately, the policy does not seem to speak to allowing registered pesticides to be used or dispensed for non-label uses or dosages. Please rest assured that AVMA will do everything possible to urge EPA to insert language that would allow veterinarians to use and dispense pesticides in conformance with their best professional judgment and expertise. It has been noted that EPA labeling for certain cattle insecticides would prohibit using those insecticides at the concentrations recommended by USDA to control scabies infestations. I believe this is one of the strong supportive arguments for allowing veterinarians to use pesticides in accordance with good medical judgment. When the proposed statement appears in the Federal Register, interested parties will have 45 days to comment on the proposal.

Another interesting clause in the drafts of the proposal indicates that the policy does not apply to state regulatory restrictions. One would generally assume that this means state regulations may be more restrictive but probably not more permissive than the federal policy. However, this should be explored since the FIFRA amendments of 1978 broaden the authority of state regulatory agencies to permit use of a pesticide within a state for special local needs. This may provide an opportunity for state VMAs to work for broader use standards for special problems that veterinarians face within a state.

If you feel somewhat confused about this system of pesticide regulation, don't feel too badly because it is evident that at least some EPA enforcement agents are more confused than you and perhaps even more confused than I am. We recently received information that an EPA enforcement agent had cited a practitioner in a southeastern state for dispensing 20% fenthion for a non-labeled use. I mentioned earlier that that particular formulation of fenthion has a New Animal Drug Application approval from FDA and, as we understand it, does not come under EPA jurisdiction at all. This incident has not been completely resolved but it is illustrative of a need to retrain EPA enforcement personnel or to perhaps replace certain ones with more competent people.

It has been suggested that pesticides for animal use should all be under the jurisdiction of FDA. AVMA's Council on Biologic and Therapeutic
Agents has rejected this idea simply because many registered pesticides now available would likely become unavailable if manufacturers or producers were required to go through the additional expense of FDA approval. Besides that, it is safe to assume that some of the registered pesticides couldn't make it through the new animal drug approval process.

AVMA has had a fair amount of success in communicating with the EPA bureaucratic structure and it's realistic to assume that we should continue to pursue adoption of EPA regulations that will allow the practice of good veterinary medicine to continue without unnecessary federal obstruction.

I would be remiss to close without touching on the need for responsible action on behalf of the veterinary practitioner. A single practitioner who misuses a pesticide or drug not only invites trouble for himself, his action can have a negative effect on the entire profession. Regulatory personnel in FDA and EPA can usually cite chapter and verse of incidents involving practitioner misuses of a particular product and are apt to use those incidents to defend a position calling for strict regulatory control for all veterinary control for all veterinary use products. The only way we can combat regulatory policies that interfere with the practice of veterinary medicine is for every veterinarian to use his/her knowledge to the best of his/her ability and to strive to use the newest information available in exercising the best professional judgment in the use of pesticides, drugs, and biologics.
KEEPING THINGS IN PERSPECTIVE

C. D. Van Houweling, D.V.M., Fairfax, Virginia

Shortly after I became Director of the Bureau of Veterinary Medicine of the Food and Drug Administration, we began reviewing the data submitted by companies on the retention of antibiotic residues in the edible tissues of animals treated with antibacterial drugs. One of the real surprises was the persistent retention of trace amounts of streptomycin and dihydrostreptomycin in the kidneys of swine and cattle. The residues were retained for the duration of the tests which, I recall, was 54 days in swine and 84 days in cattle. This naturally raised the question of the withdrawal period for these drugs. Concurrently, we began to study the significance of trace amounts of residues of streptomycin.

In an effort to evaluate this carefully, the Bureau negotiated a contract with a physician, Dr. William C. Hewitt of U.C.L.A., who had served as chairman of the National Academy of Sciences/National Research Council panel reviewing the human drugs containing streptomycin and dihydrostreptomycin. Our charge to Dr. Hewitt was to evaluate all of the literature that would have bearing on the significance of streptomycin residues in human food. Dr. Hewitt thoroughly reviewed the literature and submitted his report. We invited a group of distinguished veterinarians and physicians to meet and review it. In his report Dr. Hewitt projected streptomycin residues occurring at the unusually high level of ten parts per million (p.p.m.) and someone eating a pound of kidney. He pointed out that the streptomycin consumed would be slightly over 4.5 milligrams. He reported that in earlier days when people were treated for tuberculosis with streptomycin and dihydrostreptomycin, it was not unusual for patients to receive a gram of this drug a day for as long as a year. The only toxicity that was recognized as associated with this prolonged treatment at this higher level has been a slight impairment in hearing or damage to the eighth nerve. This early treatment represents 200 times what would be ingested from the residue in the kidney, even at 10 p.p.m. When Dr. Hewitt presented this information to the review committee, there was unanimous agreement that it was very hard to imagine any adverse effects from the amounts of streptomycin residues that had been detected as retained in the kidneys of animals. Since the data revealed that the residues were normally below 2 p.p.m. thirty days after the administration of the drug, there was a temporary tolerance established at 2 p.p.m. with a thirty-day withdrawal period for these products.

This was the first time that I had participated in a study which compared the possible effects of a drug that could be ingested, as the result of residues in tissues from animals, to the use of drugs in humans. As part of this consideration, it was also recognized that, whereas a gram a day had been given for a year or longer, the likelihood of a person encountering streptomycin residues in kidney for more than one day in succession was
extremely unlikely, and that it was even less likely that someone would eat a pound of kidney on successive days. Considering these factors, it was generally agreed that there could be no foreseeable toxic effect from these very small amounts of residue in the kidneys of animals that had been given streptomycin or dihydrostreptomycin, especially when the residues were usually in the 2 p.p.m. range and the relative dose would be at least 1000 times less than the human therapeutic dose.

Whereas this decision was based upon human use and toxicity experience, almost all toxicity decisions are made on data derived from rats and mice studies. This leads to conclusions and decisions that pose inconsistencies.

In no instance has it been more difficult to keep matters in perspective than with diethylstilbestrol or DES. You will recall that DES was approved for addition to cattle and sheep feed in 1954; it not only conserved feed, but animals gained weight more rapidly. In 1962 the Congress amended the Delaney Clause, which prohibited the addition of a carcinogenic additive to food, to allow for the use of DES in animal food. There were two requirements: DES was not to have an adverse effect on the animal, and there was to be no DES residue detected, by an analytical method approved by the Secretary of HEW, in edible tissues of animals given DES.

At the time the Delaney Clause was amended to allow the use of DES in animal feed, the level of detection for DES was approximately 2 parts per billion (p.p.b.) utilizing a mouse uterine biological assay. In time the Meat and Poultry Inspection Service improved the analytical capability to detect DES and utilized a chemical method sensitive to 0.5 p.p.b. rather than a biological assay. With the chemical method, they began to detect residues in the liver of slaughtered animals. Although its incidence varied, at its peak it was as high as 2½ percent. Meanwhile, DES has been shown to be at least a suspect carcinogen in a test conducted by Gass et al. This experimental data indicated that there were more mammary tumors in the C3H female mice fed DES at the level of 6.25 p.p.b. than in the controls. Because of this there was a clamor in the press and in the Congress for the approval of DES to be withdrawn.

The data used as evidence that DES was carcinogenic also revealed that there was no additional increase in tumors in the C3H strain of female mice when DES was increased from 12½ to 25 p.p.b. Furthermore, it is accepted that approximately 90 percent of C3H strain of mice develop mammary tumors during their normal life span whether or not they are exposed to a carcinogen. Later work conducted at the National Center for Toxicological Research revealed that when the titer of the mouse tumor virus, which is commonly found in this strain of mice, was low, DES did not cause as many tumors.

The demand for the withdrawal of DES was based upon the finding of DES residues in the liver of 1 or 2 percent of slaughtered cattle. At one of
the hearings on DES, the Department of Agriculture reported that considering the incidence of the residues to be 2¼ percent of the slaughtered cattle and the consumption of beef liver in the population, the chances of a person encountering DES in beef liver four days in succession was something less than one in several hundred million.

Later the agitation for the removal of DES from the market became much stronger when Herbst et al.\(^8\) reported, based upon a retrospective epidemiological study, that the use of three synthetic estrogens including DES in pregnant women was associated with a rare form of cancer of the vagina in their daughters. There was no discussion in this paper of the relative amounts of these estrogens women were given in the effort to avoid abortions and the amount that might be ingested from eating liver from cattle fed DES. In 90 percent of the cases,\(^4\) women received over 15,000 micrograms (mcg.) per day. The range usually was from 300 to 18,000 mcg. of one of the estrogens.

In one trial one woman received 300,000 mcg. per day. If a person ate a pound of liver with residues of 2 p.p.b., they would ingest just under 1 mcg. of DES. Therefore, the doses given women to prevent abortions were in the range of from 300 to 300,000 times greater than one would receive from liver at 2 p.p.b. with over 90 percent of the women receiving 15,000 times the amount ingested from eating liver with residues at this level. The response to these vast disparities is that no amount of a carcinogen can be considered safe. Although it may be impossible to prove that any amount of a carcinogen is “safe,” there is evidence that small amounts of substances considered to be carcinogenic do not cause cancer.

I recently wrote a paper that was published in the *Journal of the AVMA*\(^5\) on the approval of selenium as a feed additive for use in cattle, sheep, swine, and poultry. High levels of selenium in the diet had been shown to be carcinogenic; they caused liver pathology followed by hyperplasia of the liver, which in turn became neoplastic. But it was possible to approve selenium, which is an essential nutrient, as a food additive because there was a level at which it did not cause liver pathology, and therefore, not the hyperplasia or neoplasia.

A study referred to in the selenium article was by Gass\(^4\) in which he studied the incidence of mammary tumors in the C3H female mice. He tested estradiol-17β(E\(_2\)), DES, and vitamin D\(_2\). There were two significant findings from this series of experiments. The first was that the occurrence of mammary tumors in the C3H female mice was closely linked to the presence of the mouse tumor virus (MTV) in the mice used in experiment. The second was that the occurrence of mammary tumors in the MTV mice was not shown to be significantly greater whether the test substance was DES, E\(_2\), or vitamin D\(_2\). Animals given various doses of vitamin D\(_2\) had more tumors at eighteen months. Also at twenty-four months, animals given vitamin D\(_2\) had more mammary tumors and showed a decrease in tumor latent period (time to tumor). From this study one could conclude that vitamin D\(_2\) is carcinogenic in the same
strain of mice used to identify DES as a carcinogen. Everyone would probably agree that vitamin D$_2$ is safe, in fact, essential, at certain levels. As one noted oncologist is reported to have replied to the question of the carcinogenicity of selenium, "of course not, it's an essential nutrient." The same must be true of vitamin D$_2$ and other essential nutrients.

Whether or not DES has some carcinogenic potential that is distinct from its estrogenic characteristics is not certain. There is general agreement that it is the estrogenic effect that causes the mammary tumors, which were the basis for labeling it as carcinogenic. Of course, there are large amounts of estrogens in many other foods and the amounts are greatly in excess of what one could ever be expected to consume as a residue from livers. Wheat germ, for example, contains 2000 times the DES equivalent of estrogens in beef liver at 2 p.p.b. Another example, found in the CAST Report on hormones in food, pointed out that in a meal consisting of whole wheat bread, a green salad, green peas, and ground round steak from a DES implanted steer, the beef would contain by far the lowest amount of estrogenic activity.

In recent years there has been a great deal of concern and discussion about sulfonamide residues in food from hogs. The FDA and USDA have been particularly concerned about the number of swine that are found to contain over 0.1 p.p.m. of sulfonamide residue, usually sulfamethazine, in the livers and kidneys at the time of slaughter. This concern is based partly on the fact that in one experiment a rather large amount of sulfonamide fed to animals produced a thyroid hyperplasia which was considered to be carcinogenic and that there was some metastasis of this lesion to the lungs of the experimental animals which regressed when the ingestion stopped. At the present time the acceptable level of residues of sulfonamides is 0.1 p.p.m. in any animal tissue. This tolerance is applicable to duck kidney and liver as well as to beef.

The difference in consumption of these two animal food products is worth considering. There are approximately fifty million pounds of duck meat produced per year in the United States. Therefore, the average consumption of duck cannot exceed one fourth of a pound per year. The livers and kidneys of ducks are approximately 4 percent of the weight of the carcass. A person who eats the average amount of duck kidney and liver per year, would be eating approximately 4 percent of four ounces or 0.16 ounces per year. On the other hand, the average annual consumption of beef has been approximately 125 pounds per year which is 12,500 times more than duck kidney and liver. I'm sure you can agree that the relative consumption from these two sources is great. FDA has maintained, however, that one cannot consider the residue from any one source, since people do eat foods from a variety of sources. Therefore, one must consider the cumulative amount from all sources. I would like, therefore, to consider the toxicity of sulfa residues in the light of what we know about the toxicity of sulfonamides in human beings.

The toxicity of sulfonamide residues also can be compared to the
therapeutic doses of sulfonamides that have been employed in treating people over the past forty plus years. It is recorded that when people are treated with a daily dose of 4 grams and higher of sulfonamides for ten days and longer there is only an occasional sign of toxicity. Lower doses of approximately 1 to 2 grams per day have been administered to patients for many months with no appreciable ill effects. The amount of sulfonamide residue consumed by a person eating one pound of pork liver containing 1 p.p.m. (10 times the permissible level) would be only 500 mcg., 1/8000 of the normal therapeutic dose or 1/2000 of the lower prophylactic dose.

There are examples of such disparities outside of the animal drug residues. For example, the use of nitrates and nitrites for curing meat food products has come under attack in recent years. First, nitrites were shown to combine with amines of the stomach and produce nitrosamines, which have been shown to be carcinogenic. More recently, nitrites alone were reported to be carcinogenic by FDA and USDA based upon a study conducted by Newberne at the Massachusetts Institute of Technology.

Nitrites from cured meats account for between 2 and 20 percent of a human's total nitrite consumption. Tannenbaum, a well known authority, has shown that nitrites are produced in the human intestinal tract in the process of digesting proteins. He estimates that the consumption of cured meats represents no more than 2 percent of one's total nitrite consumption. The late Dr. White estimated the daily per capita ingestion of nitrates and nitrites from various sources. He found that vegetables represent the principal source of nitrates, accounting for 86 percent of the total whereas cured meats account for only 9 percent. Nitrite ingestion he found to be about 77 percent from saliva and just over 21 percent from cured meats. It is apparent that if you wish to materially affect your ingestion of nitrates and nitrites, you must quit eating vegetables and swallowing saliva.

In preparing this paper in the days preceding this meeting, I must take note of the fact that questions are being raised in regard to the reliability of the Newberne Study referred to above. It is proper to note that Dr. Newberne was conservative in his interpretation of the results of the study. It would appear from the Library of Congress review of FDA's handling of this report, Dr. Newberne's reservations about the results of the study were not heeded.

In conclusion, it has been said that there is no such thing as a poisonous substance, there is only a poisonous dose. It has been argued that there is no "no effect" dose for a carcinogenic substance, but it would appear that there are essential nutrients which are also known to be carcinogenic at higher levels. If there is a no dose response relationship for carcinogenic substances, it would be the only biological phenomenon that is not subject to such a relationship. It would seem that there must be dose responses for carcinogens as well as other toxic substances, especially if one looks at substances such as aflotoxins which are extremely potent.
carcinogens. If they are carcinogenic at any level of ingestion, the incidence of cancer would probably be higher than it is because of the almost constant exposure to aflotoxins in the various sources of food that are ingested by the human population of this land.

What I have tried to do in this presentation is to point out some of the disparities and inconsistencies that exist in FDA's food safety decisions. These disparities and inconsistencies result, to a large extent, from the use of data derived from laboratory animal studies without proper consideration of other data, such as human use experience, toxicity and food consumption. Perhaps our food safety scientists and policy makers have relied too much on laboratory animal studies in trying to assure food safety and have ignored other pertinent information.

REFERENCES

2. Unpublished data in Food and Drug Administration files.
This morning’s session started out with the presentation by Dr. C. D. Van Houweling, entitled “Putting Things into Perspective.” The Committee thanks Dr. Van Houweling for his thoughts which question the FDA Bureau of Foods’ requirements for yet more laboratory animal data on residue of antibacterials while virtually ignoring more than 40 years of human drug experience with these compounds. Hopefully, some day the health professions might question the edict that “No amount of carcinogen is safe.” The father of toxicology, Paracelsus, stated in the 5th Century that “No substance is entirely safe; it is only the dose which determines whether or not a substance is a poison.” For many so-called carcinogens, no observable effect levels (NOELS) have been demonstrated. By further study, might not NOELs be established for other carcinogens? Because of fears associated with the word “cancer,” responsible groups fearfully avoid suggesting that residues of a carcinogen be permitted in food. But as society accumulates experience and knowledge, might not this question be re-examined from time to time? Might not a risk value be determined for all substances?

The report of Dr. Homer Smith revealed that during the past year, only two new veterinary drug entities were approved by FDA, both for use in horses.

Flutrostenal, a prostaglandin-type estrus regulator was approved August 30, 1979. (Trade name Equimate)

Oxfendazole, a powder for top dressing feed for elimination of all internal parasites except bots, was approved June 27, 1979. (Trade name Benzelmin).

Dr. Cheryl Knoblock then reported on FDA’s proposal on Safety and Efficacy Data Supporting the Approval of Minor Use New Animal Drugs, published in the Federal Register July 20, 1979. These include drugs for minor species (sheep, goats, game birds, ducks, fish, rabbits), and drugs for minor uses in major species. The basis of minor use strategy is to allow extrapolation of data from one related species to another, and the use of animal models. Since the animal drug amendments, there has been no previous provision for doing this.

As proposed, sheep are to be considered minor for animal safety and
efficacy, but major for human food safety.

Generally, drug companies seeking approval will be applying for drugs already approved in other major species; if not previously approved in major species, then all data must be collected.

While BVM are sympathetic to the minor use problem, the Bureau of Foods is not as liberal, and a joint working relationship must yet be established.

The AVMA hopes that the minor species document will be adopted, but hope this is only a first step forward which will assist in opening the door for future negotiations.

This Committee has discussed the minor use problem over the past several years and are pleased with the efforts of FDA to resolve the dilemma. However, the veterinary profession and the livestock industry recognize that the problem really is not solved. As the committee chairman pointed out at our 1975 meeting in Portland, Oregon, even a major drug for a major species may be a minor drug in the eyes of a manufacturer developing a multimillion dollar drug for the human market. Therefore, even in our capitalistic society, it may be necessary for governmental agencies to assist in developing safety and efficacy data for drugs to be used in animal health and animal production.

The Environmental Protection Agency regulates the use of pesticides. Problems have occurred in regard to veterinarians repackaging pesticides for dispensing to clients and for dispensing for uses other than as registered. These problems have been partly resolved for a dispensing policy, expected to be published in final form in the Federal Register this Thursday or Friday. But many more problems are not resolved.

A chief problem with the pesticide act is that it requires pesticides be used only as provided in the labeling. This dose may not be exceeded, and the use may not be varied. In controlling such pest infestations as scabies, USDA regs, based on their studies and experience, require certain levels of a pesticide to safely and effectively control a pest, whereas labeling for a certain brand pesticide may specify the pesticide be used at a lower level. Therefore, in order to comply with USDA quarantine regulations, it might be necessary to violate the Pesticide Act. Where they conflict, it is in the public interest to observe which law or regulation?

Incidently, at last year's USAHA Meeting, the Committee on Parasitic Diseases and Parasiticides endorsed acceleration of the Scabies Program to require cattle be dipped for interstate movement in a State-Federal approved dipping vat, either at origin or destination. This Committee requested USDA to maintain scabies eradication funds equal to those awarded for FY 1979.

Veterinarians and human medical practitioners use pesticides as drugs. EPA has relinquished control of pesticides used on man to FDA. Thus far only systemically acting pesticides for animals are under FDA
jurisdiction.

It may not be difficult to persuade FDA to accept as drugs pesticides used on animals. But good disease control requires control of pests on the surroundings, also, as has been agreed by Dr. Jim Hourrigan, USDA, and by Drs. Jim Steele and Bob Anderson of the Public Health segment of our profession. This may require amending the Pesticide Act and the Food, Drug, and Cosmetic Act, and the profession fears some needed pesticides might be lost in the process. So the Committee was reluctant to act now and made no recommendations in this regard.

The AVMA Council on Biologics and Therapeutic Agents considered the same matter and recommended that Congress be petitioned to transfer the regulation of pesticides used for control of animal diseases or infestations and for the promotion of animal and the public health to some suitable agency within the Department of HEW or Department of Agriculture; such pesticides to include disinfectants and fungicides having medical, public health, and animal health implications, as well as insecticides and miticides. In order that no pesticides might be lost in the transfer of jurisdiction, the Council further recommended that enabling legislation recognize registrations already granted by EPA.

Dr. Morris Levy concluded the Committee meeting with a report of an antibiotic tissue residue investigation in cattle in five states (PA, VA, W.VA, NJ, and Dela.).

The greatest number of residues were:

- Neomycin 28 cases
- Streptomycin 24 cases
- Penicillin 11 cases
- Oxytetracycline 6 cases

Reportedly, the drugs were used in accordance with use directions. A theory as to why residues persisted is that residue studies are conducted in normal animals, but that diseased animals may not metabolize and eliminate the drug as do the normal animals, resulting in tissue residue.
STATUS OF ELECTRONIC IDENTIFICATION AND TEMPERATURE MONITORING

Dale M. Holm and Clifford T. Araki, New Mexico

ABSTRACT

The first field testing of the electronic identification system being developed by the Los Alamos Scientific Laboratory has started and five commercially produced systems are expected to be delivered within the next year for installation in two feedlots and three dairies. The major goal of the field test is to demonstrate that widespread adoption of systems having specifications approved by the National Livestock Electronic Identification Board will result in improved efficiency of production, marketing and disease control. Extensive cost/benefit analysis will be conducted to evaluate the practicality of the widespread implementation and to develop a rationale for early acceptance of electronic identification.

INTRODUCTION

A computer-compatible system for animal identification and temperature monitoring has been under development at Los Alamos Scientific Laboratory (LASL) for seven years. It was designed to play a key role in improving livestock management and serves as one of three components for an integrated system for disease control. Electronic animal identification along with a computerized disease management data base system and an automated serological test for livestock diseases is being developed at LASL to provide the means for rapid and reliable disease detection and disease traceback. We believe that application of this new technology will increase efficiency in the total livestock industry and improve disease control.

Many forms of automation are being offered to decrease labor costs and more is being learned about factors affecting livestock productivity, disease propagation and treatment. With the implementation of new technology, greater control of production and individual animal management becomes increasingly feasible. However, this entails more bookkeeping for individual animal management and increased labor costs unless automation of data collection and analysis is employed. Computer-compatible means of identifying animals and monitoring their health status is necessary to make a major improvement at all levels of livestock management.

While some commercial systems are available now for electronically identifying livestock, they suit the needs of only specialized segments of the industry, e.g., individual dairy operations of beef performance testing operations. The LASL system is intended to satisfy the needs of the total livestock industry. These needs have been formulated into a set of functional specifications that have been approved by the National Livestock Electronic Identification Board (NLEIB). This board is a
committee of the Livestock Conservation Institute and has members representing all of the major livestock groups and interests. Their specifications have been slightly relaxed for the forthcoming field test of the identification system because some of the specifications would have a high cost associated with them but would not substantially alter the validity of the tests. For example, providing the special field test equipment to identify 1,000,000,000,000,000 animals could not be justified when less than 10,000 animals were to be identified in the field test. We knew that there was little likelihood that the equipment used in the field test would be compatible with that produced in a few years for commercial sales so the additional encoding capability would be of little value.

ELECTRONIC IDENTIFICATION FIELD TEST

The LASL electronic identification system is at the stage where it needs to be tested in field environments. Within the coming year a major field test of the system will commence under the auspices of the Animal Plant Health Inspection Service (APHIS) branch of the USDA. To promote commercialization, we will purchase five systems that meet our specifications from Raytheon Service Company and we will field one of our designs that will serve as a reference. The main purposes of the field trials are to test the performance of the equipment, test its compatibility to other forms of automation and to develop improved management procedures for dairy and feedlot environments. It is expected that a cost-benefit analysis of the system can then be made for these environments to determine if greater use of individual animal management is cost effective. Analysis of the cost effectiveness of the system is an extremely important aspect of the field trials, since commercial production of the system and its use in the livestock industry is ultimately dependent upon its cost effectiveness.

Test Sites

More than 15 potential sites have been visited to evaluate their suitability for conducting field test experiments and seven were selected for further negotiations. The six systems will be distributed as follows; one remote site, two feedlots and three dairies.

Sites were chosen for the field test because of staff interest in automation and research in new forms of animal management and animal health assessment. The USDA's Jornada Experimental Range near Las Cruces, New Mexico was chosen for the remote site in testing range management applications. The University of California Imperial Valley Research Station in El Centro, California and the U.S. Meat Animal Research Center near Clay Center, Nebraska have been chosen for the feedlot applications of individual animal management and for evaluating shipping fever. Cornell University at Ithaca, New York, University of Illinois at Urbana, Illinois and Michigan State University at East Lansing, Michigan have been chosen for the dairy experiments involving
more intensive dairy animal management. Utah State University has been chosen as a first alternate site for dairy experiments. These research organizations are among the leaders in research on animal management. Their involvement in the field test provides the best possible test of the electronic identification and temperature monitoring system and provides the added benefit of advancing research in livestock management.

Electronic Identification Equipment

The LASL system is based upon an identifying transponder that has no battery (Fig. 1.) Its design is to be implanted in animals subdermally and powered through an interrogator/receiver which sends microwaves to power the identifier and receives encoded reflected signals. The system may be controlled by a computer and data may be directly input into a microcomputer or centralized computer without operator transcription. It is also possible to interface the electronics with other forms of instrumentation to not only allow computer control of the identification system but also other automated devices.

This equipment is installed at a remote site on the Jornada Experimental Range where the weather conditions are quite extreme with lots of wind, dust and large temperature extremes. The equipment includes our interrogator/receiver and 50 transponders and a TRS-80 Radio Shack microcomputer shown in Fig. 2. It is currently being interfaced with a digital electronic scale produced by Weigh-Right, Inc. A water tank provides an attraction for the animals. As they come to drink, a system of fences, gates and corrals, designed by Dr. Dean Anderson of the USDA, directs the animals over the electronic scale to automatically collect animal identification, temperature and weight. Animals can also be trapped at the water tank for physical examination and checking on the validity of the automatically collected data. Figure 3 shows one of the one-way gates designed by Dr. Anderson.

The data are currently being collected on two magnetic tape cassettes, each having part of the data. When some of the electronic communication problems have been solved, all of the data will be written on one tape. Normally, the Radio Shack keyboard and TV screen will be powered only during times when operators are at the site. Data collected will be analyzed on the New Mexico State University computer and on the LASL Central Computer by the use of telephone lines that connect the main computers with a computer terminal located in the USDA offices.

The equipment to be supplied by Raytheon will have modifications to the basic LASL design including the possible incorporation of a battery in the transponder circuitry. Batteries allow a longer transmission range or a decrease in microwave interrogation strength and a possible decrease in package size. But, batteries also present problems when considered for long term use in food animals. A maximum life expectancy of 10 years is thought to be necessary for these transponders. Whether
batteries will maintain their integrity over this period of time has not been determined. If battery integrity is not maintained, chemical leakage provides the potential danger of meat contamination and battery failure would require the added expense of replacing the unit. LASL chose a design without batteries because we believed that the disadvantages in a battery operated system outweighed the advantages. Since both designs will be used in the field trials, a comparison of their performances may lead to a better design for commercialization.

Delivery of Raytheon equipment is scheduled to commence in 8 months with the last system to be delivered about September 1, 1980. There will be about 70 identifiers for each of the feedlots, 170 for each of two dairies, and 130 for the third dairy. Equipment setup and checkout is expected to require three months before reliable experimentation with the first unit is practical. Because evaluation of the cost effectiveness of the system can only be had with extended use of the equipment, we believe at least 2 years of use with all 6 systems will be required before definitive evaluations of the system can be made.

Once the field trials have been completed and the systems prove to be cost effective, commercialization of the system will be practical. However, a necessary step in commercialization of the system will be the incorporation of integrated circuit chips into the transponders. Transponders are now made of hybrid circuitry which include discrete components and integrated circuit components. With hybrid circuitry, assembly cost is about $150 for each transponder. We expect that use of fully integrated circuit chips will decrease transponder size, increase its reliability and dramatically reduce the cost of the transponders. Hybrid circuits are currently being used because the developmental cost of an integrated circuit chip is high ($20,000 to $100,000) and manufacturers are not willing to incur such costs for an unproven product. However, once proven, we expect that transponders can be made with chip components at a price affordable to the livestock industry.

TEMPERATURE MONITORING

A temperature monitoring capability has been included in the electronic animal identification system to provide a means for assessing animal health which will complement existing methods. It potentially provides an accurate and automatic early warning system which can alert livestock managers to problem animals through management by exception.

An animal's body temperature is controlled by many factors. Thus, many disturbances to normal body function are reflected in changes in body temperature. Many of these are important to commercial livestock producers. For example, fever is a symptom of many infectious diseases. If detected and treated early, the severity of the disease could be attenuated and its spread to other animals reduced. Similar fever responses could be used to determine the effectiveness of animal vac-
cinations and changes in animal temperature have also been found to occur with estrus, parturition, and animal stress. Detection of these conditions and appropriate corrective action could be of immense economic benefit to livestock operations.

Thus, animal temperature is thought to be important in assessing animal health. However, practical use of the temperature monitoring feature of the electronic ID system needs more study. The transponder monitors subdermal temperature but it is not known how well subdermal temperatures correlate with deep body temperature. Also, the system will see application in a wide range of livestock environments from milking parlor to open range. Since much of the research published on animal temperature have been conducted in restricted or controlled environments their validity must be determined for a wider range of environments. A considerable amount of work is seen as necessary to apply the basic research conducted in this area to its practical application in the electronic ID system.

**Radiotelemetry Equipment**

Research conducted at all of the field test sites will be used to evaluate the temperature aspects of the electronic identification and temperature monitoring system. But because more work is needed to evaluate the usefulness of the temperature measuring capability of the system, a temperature-telemetry system has also been developed which provides continuous reading of an animal's temperature. LASL scientists have developed this new and improved radiotelemetry system especially for use in the field test, since extensive use of this type of system was envisioned and reliable units were deemed necessary.

The system consists of radiotransmitters, (not transponders) and a microcomputer data logging system which is capable of automatically collecting and storing temperature data. It is being developed separately from the electronic ID and temperature monitoring system because the ID system is not very suitable for continuously monitoring the temperature of freely roaming animals.

In the continuous temperature telemetry system each animal may be instrumented with one or more radio transmitters. Each transmitter has its own frequency and sends out a radio pulse about once a second. The actual time between pulses is proportional to the temperature of the sensor attached to the transmitter. The microcomputer causes the receiver to listen to one transmitter at a time, converts the time between transmissions to a temperature reading and stores the information. There can be only a few thousand different transmitters with this system so it is not suitable for animal identification. However, it is well suited to evaluating temperature at various locations in and on the animals as they move about freely. Figure 4 shows the first radio transmitter unit of the new design and Fig. 5 shows the microcomputer data logging system that is being used with it.
**Experiments**

While the major portion of the experiments on animal temperature is being planned in collaboration with researchers at other institutions, experiments have been conducted at Los Alamos Scientific Laboratory to obtain information which would be useful in preparing for the field test. To date, studies have been conducted to determine if the present location of the transponder in the animal is suitable for monitoring body temperature and to determine what effects ambient temperature has on subdermal temperature.

The transponder is currently being implanted subdermally behind the withers. This site was chosen for its ease of interrogation and not for any benefit in its temperature monitoring capability. In order to determine how well this site compares with other body sites, initial comparisons were made of body sites through the use of thermography. Thermography measures the intensity of infrared radiation emitted from the surface of an object, in this case cattle. This serves as an indicator of heat loss and allows a comparison of coat insulation with heat loss through shaved skin surfaces. Measurements of heat loss taken from an animal at different ambient temperatures indicated that among the sites compared, the withers maintained good coat insulation and a relatively high skin surface temperature at low ambient temperatures. Both factors indicate that this area may be able to maintain a relatively stable skin temperature in the face of a changing ambient temperature. These preliminary results indicate that of the sites studied, the withers is among the best sites for monitoring body temperature. However, studies using multiple body site monitoring of temperature are needed before definite conclusions are possible.

Other experiments are being conducted involving temperature monitoring in uncontrolled environments. Environmental conditions may have a considerable effect on subdermal temperature and correlations need to be found with deep body temperatures. The effect of a strain 19 brucellosis vaccination was studied on three animals in an uncontrolled outdoor environment with sunlight and rain. Figure 6 shows the relationship between deep body temperature (a), subdermal temperature (b), and ambient temperature (c) in one of these animals. Large spikes can be seen in subdermal temperature which often exceeded deep body temperature. These were often accompanied by spikes in deep body temperature which was measured by tympanic temperature. During periods of rain, large depressions in subdermal temperature are also possible. Spikes in subdermal temperature are most easily explained by the radiant effects of sunlight as they were not seen in data on the same animals in an uncontrolled indoor environment, even with activity.

Animals were vaccinated on the second day and fevers developed within the same day. Despite the effects of ambient conditions on these data, a measurable shift in subdermal temperature is apparent with
fever. Three animals were used for the experiments. All showed shifts in subdermal temperature with fever.

These data suggest that much work must still be done on the temperature aspects of the identification system. The greater variability in subdermal temperature in more extreme environments may increase the difficulty in obtaining useful data and computer enhancement of data may be needed to aid in management decisions. However, these results indicate that subdermal temperature can be useful as a monitor of animal health and in applications such as in a milking parlor, transponder monitoring may be useful without modification of the data collected.

COMPUTER DATA BASE MANAGEMENT

The advantages of electronic identification cannot be realized by the producers, markets and regulators unless the records are computerized. Therefore, computerization of records is a very important part of the field test. However, we do not need to wait for the field test to develop the data management systems for disease control. The formulation and implementation of such data bases is being done at the Los Alamos Scientific Laboratory in cooperation with USDA Personnel in Texas, New Mexico and Idaho for the control and eradication of brucellosis. The data bases were designed to store information on individual animals regarding ownership, age, vaccination status and test results. They allow rapid access of the information stored to a large number of personnel at various levels of government. The system under which these data bases were constructed also allows the production of customized reports to suit the needs of personnel from that of a staff worker tracing animal ownership to that of the state veterinarian tracing infected animals to the exposed herd. Currently, the system involves three states, but in the near future, additional states will be incorporated into the system. The plan is to eventually expand to a national system for brucellosis eradication, with the ability of documenting the life of livestock animals in greater detail. With minimum identification it is foreseen that this data base system could incorporate data on other diseases and provide a truly national system for disease control.

AUTOMATED SEROLOGY

An efficient system for disease control requires accurate and fast disease diagnosis. So the third component of an integrated system for disease management requires automated screening tests for various animal diseases. Otherwise any speed gained in computerization is lost in the time needed for the initial test analysis. Accurate diagnosis of disease or the detection of chemical residues in animal products is currently time-consuming. The problem is especially severe in slaughterhouses. LASL scientists are in the process of designing tests which are based upon an assay called enzyme immunoassay (EIA) which relies upon the specificity of antibodies to particular disease agents. In the reaction of antibodies to disease agents, a chemical complex is produced. If in a test procedure this
complex is tagged with an enzyme to produce a colored product, the intensity of the resultant color can be used to determine the presence of the agent. The advantage of the test lies in its flexibility. The processing is automatable and a single basic processing procedure can be used for a large variety of diseases. With automation, the same procedure could be used to test rapidly for a large number of diseases with very little modification to the system. The automated test is well along for hog cholera and trichinosis. Studies are currently underway for the incorporation of tests for brucellosis and certain low molecular weight chemical residues.

Automated EIA is being developed for slaughterplant applications but its use is expandable to sales barns, dairies and feedlots. Together with electronic identification of animals and biological samples, it will allow nearly full automation of disease testing systems. Computer control can automate both identification and testing procedures. Results of the tests may be analyzed and stored on site or at a centralized computer at a regulatory agency. Errors due to mislabelling, sample spillage or hemolysis could be eliminated and faster updating of data bases would be possible.

FUTURE

Experiments with the LASL system at the Jornada Range will provide a preview of the types of problems that can be expected when the commercial systems start to arrive. We anticipate a wide variety of problems that include interfacing of various types of electronic equipment, sensing of animals in crowded conditions, designing proper gates, computer programming problems and training the users of the equipment.

Limited transponder biocompatibility studies have been underway for five years, but the experiments at the Jornada Range will entail a larger number of animals and test the equipment under more severe conditions. It is anticipated that temperature monitoring experiments with the radio transmitters will start early in 1980 at three locations so the delay in arrival of the commercial electronic identification equipment will not seriously impair those experiments.

Three options will be available to the experimenters for data analysis. Some data analysis can be done at the experimental site with a small computer associated with the keyboard terminals. More powerful capabilities will be available at Los Alamos Computing Center and the users may wish to use a large computer associated with their particular site. Prototype programs for these various options will be developed with the LASL system at Jornada. This will include specific subroutines for data analysis and preliminary routines for cost-benefit analyses.

Although preliminary experimental plans are in hand, the memoranda of understanding must be negotiated with the test organizations and meetings held to work out cooperative agreements for sharing data and
comparing results. We anticipate that the first commercial unit will be installed in a dairy. Remaining units will then be alternately installed in feedlots and dairies until all five systems have been installed. This will provide the opportunity to make modifications to the second unit in each of the two classes.

We expect it will take about one year before any significant amount of useable data starts coming in from the field test and another two years to accumulate enough data to form a conclusion as to the success of the field test in demonstrating cost effectiveness. In 1983, if things go reasonably well, we will be planning for commercial production of equipment having the functional specifications of the NLEIB. Those experiments under the sponsorship of APHIS could be phased out after that time while more fundamental research experiments could be taken up by the Agriculture Research branch of Science Education Administration. The main APHIS effort could then be directed toward the development of a fully integrated disease control system that will include the large computer data base, automatic disease detection and the incorporation of many data input terminals throughout the country. The integration of a national disease control system is likely to take many years to implement but work in that direction needs to be seriously undertaken as soon as the future of a national system is assured. In the meantime work needs to be continued relative to a national disease control system, so that serious unforeseen problems do not crop up.

We believe that multiple suppliers of equipment will materialize as soon as a market has been established by the field tests. The experimental results should provide enough information to design a fully integrated chip for the transponder electronics which can fulfill the needs of the National Electronic Identification System. We also believe that only a very small amount of foresight will be needed to incorporate a national system into an international system. Thus, we can foresee the first implementation of an international system of animal electronic identification in about 1983 or 1984.

There is a considerable amount of interest in applying the basic technology developed for animal identification to a variety of other problems such as vehicle identification, credit cards, containerized cargo, luggage and inventory control. Application of electronic identification to these items may become widespread prior to the application in livestock identification. However, it is our intent to encourage those interested in these applications to design their transponders so that they will also be useful to the livestock industry. If we are successful, this will also help in the early cost reductions of livestock identification transponders. Electronic identification is no longer a "pie-in-the-sky" concept. A few years may make it a commercial reality.
The LASL 1978 model electronic identifier. The hybrid circuitry at the left has a metal lid welded on prior to application of the white biocompatible encapsulation.
The electronic identification equipment assembled for the Jornada site. It includes the LASL interrogator/receiver, a Radio Shack TRS-80 microcomputer with a printer, magnetic tape cassette, keyboard and TV screen.
Figure 3

Electronic scales at the Jornada Range with a one-way gate that cattle must pass through on their way to water.
A new LASL model radio transmitter for temperature monitoring. With only slight modification, these units can be used for external or subdermal monitoring.
Figure 5

The radio receiver and minicomputer terminal for continuous temperature monitoring experiments.
Three temperature recordings related to one animal to demonstrate the relationship between (a) deep body, (b) subdermal and (c) outdoor ambient temperature. The animal was vaccinated in the middle of the second day.
REFERENCES


REPORT OF THE COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: J. Ralph Bishop, Tipton, Ind.
Co-Chairman: Lee S. Garner, Albuquerque, New Mexico.


With the predetermined goal of identifying weaknesses on currently used systems of identification with the aim of finding recommendations for action that would correct the weaknesses, the Committee first reviewed the current situation as brought forth by representatives of several segments of the livestock industry.

After a short briefing on the status of the development of electronic identification now entering the field trial phase, the Committee was briefed on the current status of other APHIS programs and projects by Dr. E. C. Roukeman.

In his comments, he emphasized the decreased effectiveness of the MCI Program because of the drop of $3\frac{1}{2}$ million animals screened in total and a drop of 4 million screened at slaughter, compared with 1976. By deduction, it is reasonable to conclude that about 1500 Brucellosis-infected herds are being missed. He then commented on various procedures followed and devices used for identification with indication on the effectiveness of each. He enumerated the number of states which recognize the various means of identification and the possible need for improved coordination and identification problem-solving at the local and state level.

Dr. D. F. Leese of FSQS discussed the five proposed Food, Safety and Quality Amendments of 1979 - Title 3 - Residues Prevention, with the qualifying admonition that this legislative package was not yet fully cleared within USDA and may therefore not be in final form. As to that part of the legislative package dealing with animal identification and quarantine in relation to residue prevention, he pointed out that if there was reason to believe that a problem existed, all livestock would be quarantined at that location or products would be held. Whoever had the livestock or product in their custody would be responsible but with there being a right of appeal.

He went on to explain the responsibility of the seller to advise the
authorities if contamination was suspected after the sale and the authority to condemn a carcass if it is not identified in a manner to permit traceback for 90 days.

In response to this presentation, the Committee acted to request its Chairman to arrange to have USDA-FSQS consult with major livestock organizations and follow through to the point that the draft of proposed legislation meet the approval of the USAHA Identification Committee and major livestock groups.

In attempting to assess the effectiveness of existing Systems of Identification:

1. Richard Sechrist of National Dairy Herd Improvement Association determined that about 42% of all dairy cattle were identified in some way thru one or more national systems with apathy and lack of direct monetary gain to the producer being the primary deterrent to more complete and precise identification.

2. Burton Ellor of the National Cattlemen's Association questioned the need for total individual animal identification, in that the beef industry had little problem with residues, beef buyers and/or packers knew the source of fat cattle by lot, trader cows were not a significant factor, most beef cattle were identified through private systems for management purposes and most animals in western states were identified by brand.

3. Harold Minderman of Iowa Farm Bureau related the incentives created in the swine industry by federal and state programs as that for Hog Cholera eradication and Brucellosis eradication. From these, demonstrated value in identifying feeder pigs came as a spin-off.

   However, programs involving traceback for residues has developed into a deterrent to identification and most expedient corrective action would be a modification of allowable tolerance levels to a point reasonable yet safe.

   He enumerated the various types of identification, recognizing that the back tattoo, while the most common between producer and packer, had value primarily for traceback, was reduced in effectiveness by skinning. Workable electronic identification would be generally accepted by the swine industry as a uniform system, he added.

4. R. B. McCreight, American Stockyards Association, pointed out that usually the only cattle entering market channels identified as to ownership are branded cattle. After entering the market, the primary identification of cattle is by back tag, to accommodate the Brucellosis program and amounts to a policing action, as the case of applying tattoos to sows and boars, with the latter having no market management benefit. He emphasized that identification by the first owner and not by a disinterested third party is the only fail-safe
identification procedure.

5. Dewey Bond, American Meat Institute, reported that cattle are generally identified at least to feedlot when arriving at slaughter, and the majority of hogs can be traced to farm of origin; however, some have no identification. Of particular interest was the cattle packers' unanimous opposition to the proposed mandatory identification legislation, with substantiating reasons put forth. While hog packers supported the proposal 2 to 1, they recognized problems as gaining cooperation of sellers and market agencies, added administrative expense and maintaining identity through slaughter.

An educational program was recommended in connection with feedback to producers as an incentive.

6. Dr. W. C. Ray, USDA-APHIS, explained the pilot project of computerizing animal health records in order that the massive amounts of information would be retrievable and computerized systems that would take data from the field on each animal, in order that information could be retrieved for any number of purposes. In this, a major problem was the inconsistencies in, or varied systems of identification. In the 5 pilot states, the data will include herd tests, vaccination, market cattle ID, BRT results and epidemiological information for program evaluation.

7. Dr. G. W. Snyder, USDA-FSQS, pointed out that the responsibility of his staff involved post mortem procedures and with complete identification, the procedures could be tailored to the type of animal and its origin.

8. Dr. Keith Farrell, USDA-ARS, introduced Dr. Waldron, President of the Idaho Horse Council, who expressed concern about the efforts of preservationists to stop legitimate horse slaughtering.

9. Dr. Harry Gyer, USDA-SEA, suggested that the Committee give serious consideration to what role identification can play in being a factor in assessing various causes of morbidity and mortalities which will help identify areas of needed research and provide information which will justify the kind of budgetary expenditure in areas of research that are needed.

In acting on Resolutions and Motions, the Committee approved a Resolution calling for records in each state that identifies the apparent reason for each unsuccessful Brucellosis reactor traceback and that they can be summarized quarterly, annually and nationally, as a basis for program improvement.

In addition, the Resolution called for methods of maintaining Brucellosis surveillance where multiple dealer and/or market movement should receive special study with a view of developing a system or systems which is practical for (1) the Brucellosis Program, (2) dealer and
The committee acted to suggest:

1. That states with relatively large livestock populations and/or extensive marketing and slaughtering facilities establish a state-federal veterinary position of Animal Identification and Disease Surveillance Coordinator. This individual will be responsible for planning, organizing and implementing an effective program. Non-veterinary Animal Identification Program Specialist positions would also be established to assist the coordinator where the volume of activity justifies such action in order to provide adequate program support at the field operating level.

2. That each state or federal area establish an Animal Identification and Disease Surveillance Oversight Committee. Membership of the Committee will include the State Veterinarian, Federal Area VIC, Representative of the Livestock Market Association, representative of the meat packing industry, producer representative (cattle), producer representative (swine), Extension Service Representative, Area Inspector in Charge, Federal Meat Inspection, State Director, State Meat Inspection, and the Animal Identification and Disease Surveillance Coordinator. The latter designated person will serve as the Executive Secretary and Program Chairman of the Committee. In some states, Committees already exist which can accommodate this function.

In other action, the Committee requests that in the procurement of identification materials and devices that a prospective vendor be required to establish financial capability, that the purchase contract contain satisfactory specifications to assure adequate quality with appropriate quality control procedures and that due consideration be given to past performance of such prospective vendor.

It then instructed the Chairman of the Committee, through the use of the members of this Committee, to institute a program of reviewing the system of keeping identification records and distribution of identification materials in the offices of State Veterinarians, Extension Dairymen and DHI organizations, including their systems of accountability.

Mr. Chairman, we present this report for further consideration of the Executive Committee.
THE EFFECT OF EXPOSURE TO MITES, 
PSOROPTES OVIS (ACARINA: PSOROPTIDAE), 
ON CALVES OF VARYING SUSCEPTIBILITY TO COMMON SCABIES

William P. Meleney, DVM, and William F. Fisher, MS
Scabies and Mange Research Unit, U.S. Livestock Insects Lab.,
Agricultural Research, Science and Education Administration,
U.S. Department of Agriculture, P.O. Box 232, Kerrville, TX 78028

ABSTRACT
During the winter of 1976-77, 71 predominantly Hereford beef calves were exposed to psoroptic scabies by contact and by mechanical transfer. Two calves died and the other 69 were divided into 3 groups based upon their susceptibility to scabies as judged by monthly examinations and weighings. Five similar Hereford calves were not exposed to scabies, but were weighed and examined monthly.

Group A (highly susceptible) consisted of 38 calves so heavily infested at the end of 2 months' exposure that they were removed from the larger group and treated with various acaricides. Group B (moderately susceptible) consisted of 9 calves that became heavily enough infested to need treatment at the end of 4 months. Group C (refractory) consisted of 22 calves that developed clinical lesions of scabies but not of sufficient severity to warrant treatment. The controls were designated Group D.

Groups A, B, C, and D gained 17.0, 34.9, 55.3, and 51.8 lb, respectively, in 2 months. Groups B, C, and D gained 32.2, 79.3, and 125.8 lb, respectively, in 4 months.

INTRODUCTION
Psoroptic scabies of cattle, caused by the mite Psoroptes ovis (Hering 1838), which also attacks sheep, horses, and bighorn sheep in this country, has become an increasingly common and alarmingly costly parasitic condition among feedlot and range beef cattle in the western and mid-western United States over the past decade.

Very few cost-related investigations have been conducted on this disease in cattle, even though such studies could greatly bolster the push toward nationwide eradication of scabies. The single most widely quoted study was published 2 decades ago when prices and costs were much lower than today.

In the process of obtaining suitably infested calves for the evaluation of candidate acaricides during the winter of 1976-1977, records of weights were kept on calves, exposed and not exposed to psoroptic scabies, at the Parasite Research Laboratory at Albuquerque, New Mexico.

This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement by the USDA.
EXPOSURE TO MITES ON CALVES

MATERIALS AND METHODS

Seventy-six calves, 6-8 months old, of predominantly Hereford breeding, but some crossed with Angus or other beef breeds, and weighing between 154 and 445 lb, were used in this study. The calves had been purchased from 2 ranchers in New Mexico or had been raised on the Parasite Research Laboratory (PRL) premises. During September 1976, the calves were assembled and were immediately placed in a 1.3-acre drylot with scabies infested cattle where they were fed alfalfa hay and water ad libidum, and had access to commercial salt-mineral blocks. Five Hereford calves were segregated as unexposed controls, and were confined to a separate 0.65-acre drylot where they received the same type of feed and water. These controls were selected from the calves purchased from one of the 2 ranch sources. At no time were the control calves allowed to come in contact with the infested calves.

On Sept. 14 all calves were ear-tagged and weighed. Each month thereafter calves, except those treated with experimental acaricides and confined to special isolation pens, were weighed again. On these occasions, control calves were weighed first, minimizing the chance of infestation through contact with fences, chutes, pens, and scales used to handle and weigh infested calves the previous month. However, no such chance could be taken with the infested calves that had been treated with test materials. If acaricide-treated animals had been found harboring live mites, we could not have been able to say whether mites had survived the treatment or had been acquired subsequently through contact with a contaminated fomes.

From September to November, all calves came down with severe cases of pink-eye (infectious keratoconjunctivitis); as a result, some became blind and were moved to an adjacent 1.3-acre drylot so they would not have to compete for feed with sighted calves.

As calves became clinically infested with scabies, they were selected from one of the infested groups and were treated with various experimental acaricides. At each monthly weighing (Table 1) only controls and calves not previously selected for treatment were weighed.

On 3 occasions, one each in October, November, and December, scrapings were taken from some of the heavily infested cattle and transferred to the backs of calves that had not yet developed clinically detectable scabies.

RESULTS

Between the second (Oct. 19) and third (Nov. 15) monthly weighings 2 calves in the exposed group died and 38 were selected for treatment (Group A). The remaining 31 exposed calves and the 5 controls (Group D) were weighed on Dec. 14 and Jan. 11; and after the January weighing, 9

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*Mooreman Mfg. Co., Quincy, IL 62301*
more calves were selected for acaricide treatment (Group B). The 22 untreated calves remaining in the exposed group were designated Group C.

The high, low, and mean of the initial weights and the high, low, and mean of the pounds gained each month, during the first 2 months, and during the second 2 months by each of the 4 groups of calves are given in Table 1.

Group A had the greatest range between the initial weights of the lightest and heaviest calves, 291 lb (this is not unexpected since it was the largest group); Group B which consisted of only 9 calves, had a range of 141 lb; Group C had a range of 213 lb; and in Group D, the control group, the range was only 86 lb. The average gain in weight in Group A during the first 2 months was 17.0 lb. They were not weighed thereafter. The calves in Group B gained 34.9 lb during the first 2 months (twice as much as the calves in Group A), but only 32.2 lb during the entire 4-month period of the test. The 22 calves in Group C, which never became severely enough infested with scabies to be used in any chemical trials, gained an average of 55.3 lb the first 2 months and had an average total gain of 79.3 lb during the entire test. The 5 control calves, which were not subject to mite worry or attack at all, gained 51.8 lb the first 2 months and 125.8 lb in 4 months.

The weights of the calves in each group at each of the 4 monthly weighings as well as the data summarized in Table 1 were subjected to an analysis of variance, and the data summarized in Table 1 were compared between groups by using the Student-Newman-Keuls Test. We found no significant difference at the 95% level of confidence between the initial weights of groups of calves. Significant differences between the mean values in each column given in Table 1 are indicated by the same lower case letters appended to the values.

We recognize that not all calves were subjected to the same degree of infestation pressure with psoroptic scabies mites. However, they were exposed to enough pressure to develop easily recognizable, clinical cases of scabies in a majority of cases (68%). Those calves subjected to additional pressure, in the form of 1 or more mechanical transfers of scabs and mites scraped from donor cattle were not necessarily those that came down with clinical scabies in January; many of them escaped clinical infestations altogether.

DISCUSSION

The 3 groups of calves exposed to psoroptic scabies may be designated "highly susceptible" (Group A), "moderately susceptible" (Group B), and "refractory" (Group C). It should be noted that groups were compiled in retrospect and that exposed calves were kept together in 1 of the 2 drylots until selected for treatment or until the end of the scabies season.

Calves were exposed to and almost all came down with severe bacterial
pink-eye. This disease, coupled with the adjustment to a new environment and hay rack feeding, probably contributed to the relatively low weight gains recorded in the 4 groups of 4.0, 6.0, 14.9, and 11.6 lb, respectively, during the first month of the test. December and January of the winter of 1976-1977 were very cold and windy; this stress factor probably kept all calves from gaining as much weight as they would have in less severe weather. Groups B and C, however, also had to contend with the added stress of mite worry and attack, which explains why they gained so much less weight than the unexposed controls in Group D.

The weight gains during the first 2 months of the test follow the scabies susceptibility pattern of the 3 groups of exposed calves. The similarity in weight gains made in Groups C and D illustrates the degree of protection enjoyed by the refractory group (Group C) and underscores the undesirable effects of scabies mites on the more susceptible individuals.

Group A was not weighed at the end of 4 months, but the marked differences in the amount of weight gained by the other 3 groups demonstrate the role played by resistance and non-exposure in protecting calves from the ill effects of scabies and allowing them to put on money-making pounds of flesh. During the last 2 months of the test, Group B lost 2.7 lb, Group C gained 24.0 lb, and Group D gained more than 3 times as much, 74.0 lb. Cumulative data on weight gains are presented graphically in Fig. 1.

This study is a clear demonstration of the debilitating effect of psoroptic scabies mites on short-yearling beef cattle under the conditions described. If the importance and seriousness of scabies infestations is minimized, and if State and Federal programs to eradicate the disease are not supported or are de-emphasized, cattlemen, feeders, traders, and consumers will all suffer the consequences.
Table 1. Initial Weight and Gain or Loss (in pounds) by 69 Calves Exposed and 5 Calves Not Exposed to Psoroptic Scabies for 2 or 4 Months.

<table>
<thead>
<tr>
<th>Initial weight (lb)</th>
<th>Gain or loss (lb)</th>
<th>mos. 1 &amp; 2</th>
<th>mos. 3 &amp; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st mo.</td>
<td>2nd mo. (cumulative)</td>
</tr>
<tr>
<td>Group A (38 calves)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 238</td>
<td>-4.2a</td>
<td>20.9a</td>
<td>16.7ab</td>
</tr>
<tr>
<td>High 445</td>
<td>71</td>
<td>71</td>
<td>91</td>
</tr>
<tr>
<td>Low 177</td>
<td>-105</td>
<td>-89</td>
<td>-91</td>
</tr>
</tbody>
</table>

| Group B (9 calves)  |                   |            |                      |          |                      |       |
| Mean 246            | 4.3               | 30.6       | 34.9                 | 1.1ab    | -3.8a                | -2.7a  |
| High 337            | 22                | 55         | 58                   | 16       | 22                   | 23    |
| Low 196             | -9                | 14         | 12                   | -13      | -31                  | -36   |

| Group C (22 calves) |                   |            |                      |          |                      |       |
| Mean 248            | 14.9a             | 40.4a      | 55.3a                | 19.9a    | 4.1b                 | 24.0a  |
| High 393            | 42                | 69         | 99                   | 51       | 31                   | 72    |
| Low 180             | -9                | 10         | 18                   | -1       | -17                  | -12   |

| Group D (5 calves)  |                   |            |                      |          |                      |       |
| Mean 225            | 11.6              | 40.2       | 51.6b                | 24.0b    | 50.0ab               | 74.0a  |
| High 264            | 36                | 56         | 92                   | 36       | 70                   | 106   |
| Low 178             | 0                 | 27         | 27                   | 14       | 38                   | 52    |

*Same superscript within a column indicates statistical difference between means (P < 0.05).
Fig. 1. Cumulative mean gain or loss (in pounds) in four groups of beef calves exposed or not exposed to psoroptic scabies for 2 or 4 months.


The United States-Texas Tick Program (Boophilus annulatus and B. microplus) in FY 1979 continued to be involved with stray or smuggled livestock crossing the international border between Mexico and the State of Texas. Texas' law requires the quarantining of premises due to "exposure" from these animals. The following number of animals were apprehended by the tick force in FY 79:

<table>
<thead>
<tr>
<th>Mexico to USA</th>
<th>USA to Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 cattle</td>
<td>20 cattle</td>
</tr>
<tr>
<td>54 horses</td>
<td>13 horses</td>
</tr>
<tr>
<td>1 mule</td>
<td></td>
</tr>
</tbody>
</table>

Forty-four (44) percent (12 of 27) of the Mexican cattle were infested with *Boophilus* ticks.

We had a total of 28 new infestations in FY 79. Dividing the infestation into two historical categories, the Quarantine Zone next to the Rio Grande, we had 22 new infestations. Our second category, the final area, which is the remainder of the state, had 6 new infestations.

Wildlife survey activities were expanded in FY 79 to define the extent to which *B. annulatus* infest white-tailed deer (Odocoileus virginianus) and other wildlife within the Webb County, Texas problem area, and to determine the geographic distribution of wildlife infested with *B. annulatus*.

All survey activities were conducted by the Southeastern Cooperative Wildlife Disease Study (SCWDS), Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, Georgia through cooperative Agreement No. 12-16-51057.

Fifty-two (52) "pastures" were involved. White-tailed deer were sampled from April 10-June 20, 1979. Two hundred ninety-seven (297) deer (7.2 percent of the estimated total deer population) were collected. Twenty-nine (29) deer from 10 pastures were infested with *B. annulatus*.

The following table illustrates the relationship of infested pastures to cattle and deer.
Surveillance activities have also been completed on the rodent phase and the rabbit populations.

The rodent collections included the following animals:

- hispid cotton rats (*Sigmodon hispidus*)
- hispid pocket mice (*Perognathus hispidus*)
- cactus mice (*Peromyscus eremicus*)
- Mexican ground squirrels (*Citellus mexicanus*)
- southern plains woodrats (*Neotoma micropus*)
- northern grasshopper mice (*Oncychomys leucogaster*)
- Fulvous harvest mice (*Reithrodontomys fulvescens*)
- house mice (*Mus musculus*)
- white footed mice (*Peromyscus leucopus*)
- Merriam pocket mice (*Perognathus merriami*)
- brush mouse (*Peromyscus boylei*)
- pygmy mouse (*Baiomys taylori*)

No infestation was detected among the rodent population.

The rabbit surveillance included the desert cottontail rabbits (*Sylvilagus auduboni*) and the blacktail jackrabbits (*Lepus californicus*). No infestation was detected among the rabbit population.

Javelina (*Pecari tajacu*) and coyote (*Canis latrans*) surveillance is in progress. As of October 12, 1979 one coyote was found to be infested with one *B. annulatus* attached near the ear. Twenty-five (25) coyotes have been sampled. Fifty-four (54) javelina have been sampled and all are negative for *B. annulatus*.

We believe the relationship between these non bovine-equine hosts is similar to the relationship between *B. annulatus* and sheep and goats. That is, other animals become infested with fever ticks in direct relationship and proportion to the rate of infestation of cattle.

Experimental and field experience have shown that dipping of the cattle and horses will eradicate *Boophilus* ticks from the alternate hosts that cohabitate within the infested area.

The following animals have been found to be alternate hosts for *B. annulatus* in Webb County, Texas:

- white-tailed deer (*Odocoileus virginianus*)
- nilgai (*Boselaphus tragocamelus*)
- coyotes (*Canis latrans*)

Mr. E. Ahrens, Regional Entomologist and Dr. J. H. Gray, Regional
Epidemiologist, visited the Mexico Tick Program in FY 79. The Mexico Tick Program is ambitious and extensive. There are approximately 27,000 dipping vats, with the average vat size approximately 10,000 liters (2,642 gallons). The tick campaign employs approximately 3,200 field people. All areas of Mexico infested with Boophilus ticks are actively involved in some stage of the program. The campaign has four basic stages as follows:

1. Promotion — The emphasis during this stage is construction of dipping vats and the education of the livestock owners concerning the tick campaign.

2. Control — Commences with voluntary cooperation of dipping infested animals at 14-day intervals. Establishment of a tick surveillance system.

3. Eradication — Compulsory dipping of livestock.

4. Free — The free stage is declared after an area has gone two winters and one summer without detection of Boophilus ticks.

Movements of cattle are controlled by quarantine stations and “line vats” placed along the major routes of commerce. If cattle are found infested with ticks at one of these facilities, the animals are dipped, held 3 days, and redipped.

A new research laboratory is under construction in Cuernavaca which will employ approximately 135 people. The laboratory will have two dipping vats and one spray-type treatment area. The laboratory will be for basic tick research and other livestock pest research.

Until Mexico accomplishes its Boophilus tick eradication goals, the United States-Mexico border area will continue to face the problem of reintroduction of Boophilus ticks.

SCREWWORM (SW) ERADICATION PROGRAM

The major objective of the screwworm (SW) eradication program is to eradicate the parasite from the United States and Mexico down to the Isthmus of Tehuantepec, and there establish a barrier to prevent reinfestation of free areas to the north. Departments of Agriculture from both countries participate in the endeavor along with livestock industries and local government officials.

Sterile fly production was increased from a maximum of 200 million weekly up to 500 million weekly by the addition of a new production facility at Tuxtla Gutierrez, Chiapas, Mexico. Limited production began in Tuxtla late in 1976, and reached a level of 400 million for a short period early in 1979, but soon was reduced to 300 million again due to budgetary restrictions.

The Screwworm Adult Suppression System (SWASS) was used in 1979 as an aid to the sterile fly technique in widespread application other than in developmental tests. SWASS is an attractant-bait-toxicant product
designed for aerial distribution in areas of wild SW fly activity to reduce the wild fly population down to low levels where sterile flies will be the most effective.

Baja, California, received first priority for eradication when the effort in Mexico was initiated. The last case in the peninsula was collected on June 30, 1979, and it appears eradication of self-sustaining populations has been achieved. A quarantine line to control livestock movements between the Mexican mainland and the peninsula has been established.

Releases of sterile flies during 1979 was concentrated in southwestern United States and northern areas of Mexico. After a difficult beginning with heavy infestations in many locations, the program gained control of the native fly populations and record low numbers of cases resulted.

The Mexico States bordering the United States will be freed of self-sustaining populations of native SW during 1980 provided sufficient funds are available to maintain scheduled production levels; permission is granted by the Mexican Government to use SWASS throughout Mexico; and weather conditions are favorable for the eradication effort.

Continued gains in moving the eradication effort southward in subsequent years will depend primarily on receiving sufficient funds to overcome the increased costs of labor and materials in Mexico and the United States. Additional improvements in program techniques and development of new systems are also vital to the success of the campaign. Extensive research is now underway to determine the possibilities of genetic differences in SW flies. A university group and government researchers are cooperating in the study.

The following cases have been reported through October 17 of this year: Arizona—33; California—2; New Mexico—13; and Texas—31. Last year during the same time span the following cases were reported: Arizona—3,801; California—112; New Mexico—1,264; and Texas—994.
### ARIZONA (3)
- Maricopa (2)
- Navajo (1)
- CALIFORNIA (21)
  - Fresno (2)
  - Kern (1)
  - Merced (1)
  - Modoc (1)
  - Monterey (4)
  - Sacramento (1)
  - San Joaquin (1)
  - San Luis Obispo (5)
  - Santa Barbara (1)
  - Shasta (1)
  - Stanislaus (1)
  - Tulare (1)
- COLORADO (31)
  - Baca (2)
  - Cheyenne (1)
  - Crowley (1)
  - Kiowa (3)
  - Kit Carson (6)
  - Logan (2)
  - Morgan (2)
  - Otero (4)
  - Phillips (1)
  - Prowers (2)
  - Pueblo (2)
  - Weld (4)
  - Yuma (1)
- IDAHO (2)
  - Custer (1)
  - Gooding (1)
- ILLINOIS (2)
  - Bureau (1)
  - Henry (1)

### IOWA (24)
- Clay (1)
- Crawford (1)
- Dallas (1)
- Decatur (1)
- Emmet (1)
- Fayette (1)
- Franklin (1)
- Hamilton (1)
- Hardin (1)
- Harrison (1)
- Humbolt (1)
- Ida (2)
- Iowa (1)
- Lyon (1)
- O'Brian (3)
- Osceola (1)
- Plymouth (1)
- Sioux (2)
- Wayne (1)
- Winneshiek (1)

### NEBRASKA (29)
- Buffalo (2)
- Cherry (1)
- Cheyenne (2)
- Cuming (2)
- Dawson (4)
- Dundu (1)
- Gosper (1)
- Greeley (1)
- Hall (1)
- Hamilton (1)
- Howard (2)
- Lincoln (2)
- Morrill (2)
- Perkins (1)
- Scotts Bluff (2)
- Sioux (2)
- Thomas (1)
- Washington (1)

### SOUTH DAKOTA (25)
- Beadle (1)
- Brown (1)
- Campbell (1)
- Corson (2)
- Charles Mix (3)
- Dewey (4)
- Edmonds (1)
- Faulk (1)
- Hand (2)
- Kingsbury (2)
- Lincoln (1)
- Lyman (1)
- McPherson (1)
- Moody (1)
- Spink (1)
- Walworth (1)
- Ziebach (1)

### KANSAS (18)
- Finney (2)
- Hamilton (1)
- Lane (2)
- Logan (1)
- Meade (1)
- Norton (1)
- Osborne (1)
- Reno (1)
- Rush (1)
- Scott (3)
- Wichita (4)

### NEW MEXICO (17)
- Colfax (2)
- Lea (2)
- Roosevelt (9)
- Santa Fe (2)
- Union (2)

### OKLAHOMA (12)
- Cimarron (2)
- Craig (1)
- Garvin (2)
- Stephen (1)
- Texas (5)
- Tillman (1)

### NEVADA (1)
- Pershing (1)

### TEXAS (32)
- Brisco (1)
- Carson (1)
- Castro (3)
- Dallam (2)
- Deaf Smith (3)
- Floyd (1)
- Hale (1)
- Hays (1)
- Hartley (2)
- Hutchinson (3)
- Oldham (1)
- Potter (1)
- Randall (4)
- Sherman (4)
- Swisher (4)

### WYOMING (11)
- Converse (2)
- Goshen (3)
- Natrona (5)
- Niobrara (1)
REPORT OF THE COMMITTEE ON
PARASITIC DISEASES AND PARASITICIDES

Chairman: R. L. Pyles, Albuquerque, New Mexico

Co-Chairman: John F. Hudelson, Denver, Colorado


The Committee met on Wednesday, October 31, 1979, with 32 members and guests present.

Dr. Cheryl Knobloch, Veterinary Communications Officer, Bureau of Veterinary Medicine, FDA, outlined to the Committee, efforts aimed at a solution to the liver fluke problem caused primarily to the loss of the use of Hexachlorethane in 1971 after it was found to be a highly potent carcinogen. A new animal drug, Albendazole, will be made available at a rate of up to one million doses every six months for use by veterinarians in areas where liver flukes are a great problem. Serious food problems, in connection with this product, needs to be answered and FDA will monitor the use of this product. Usage will be limited to brood beef cows, initially. The present feeling is that this product may be the answer to the liver fluke problem.

Dr. W. P. Meleney, Leader, Scabies and Mange Research, SEA, USDA, Kerrville, Texas, gave a paper on "Effect of Exposure to Psoroptic Scabies Mites on Calves of Varying Susceptibility to Common Scabies."

Dr. J. R. Pemberton, Head, Chemistry Section, SSL, NVSL, Ames, Iowa, discussed problems of the national scabies eradication program from the laboratory's point-of-view. His comments, highlighted with slides, emphasized the great need for better care of the products used; mixing; sample collection and identification for submission to the laboratory; the need for use of proper dipping vat sample bottles; use of the proper forms and good record keeping. The report re-emphasized the need for improvement in our overall dipping vat management practices.

Dr. J. H. Gray, Regional Epidemiologist, VS, APHIS, USDA, Austin, Texas, gave a paper on the FY 1979 activities in the United States - Texas Tick Program (Boophilus annulatus and B microplus).

Drs. J. L. Hourrigan, G. O. Schubert and J. Novy, V.S. APHIS., USDA reviewed the national programs on cattle scabies; the international program on screwworms and the tick eradication program in Puerto Rico.

In the scabies program, veterinary services has purchased 10 more spray-dip machines, 10 portable swim vats, 7 portable cage vats and 17
trailers with tanks. Most have now been delivered to the field stations for use. Approximately 44 persons have been trained and approved to use the field test kits for Prolate and Co-Ral dips. Preliminary information on withholding times of dipped cattle from streams and ponds indicates toxaphere dipped cattle should be withheld for 7 days; prolate for 4 days; Co-Ral for 3 days; Ciodrin for 3 days and Delnav for 3 days.

A copy of each of the various papers presented to the Committee are made a part of this report.

The Committee received, considered and voted favorably on four resolutions. These resolutions, briefly outlined below, were properly prepared and presented to the Resolutions Committee for its consideration.

Psoroptic Scabies Control — requests that USDA, APHIS, VS, maintain the present quarantine and dipping regulations and the funding at a level, at least equal to those maintained during the past two years, until psoroptic cattle scabies is finally eradicated.

Psoroptic Scabies Research — requests USAHA to go on record as favoring increased financial support by USDA/AR and CR of ongoing research projects involved with Psoroptes mites.

Research on ticks in Puerto Rico — requests USAHA to urge USDA-SEA/AR to establish a temporary research laboratory in the Commonwealth to study the biology, life history, etc. of both B. microplus and A. variegatum.

Integrated Pest Management of Livestock Pests — requests USAHA to support the budget increase proposal for Integrated Pest Management of insects affecting livestock and poultry and urges USDA-SEA to fund this proposal as soon as possible.

CATTLE SCABIES PROGRAM

The total number of reported cattle scabies outbreaks in FY 1979 showed a 24 percent decrease over FY 1978. FY 1979 had 238 outbreaks and FY 1978 had 313. There was a total of 16 States and 137 counties involved. Enclosed is a list of States and counties showing the total number of outbreaks in each.

The Veterinary Services (VS) budget for FY 1979 had been increased to a total of $5,425,000, allowing VS and States to enter into cooperative agreements to increase the scabies work force. Additional equipment was contracted for in order to provide more and better portable treatment facilities. This included 10 spray-dip machines, 10 portable swim vats, 7 portable cage vats, and 17 tanks and trailers.

Part 73, Title 9, Code of Federal Regulation revisions were started which will, if adopted, provide for State-Federal approved treatment facilities. These facilities will hopefully improve the precautionary
treatment of cattle entering those States that require such treatment before moving.

A committee of the National Academy of Sciences was contracted to review scabies research and recommended future research. The final report was due at the end of FY 1979, but a request for a 90-day extension was granted. The final report will not be available until the first quarter of FY 1980.

A National Scabies Epidemiologist was assigned to the Sheep, Goat, Equine, and Ectoparasites Staff in Hyattsville, Maryland. VS Memorandum 556.11 was prepared and distributed outlining the National Scabies Program. This program called for assigning Regional Scabies Epidemiologists and a State Coordinator for each State experiencing scabies outbreaks. These assignments were made.

Hopefully, by instituting the National Scabies Program, the increase in cooperative agreements, adequate treatment facilities, and an increase in scabies epidemiology, a continued decrease in scabies outbreaks will eventually lead to eradication.
PREVENTION AND CONTROL OF AVIAN INFLUENZA IN TURKEYS

A. K. Bahl
Cuddy Farms, Marshville, North Carolina
Althea Langston
Veterinary Services, APHIS-USDA, Des Moines, Iowa
R. A. Van Deusen
National Veterinary Services Laboratories, APHIS-USDA, Ames, Iowa
B. S. Pomeroy, John Newman, Daniel Karunakaran, David Halvorson
Avian Disease Research Program, College of Veterinary Medicine
St. Paul Minnesota 55108

Avian influenza has become an increasingly important problem facing the poultry industry, particularly the turkey industry. The first reported isolation of avian influenza virus from North America (other than fowl plague) was made from ducklings in Manitoba in 1953, but it was not until 1967 that the isolate was identified as Type A influenza virus. The first isolation from turkeys was made in 1963 in Canada, and was followed by other isolations reported by Lang et al. The first reported isolation in the United States was made in California in 1964 by Bankowski and Mikami. Olesiuk et al. reported an isolation in Massachusetts from turkeys in 1965, followed by isolations in 1965-66 from turkeys in Wisconsin. Since 1966 numerous reports have been made, indicating isolations from many species of birds. Easterday and Tumova have indicated that viruses or their antibodies have been demonstrated in at least 50 avian species.

HISTORY OF AVIAN INFLUENZA OF TURKEYS IN MINNESOTA

Avian influenza was first recognized in Minnesota in the fall of 1966 by serological procedures and virus isolation was made in 1967 (HAV₄N₅). The outbreak occurred in a range flock which showed depression, loose droppings and absence of respiratory signs. This outbreak was first reported as a clinical outbreak of bluecomb disease (Coronaviral Enteritis) but, in fact, proved to be avian influenza. In 1965-66 avian influenza had been diagnosed in turkeys in western Wisconsin, but there was no association between the two outbreaks except that the same subtype was involved (HAV₄N₅). In 1967 Kleven et al. studied an extensive involvement of turkey breeder flocks and artificial insemination crews were implicated in the spread.

Since 1966 the disease has been recognized each year in market and breeder flocks, but it was not until the fall of 1971 that the agar gel precipitin (AGP) test was used in place of HA antigens as a screening test to identify flocks exposed to influenza A viruses. Virus isolations have been attempted, but only a few were successful because in many instances it was late in the outbreak when these attempts were made. In the fall of 1971 an extensive outbreak of avian influenza was recognized in a high-density turkey raising area of Minnesota and resulted in heavy
condemnations owing to airsacculitis and septicemia-toxemia in both fryer-roaster and heavy turkey flocks.

In 1972 Bahl began intensified epidemiological investigations of natural outbreaks as well as field studies on the isolation of influenza viruses from migratory waterfowl and gulls. This study was completed in 1977.4,9,15

In 1978 an epidemiologist from APHIS-USDA Veterinary Services (Althaea Langston) was assigned to the project. In the meantime, National Veterinary Services Laboratories, APHIS-USDA, Ames, Iowa, expanded their capabilities to isolate and serotype field isolates and do serological profiles of serum samples. Also, the Influenza Laboratory at St. Jude Children's Research Hospital, Memphis, Tennessee (Virginia Hinshaw) has been of great help in identification of and cloning virus isolates. It has been extremely difficult for the research facility at Minnesota to develop the laboratory techniques that are available at NADC and St. Jude Children's Research Hospital.

The 1978-79 outbreak became the most extensive and costly outbreak in the history of the disease in Minnesota. Approximately 130 market flocks involving 2,137,989 turkeys and 11 breeder flocks involving 27,680 hens were infected.13

Halvorson et al.15 reported on the isolation of HAV₃N₁ from a caged laying chicken flock experiencing a drop in egg production. This is the only incidence of avian influenza in chickens in Minnesota even though outbreaks in turkeys have occurred in close proximity to chicken farms. Laying and broiler flocks are scattered in the areas where there is a heavy concentration of turkeys.

The following serotypes have been isolated from turkeys and chickens since 1972.

<table>
<thead>
<tr>
<th>Year</th>
<th>HAV Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>HAV₃N ?</td>
</tr>
<tr>
<td></td>
<td>HAV₃Neq₂</td>
</tr>
<tr>
<td>1973</td>
<td>HAV₃N₂</td>
</tr>
<tr>
<td>1974</td>
<td>HAV₃Neq₂</td>
</tr>
<tr>
<td></td>
<td>HAV₃N₁</td>
</tr>
<tr>
<td>1975</td>
<td>HAV₃N₂</td>
</tr>
<tr>
<td>1976</td>
<td>HAV₃N ?</td>
</tr>
<tr>
<td>1977</td>
<td>HAV₃Neq₂</td>
</tr>
<tr>
<td></td>
<td>HAV₃N₂</td>
</tr>
<tr>
<td>1978</td>
<td>HAV₃Neq₂ (1)*</td>
</tr>
<tr>
<td>1979</td>
<td>HAV₃N₁ (72 + )*</td>
</tr>
</tbody>
</table>

* Number of isolations from flocks

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian Influenza Serotypes Isolated from Turkeys and Chickens in Minnesota</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>1972</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1973</td>
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<td>1974</td>
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<td></td>
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<tr>
<td>1978</td>
</tr>
<tr>
<td>1979</td>
</tr>
</tbody>
</table>

chicken flock
AVIAN INFLUENZA IN TURKEYS 357

ECONOMIC IMPORTANCE OF AVIAN INFLUENZA

Berg, Halvorson and Newman\(^\text{18}\) did an extensive economic study of the 1978-79 avian influenza outbreak in Minnesota. The summaries of the losses are reported in Tables 2 and 3.

### TABLE 2
Summary of the Losses Caused by an Outbreak of Avian Influenza in Minnesota 1978-79

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flocks involved</td>
<td>130</td>
</tr>
<tr>
<td>No. of poults started infected with AI</td>
<td>2,137,989</td>
</tr>
<tr>
<td>No. of poults died from AI</td>
<td>356,441</td>
</tr>
<tr>
<td>Percent mortality</td>
<td>16.7%</td>
</tr>
<tr>
<td>Value of birds that died</td>
<td>$2,171,355</td>
</tr>
<tr>
<td>Value for loss in weight</td>
<td>$ 555,020</td>
</tr>
<tr>
<td>Loss of profit</td>
<td>$ 174,488</td>
</tr>
<tr>
<td>No. of birds marketed</td>
<td>1,208,238</td>
</tr>
<tr>
<td>No. of birds condemned</td>
<td>81,535</td>
</tr>
<tr>
<td>Percent of condemned turkeys</td>
<td>6.7%</td>
</tr>
<tr>
<td>Value of condemned turkeys</td>
<td>$ 759,966</td>
</tr>
<tr>
<td>Medication costs</td>
<td>$ 135,480</td>
</tr>
<tr>
<td>Extra clean-up costs</td>
<td>$ 38,743</td>
</tr>
<tr>
<td>Other costs</td>
<td>$ 112,859</td>
</tr>
<tr>
<td><strong>Total loss from AI</strong></td>
<td><strong>$3,947,911</strong></td>
</tr>
</tbody>
</table>

### TABLE 3
Breeder Hen Losses from Avian Influenza in Minnesota

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flocks infected</td>
<td>11</td>
</tr>
<tr>
<td>No. of hens involved</td>
<td>27,680</td>
</tr>
<tr>
<td>Percent mortality</td>
<td>10.2%</td>
</tr>
<tr>
<td>No. of eggs lost</td>
<td>486,700</td>
</tr>
<tr>
<td>Value due to mortality</td>
<td>$ 49,585</td>
</tr>
<tr>
<td>Loss of egg production (value)</td>
<td>$184,946</td>
</tr>
<tr>
<td><strong>Total loss</strong></td>
<td><strong>$234,531</strong></td>
</tr>
</tbody>
</table>

The cost of depopulation of farms where avian influenza had occurred and the loss of income from leaving the facilities empty were not calculated. The highest total mortality reported was 76.6% and the highest total condemnation loss was 73.1% of the birds marketed.

**SOURCE OF INFECTION**

In Minnesota heavy market turkeys are raised on range from May 1 to November 1 with exceptions depending on weather conditions. The fryer-roaster turkeys are raised in confinement. In 1971-72 fryer-roaster
Turkeys were involved in the avian influenza outbreak as well as heavy turkeys, and the same was true in 1978-79. These turkeys are raised in an area adjacent to the area where heavy production of market turkeys occurs. In the intervening years (1972 to 1978) no outbreaks occurred in the fryer roaster growing areas.

One aim of the epidemiological study in Minnesota was to determine some of the factors relating to the primary source of the infection and the way in which it spreads within the area. One hypothesis is that the virus is constantly present in the turkey population and it reaches an explosive stage under certain stress conditions, producing clinical disease and serological response. The second hypothesis is that the virus is introduced occasionally from external source(s) at a primary point(s) and it spreads from the primary introduction to secondary flocks.

Since 1972 breeder flocks (150-200 per year) at 16-20 weeks of age have been serologically tested using the AGP test, and no blocks have been identified as serologically positive resulting from apparent infection. Extensive studies (serological and tracheal swabs) have been made of turkeys on farms where previous flocks have been positive and the farms have been depopulated before new birds were started. The new flocks have remained negative for several months. The weakness in the data may be that cloacal swabs were not collected at the same time as tracheal swabs and blood samples. The flocks remained serologically negative and no avian influenza virus isolations were made. In one year (1975) only one infected breeder flock was identified which was not in the area where the disease commonly occurs in west central Minnesota.

Bah18J6 reported on his studies of sampling migratory wild ducks on two wildlife refuges in northern Minnesota over a four-year period (1973, 74, 75, 76). In 1973 HAVs and HAVv were isolated, and in 1974, HAVs, HAVv, HAVv, and HAVv were also identified. In 1975, HAVs, HAVv, HAVv, and HAVv were isolated. In 1976, HAVs, HAVv, HAVv, HAVv, and HAVv were identified. A variety of neuraminidases were also identified, NAVv, NAVv, NAVv, NAVv, Neq, N2.

HSW1N has been reported isolated from aquatic birds in Quebec in 1977. It would appear from reports that various combinations of H and N antigens have been detected among the isolates from birds worldwide. If one monitored a wild duck population over an extended period, various H and N antigens would be detected, indicating new introductions of avian influenza viruses and possible recombinations of the viruses.

The data certainly point to the fact that wild aquatic birds are heavily infected carriers of various H and N antigens primarily in the intestinal tract. These birds, by fecal excretions may pollute ponds, lakes and streams in the vicinity of turkey operations or may actually land in fields adjacent to turkey ranges in search of food. Migration of gulls takes place earlier and at the same time as migration of ducks and other water fowl. Bah1 has been able to experimentally infect mallard ducks, Franklin's
Gulls, redwinged blackbirds and starlings with a turkey isolate. These birds shed the virus for a limited time. Also, using a duck isolate, turkeys were readily infected and transmitted the infection to contact turkeys.

MODES OF SPREAD

Primary focus: Once the virus is introduced into a turkey flock it readily spreads in that flock, and on a farm with multiple ages, it is readily transmitted to flocks in different buildings. In active cases the virus is eliminated in respiratory and intestinal excretions. How long the virus is excreted in an infected flock is not known. Once a flock is known to be infected, it should be considered infected for the life of that flock. It would appear that a number of vectors are implicated in the spread of the infection from farm to farm. In the 1978-79 outbreak one serotype HAV<sub>e</sub>N<sub>1</sub> was involved in practically all outbreaks with isolated instances of other serotypes HAV<sub>v</sub>N<sub>e</sub>q<sub>2</sub>, HAV<sub>e</sub>N<sub>v</sub>, HSW<sub>i</sub>N<sub>1</sub>, HAV<sub>e</sub>N<sub>e</sub>q<sub>1</sub> and HAV<sub>e</sub>N<sub>v</sub>.

1. Man — Movement of personnel on the same farm and from farm to farm is conducive to the spread of the infection. In several instances artificial insemination crews were implicated in the spread of the infection from one breeder flock to another. For example, in 1972 an outbreak of avian influenza occurred in 13 of 24 breeder flocks of one hatchery.4

2. Transfer of turkeys — Transfer of turkeys from farm to farm in the incubative stages of the disease has been a means of spreading the disease.

3. Wild birds — Around turkey farms there are numerous species of wild birds that potentially may spread the infectious agent—gulls, starlings, redwinged blackbirds, etc.

4. Water — Ponds, lakes and streams may become polluted with bird feces and be a potential source of infection to turkeys on range. Avian influenza virus has been isolated from pond water.

5. Fomites — a) Processing trucks; b) turkey loaders moving from farm to farm; circumstantial evidence certainly suggests that these vehicles become contaminated from hauling infected turkeys and transfer the infection from farm to farm; c) feed trucks and personnel may be potentially involved in the mechanical transfer of the agent from farm to farm. Halvorson et al.15 believed the feed truck and personnel may have been the source of the introduction of avian influenza to the chicken flock. Trucks and personnel from the same feed company serviced infected turkey flocks.

CONTROL

1. Depopulation — The controlled marketing of infected flocks after they have recovered from acute infection (4-6 weeks) will minimize the condemnations and has been successful in elimination of the disease from farms.
2. Cleaning and disinfection — After the farm has been depopulated, the buildings are cleaned and sanitized. Under varying Minnesota weather conditions it is difficult to speculate how long the virus will survive in turkey buildings and on range.

3. Rest period — The farm should be depopulated at least two to four weeks following cleaning and disinfection before being repopulated.

4. Repopulation — In almost every instance where the above procedures have been followed, no evidence of reinfection has occurred in subsequent flocks. However, infection has occurred months later on some farms, suggesting a new introduction.

5. Security program — Controlled movement of people and equipment is paramount to prevent introduction of avian influenza. A strict security program must be enforced.

6. Control of wild birds — Turkeys on range are constantly exposed to wild birds and there is constant threat of the introduction of avian influenza. The only solution to this problem is complete confinement of turkeys in bird-proof buildings.

7. Vaccination — A killed autogenous oil emulsion vaccine has been effective under experimental conditions in reducing the mortality and morbidity losses as well as protecting against a drop in egg production. The current plan in Minnesota has been to use an autogenous vaccine in breeder flocks and to limit the use to market flocks in the primary introduction of the infection. Since HAV₄ has been the predominant type over the period of this report, the primary concern is for this type. However, other serotypes have been encountered, such as HAV₂, HAV₄, HAV₅, and HAV₆. It may mean the stockpiling of autogenous vaccines for these other types for use when needed.

PREVENTION

Prevent introduction

1. Complete confinement — It will require time and expenditure of millions of dollars for the Minnesota industry to move to complete confinement programs. The turkey breeder industry has virtually accomplished this end. The commercial broiler and egg-laying chicken industry in Minnesota has also gone this route.

2. Security program — The processing, feed manufacturing and producer groups know what is needed as to security programs but become very lackadaisical in maintaining a rigid sanitation and security program until an outbreak occurs.

3. Wild bird control — The wild bird reservoir of the infection will not go away. This fact has to be accepted as a constant factor. The need is to prevent direct and indirect contact with wild birds, particularly water fowl. This can be accomplished only by complete confinement
and bird control programs.

4. Vaccination — For areas and farms that have perennial problems with avian influenza, a vaccination program may be indicated. Once an outbreak is determined, then preventive vaccination programs may be carried out. This requires a constant surveillance program with rapid identification of influenza isolates so an autogenous vaccine may be used immediately to prevent the spread of the infection.

DISCUSSION

In the past few years Australia (1975) and England (1979) have reported outbreaks in chickens and turkeys with highly virulent strains of influenza A virus. The isolates from turkeys in Canada and the United States have varied in virulence, but under field conditions complicated infections are not uncommon (colibacillosis, Newcastle disease, pasteurellosis). The losses have been as high as 41% in breeder flocks and 76% in market flocks. Condemnations in flocks in the active stages of the disease may be as high as 75%. In breeder flocks in production there is a dramatic effect on egg production, increased cull eggs and lowered fertility and hatchability. The loss to the turkey industry in Minnesota in the 1978-79 outbreak was approximately $5 million. One can only shudder at what the cost might be if a highly virulent strain became involved in a high density turkey raising area such as west central Minnesota where 75% of the turkeys in Minnesota are raised.

In the 1977 report of the Committee on Transmissible Diseases of Poultry (USAHA) recommendations of the subcommittee on avian influenza were made. These recommendations were submitted earlier to Veterinary Services, APHIS-USDA when the subcommittee was part of the Advisory Committee on Poultry Health to the Secretary of Agriculture. It is time for a frank discussion of the avian influenza problem not only in this country but on an international basis. International authorities become greatly concerned when the term “fowl plague” is used to identify an outbreak of highly virulent form of influenza A virus involving HAV, serotype. Isolations of low virulent HAV, serotypes have been made in the United States and England. It is paramount that avian influenza isolates be rapidly serotyped and tested for virulence in susceptible chickens and turkeys. Allan et al. and Alexander et al. have studied intravenous pathogenicity and intracerebral pathogenicity index tests to measure the virulence of avian influenza viruses. The intravenous pathogenicity index test has been used by National Veterinary Services Laboratories.

SUMMARY

Since 1966, avian influenza has been recognized in turkeys each year in Minnesota. Other states and Canada have reported isolations and
serological evidence of influenza A infections. Isolations from wild waterfowl in Minnesota and in other areas have indicated a variety of serotypes, including HAV₂, HAV₃, HAV₄, HAV₅, HAV₆, HAV₇. Isolations from turkeys have included HAV₂, HAV₄, HAV₅, HAV₆, HAV₉, HSW₁, with a variety of neuraminidases. A widespread outbreak occurred in 1971 and an extensive outbreak occurred in 1978-79 involving over 130 flocks. The latter outbreak involved over 2,000,000 turkeys and a loss of approximately $5 million. With the large reservoir of infection in migratory waterfowl, the evidence suggests that wild waterfowl are a potential source of avian influenza viruses to turkeys on range. In contrast, only one outbreak in chickens in Minnesota has been identified in the period from 1966-1979. The commercial broilers and egg production chickens are raised under complete confinement.

ACKNOWLEDGMENTS

I must first certainly acknowledge the excellent services and advice of Dr. B. C. Easterday, University of Wisconsin, Madison, Wisconsin, that were very helpful in the development of the work on avian influenza at Minnesota. Dr. Virginia Hinshaw at the Influenza Laboratory at St. Jude Children's Research Hospital, Memphis, Tennessee, has been extremely helpful in the identification and cloning of virus isolates. The excellent cooperation of the staff of the Minnesota State Livestock Sanitary Board and Veterinary Services—APHIS-USDA is appreciated.

REFERENCES

AVIAN INFLUENZA IN TURKEYS


CONTROL OF *MYCOPLASMA GALLISEPTICUM*
IN COMMERCIAL LAYING CHICKENS USING ARTIFICIAL EXPOSURE
TO CONNECTICUT F STRAIN *MYCOPLASMA GALLISEPTICUM*

Carpenter, T. E.; Miller, K. F.; Gentry, R. F.; Schwartz, L. D.;
Mallinson, E. T.

INTRODUCTION

The losses in reduced feed and egg production efficiency and increased medication costs make *Mycoplasma gallisepticum* (MG) one of the costliest disease problems confronting the poultry industry today. A paper reviewing the 25 year history of MG immunization in the United States and presenting results and statistical analyses of a large, 2 year field study involving over 143 Pennsylvania commercial poultry farms has recently been presented. This paper presents further details on the Pennsylvania study, particularly regarding an economic evaluation for the poultryman, and the study's implications for local and national prevention, control and eradication of MG.

STATISTICAL ANALYSIS

Production data from the aforementioned 143 flocks were analyzed using the analysis of variance and Duncan's multiple-range test techniques. The flocks were divided into three groups: infected with MG (MG(+)), inoculated with Conn. F-strain (MG inoculated) or free from infection (MG(-)). The production of these three types of flocks were analyzed according to a 45 week production period.

In addition to measuring the impact of MG on production, these flocks were evaluated for production differences according to the strain of the flock as well as flock strain-MG interaction.

The results showed that there were significant (p < .05) differences between the three types of flocks. Hens in the MG(-) flock had an increased production of 10 eggs when compared with the MG inoculated flocks and 21 eggs greater when compared with the MG(+) flocks. Similarly, the average production for the artificially exposed birds was significantly (p <.05) greater (11 eggs/hen housed) than that of the birds from flocks naturally infected with MG.

When the production data for the various strains were compared, significant (p < .05) differences were observed. However for this paper, the importance of strain differences was that there was no strain-MG interaction. That is, no strain was any more or less affected by natural exposure to, or inoculation with Conn. F-strain, MG. Therefore strain differences could be ignored.

Flocks which were artificially exposed to MG, via inoculation, received this exposure at different periods prior to lay. In order to determine if there was any effect related to the time of exposure, the exposure times
were separated into three groups and their respective production levels were compared:

<table>
<thead>
<tr>
<th>Flock age when inoculated</th>
<th>Average production</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 - 12 weeks</td>
<td>214 eggs</td>
</tr>
<tr>
<td>13 - 18 weeks</td>
<td>214 eggs</td>
</tr>
<tr>
<td>after 19 weeks</td>
<td>211 eggs</td>
</tr>
</tbody>
</table>

These differences were not significant ($p > .05$).

POULTRYMAN DECISION MAKING

Given this information, the poultryman is now faced with the question of what is the most economically optimal action which he should take concerning MG. This question may be answered with the assistance of decision analysis. This process will allow the poultryman to quantify the risks involved with maintaining a flock free of MG as well as the benefits to be derived from each of the alternative programs. Once quantified, these alternatives may then be compared and that alternative with the greatest expected monetary return chosen.

In order to quantify these alternatives, a number of assumptions must first be made:

1. The price of an egg is 4¢.
2. The cost of inoculating birds with Conn. F-strain is 3.5¢/bird.
3. The net benefits per hen housed for each of the alternatives are:
   
   \[
   \text{MG}(+) = 0.0¢ \\
   \text{MG inoculated} = 10 \text{ eggs at 4¢/egg less 3.5¢ per inoculation} = 36.5¢ \\
   \text{MG}(-) = 21 \text{ eggs at 4¢/egg} = 84¢.
   \]

With these assumptions, the poultryman may now decide the minimum level of risk of exposure at which his flock would need to be before it would be optimal for him to vaccinate his birds against MG. This level will be referred to as the break even point (BEP). In order to calculate the BEP, we must determine the risk of infection where the expected monetary returns for the inoculation alternative are equal to those for the eradication effort.

While the expected benefits to be derived from an inoculation program are constant, the expected benefits from the eradication effort will obviously depend on how successful the poultryman is in keeping his flock free of MG infection. This probability of success may then be compared to the BEP and the poultryman will then be able to decide for himself whether the risk of infection he faces is greater or less than the level of risk calculated at the BEP. In this way he may determine what is the most economically optimal alternative for this situation.

The BEP may be calculated as follows:
Let, \( \text{Prob}(\text{MG}(+)) = \) the probability of a flock becoming infected. 
Thus, \( 1.0 - \text{Prob}.(\text{MG}(+)) = \) the probability of a flock remaining MG(-).

Since the BEP is where the risk of MG infection and the expected revenue associated with it as well as the probability of maintaining a flock MG(−) and the expected revenue associated with it are equal to the net benefit derived from a controlled exposure program, this point may be determined by solving the following expression:

\[
\text{Prob}(\text{MG}(+)) \times 0^c + (1.0 - \text{Prob}(\text{MG}(+)) \times 84^c = 36.5c \\
\text{Prob}(\text{MG}(+)) \times 84^c = 36.5c \\
84^c - 36.5c = \text{Prob}(\text{MG}(+)) \times 84^c \\
(84^c - 36.5c)/84^c = \text{Prob}(\text{MG}(+)) \\
0.57 = \text{Prob}(\text{MG}(+))
\]

Therefore, the break even point occurs when the risk of a flock becoming infected with MG is 0.57 (57%). At a level of risk greater than 57% it would be more economically advantageous to the poultryman if he were to inoculate his birds. However, if the risk of infection were less than 57% the optimal choice would be to attempt to maintain an MG(−) flock. Or simply, in a high risk region, inoculate! If in a low risk region, eradicate!

This break even point may also be determined graphically (figure 1). Note the BEP occurs at \( \text{Prob}(\text{MG}(+)) = 0.57 \). To the left of this point (low risk region) the greater net benefits are derived from a eradication program, with the maximum being 84¢/hen housed. Following the "eradication line" to the right we pass through the BEP and into the high risk region where the optimal action is controlled exposure, with a constant expected net benefit of 36.5¢/hen housed.

With this graph and knowledge of the break even point, the poultryman has the ability to evaluate his situation and make a rational decision concerning the economics of MG control on his premises.

NATIONAL IMPLICATIONS

MG is a common problem affecting a large percentage of the total number of laying hens. Although the true prevalence rate of MG in layers in the U.S. is unknown, estimates supplied by several authorities suggest that 50% of the layers in the U.S. are infected with MG. With this prevalence and the estimated loss of 21 eggs per infected bird, the annual losses caused by MG may be as high as $118 million. This is similar to the widely predicted annual price tag "to live with" exotic Newcastle disease. The only difference is that MG is a very real annual cost while the losses which would be caused by Newcastle disease are currently unrealized.

MG is consequently a major, is a major, measurable disease with current annual economic losses of $118 million, shared by the poultry industry and the consumer, which should warrant greater priority and funding than has been ascribed in the past. Its control therefore presents
a significant opportunity for veterinarians to serve both industry and the public.

Observations made during this\textsuperscript{a} and other field studies indicate that the following areas must be markedly strengthened at both the state and federal levels before we may expect major improvements concerning MG.

1. Development of ways to provide official recognition and MG negative classifications to started pullet flocks originating from multiplier breeder flocks and hatcheries with “U.S. (NPIP) \textit{M. gallisepticum} and \textit{M. synoviae} Clean” classifications. This form of recognition for qualifying pullet flocks, providing pullet buyers with necessary safety assurances, is currently unavailable and its absence has resulted in financially disastrous, unwarranted, persistent spread if infection to many MG-laying operations.

2. Further study of Conn. F-strain MG and/or medication technique to reduce the financial losses of MG and convert large multiple age laying complexes from dirty to clean. Artificial exposure, with or without antibiotic medication has been found to reduce the risks of MG transmission to non-infected pullets housed on infected premises. Such a reduction of risk as discussed above pave the way for eradication on the farm and subsequently improved egg production and profit margins.

3. Improvement of serological detection procedures to more adequately assure correct classification of breeder flocks and thus protect the table egg producer, the person who also has a lot to lose or gain. The existence of MG infections defying easy serological detection and consistent cultural confirmation was reported over 15 years ago\textsuperscript{b} and continue to be recognized as an important problem to regulatory agencies and poultry breeders.\textsuperscript{c}

The opportunity for major progress with significant economic returns awaits our concentrated action. The time appears to have come to develop channels of communication to Washington to assure funding for expanded state and federal programs for avian mycoplasma prevention and control! Government agencies should be encouraged to propose new budgetary allocations specifically aimed at significant improvements in MG prevention and control. Coordinated contact with key congressmen and appropriation committees by USAHA and numerous state and national veterinary and poultry industry associations will be crucial in any attempt to develop properly staffed and funded programs.

\textbf{SUMMARY}

A two year study was performed in the state of Pennsylvania to evaluate the impact of \textit{M. gallisepticum} (MG) infection and controlled exposure to Conn. F-strain inoculum on the egg production of laying hens. The results showed that natural exposure to MG caused a decrease
production of 21 eggs/hen housed, compared with hen production in noninfected flocks. While inoculation with Conn. F-strain resulted in lowered egg production, 10 eggs/hen housed, compared with hen production in noninfected flocks, it did account for an increased production of 11 eggs/hen housed when compared with hens from flocks which were naturally infected with MG.

These findings were evaluated to determine their importance to both the individual poultryman and the nation as a whole. It was determined that the poultryman would benefit from flock inoculation with Conn. F-strain if his flock were in an area with the risk of infection with MG exceeding 57%. Below this level of risk, he would be better off to attempt to maintain a flock free of MG.

On a national level, the implications of MG were that it was costing the industry and consumer as much as $118 million per year. Losses affecting the broiler industry or other losses in the layer industry such as morbidity, mortality or feed conversion, which would exacerbate this estimate were not included in this paper. Nonetheless, they should be taken into consideration when evaluating the impact of MG in the United States.
Figure 1. The expected net benefit received from increased production due to maintaining a flock free from infection with *M. gallisepticum*.
ACKNOWLEDGEMENTS

The assistance of Mrs. Barbara A. Bingaman in coordinating flock performance records, Mrs. Sandra L. Grigor in the quantitative evaluation of egg production data and Ms. Patricia A. Thomas in the statistical evaluation is sincerely appreciated.

REFERENCES


REPORT OF THE COMMITTEE ON
TRANSMISSIBLE DISEASES OF POULTRY

Chairman: Raymond A. Bankowski, Davis, CA

Bobby Baros, TX; Everett S. Bryant, CN; Francis G. Buzzell, ME; Morris S. Cover, MO; G. A. Erickson, GA; H. E. Goldstein, OH; L. C. Grumbles, TX; Robert L. Hogue, IN; D. D. King, MD; Thomas L. Landers, AR; Hiriam N. Lasher, DE; E. T. Mallinson, PA; R. McCapes, CA; C. D. Murphy, IL; H. E. Nadler, NY; T. D. Njaka, W. Va.; W. C. Patterson, GA; J. E. Pearson, IA; I. L. Peterson, MD; G. F. Pierson, MD; Ben S. Pomeroy, MN; James B. Roberts, OK; T. B. Ryan, NC; Raymond Schar, MD; John A. Smiley, ME; and H. W. Towers, DE.

The Committee met at 1:30 pm, October 30, 1979. Seventeen members and eighteen guests attended.

NEWCASTLE DISEASE

During fiscal year 1978, domestic surveillance of poultry did not reveal any cases of exotic Newcastle disease in poultry in the continental United States. Increased effectiveness in border and port enforcement and alert surveillance by field personnel aided in the effort. In the early part of 1979, two infections were detected in caged pet birds which were recently introduced into the United States.

On February 20, 1979, exotic Newcastle disease was confirmed at the National Veterinary Services Laboratories (NVSL), Ames, Iowa, from specimens submitted from a citron crested cockatoo at Stanton, Orange County, California. The disease was traced to a bird-holding facility to house birds as they were released from a privately-owned import quarantine station, both under the same ownership and management. Sales and shipments of birds from the holding facility were traced and evaluated. As a result, birds on ten commercial and seven privately-owned premises in Los Angeles, Orange, San Beranrdino, and Riverside Counties, California, were positive for exotic Newcastle disease. In addition to California, movements of birds from infected premises were traced to Arizona, Illinois, Minnesota, Nevada, Oregon, Texas, Utah, and Washington.

On March 24, 1979, another case of exotic Newcastle disease was confirmed in the holding facility used to house birds after release from a USDA-approved, privately-owned import quarantine station at Miami, Florida. A total of 109 shipments were made from this infected facility to 29 states and the Commonwealth of Puerto Rico. As a result of these tracings and evaluations, positive cases were disclosed in Illinois, Michigan, North Carolina, Ohio, and Texas. No commercial poultry were involved in either the California or Florida outbreaks. Since there is no known treatment for this disease, the infected cage birds involved were appraised & humanely destroyed and the premises cleaned and disinfected. The cost to eliminate these outbreaks to APHIS was estimated to be $1.8 million.
Puerto Rico currently is under Federal quarantine for exotic Newcastle disease. Dr. D. C. Johnson reported on a surveillance for exotic Newcastle disease on the Island, which began on June 4, 1979. After an extensive study involving 64 laying flocks, an equal number of broiler flocks, 105 fighting cocks, and 217 backyard flocks, no evidence of VVND was detected. It will be recommended that the Commonwealth of Puerto Rico be released from quarantine for exotic Newcastle disease.

AVIAN INFLUENZA

Outbreaks of Avian Influenza were encountered in three different areas of the country, North Central area (Minnesota, South Dakota, Wisconsin), California, and Texas. Minnesota experienced an extensive outbreak involving more than 120 market flocks, 11 breeder flocks, and one chicken operation involving over two million market turkeys, 27,600 turkey breeders, and 185,000 laying chickens.

The dominate serotype in Minnesota was Hav6N1 with single isolations of Hav4Neq2, Hav6Neq2, Hav9N2, and Hsw1N1. The loss attributed to the outbreak was approximately $5,000,000. A bivalent (Hav6, Hav4) oil emulsion killed (BPL) vaccine was prepared by a commercial laboratory and used extensively in breeder & market flocks. Monovalent vaccines have been made available for future use (Hav2, Hav4, Hav6, Hav9) for use singly or in combination.

In California, Hav5 Hav2 has been involved in seven breeder flocks involving over 25,000 birds. The predominate signs were marked drop in egg production, respiratory distress, anorexia and depression. There was some mortality with high morbidity. An autogenous inactivated (Binary Ethyleneimine) oil-emulsion vaccine was made by a commercial company.

In Texas, four turkey breeder flocks on two farms involving 35,000 birds were involved with drop in egg production, anorexia and depression and respiratory signs. The serotype involved was Hav1Nav1. A killed (BPL) vaccine was prepared by a commercial laboratory and used on breeder flocks. The Hav1 isolate was found nonpathogenic to young chickens and turkeys.

In view of the seriousness of avian influenza in turkey flocks in the U.S. during the past few years and in chickens and turkeys throughout the world, the Committee adopted a resolution urging the USDA and USAID to develop an international symposium on avian influenza. The later is to adopt a common understanding of the epizootiology identification and classification of strain virulence as well as other aspects on prevention and control of the disease. A more detailed report on avian influenza in turkeys in the U.S. was presented at the general session by Dr. B. Pomeroy, et al., and is published in these proceedings.

Dr. J. E. Pearson of the National Veterinary Services Laboratory, Ames, Iowa, submitted more detailed information on hemagglutinating (HA) agents other than Newcastle disease virus which were isolated from
samples from birds from quarantine stations. Very few of the isolates have been identified. On inoculation, none of them were pathogenic for young chickens and turkeys, however, the Committee is concerned that there is no information on their effect on poultry health and particularly on egg production. The number of hemagglutinating isolates from the various species were as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Isolations 1978</th>
<th>Number of Isolations 1979</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan 1 - Dec 31</td>
<td>Jan 1 - Oct 1</td>
</tr>
<tr>
<td>Finch</td>
<td>782</td>
<td>413</td>
</tr>
<tr>
<td>Parrot</td>
<td>118</td>
<td>175</td>
</tr>
<tr>
<td>Parakeet</td>
<td>111</td>
<td>10</td>
</tr>
<tr>
<td>Conure</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Canary</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Cockatoo</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Cockatiel</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Mynah</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Macaw</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Rosella</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Hornbill</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Waxbill</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Crane</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Song Sparrow</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Cardinal</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pekin Robin</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Lovebird</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>1083</td>
<td>635</td>
</tr>
</tbody>
</table>

In 1978 there were 35,501 samples tested; and so far in 1979, there have been 20,274 samples tested. Of these, approximately 3.2% of the samples tested each year were positive for HA viruses.

ADENOVIRUS

In spite of surveys indicating that antibodies to adeno-virus 127 are widespread in duck populations in the U.S., the consensus of the Committee was that the condition known as "egg drop syndrome 76" of Europe is not known to exist in the U.S.

Experiments at the Southeast Poultry Laboratory (SEPRL), Athens, Georgia, have shown that an isolate of Adenovirus from Missouri ducks, serologically similar to the agent causing ED-76, did not cause the classical egg drop syndrome in broiler, breeder, or egg type chickens. The classical syndrome was reproduced with the inoculation of Adenovirus 127 from Great Britain. The U.S. duck adenovirus protected chickens against an egg drop induced by subsequent challenge with
Adenovirus 127. Sera from chickens previously infected with Adenovirus 127 had extremely high HI titers. Some unexposed normal commercial chickens had titers of 1/20 or less.

Similar experiments with laying white Leghorn chickens were conducted at the University of California, Davis, California. An adeno-virus, which is serologically related to Adeno 127, was isolated from commercially raised white-pekin ducks did not cause clinical signs or a significant drop in egg production in two trials.

CERTIFIED VVND NEGATIVE FLOCKS

A report of the January 25, 1979, meeting of the subcommittee on VVND-Negative Flock Certification Program for chickens was given. It is the concensus of the subcommittee that a program of the type recommended for turkeys was desirable and feasible for egg and broiler type primary breeding flocks. Additional meetings of the subcommittee are recommended to develop a specific chicken program for consideration by the full committee.

In the absence of a publication of an official program from USDA regarding the 1978 USAHA recommendation to USDA-APHIS for the adoption of a VVND-Negative Flock Certification Program for Turkey Primary Breeding Flocks, the Committee reinforces its recommendation that the USDA made available to the industry and State Regulatory agencies a program of standard rules and regulations for certification of primary turkey breeders flocks as adopted and outlined in our last years (1978) report.

SALMONELLA GALLINARUM — FOWL TYPHOID

This Committee directed its attention to the Data Report on Salmonella Gallinarum isolations as reported to NPIP in the 1969-1978 time period. As a result of this data, it is apparent that the organized poultry industry of this nation under the direction of the NPI plans have reduced the incidence of Salmonella gallinarum to an all-time low rate.

This committee is concerned with the increased incidence of fowl typhoid reported in other countries either from natural exposure or from the use of vaccines, and recommends that APHIS, Veterinary Service, USDA, take every step possible to prevent the spread of this disease within our nation, and to eliminate the threat of S. gallinarum being introduced into our country from the outside.

The Committee recommends that the General Conference Committee of the National Poultry Inspection Plans Program direct their attention to establishing the criterion for total eradication of fowl typhoid, and attempt to stimulate the state regulatory officials cooperating with APHIS, Veterinary Services, to engage in programs that will ultimately eradicate this disease.

CAGE AND AVIARY BIRDS

The report of the Subcommittee on cage and aviary birds presented
both areas of positive development and points of urgent concern among aviculturists of the estimated $500 million pet bird industry. The following outlines major findings and recommendation for the future.

**Positive Developments**

1. Active support of pet and exotic bird disease research on Pacheco's Disease vaccination, psittacosis, salmonellosis, bird pox, and colibacillosis, by several major aviculturists associations and institutes.

2. Universities, colleges, aviculture and veterinary associations have begun vigorous promotion of continuing education in aviculture and avian medicine.

3. Mutually satisfactory accords have been achieved between government and aviculture on: the handling of future outbreaks of exotic Newcastle disease; and the effective captive propagation of rare or endangered species.

4. A positive, promotional rather than negative restrictive strategy towards a national supply of safe, healthy birds is being developed. The American Federation of Aviculture and the Pennsylvania Department of Agriculture are initiating ambitious programs to promote domestic production of cage pet and exotic birds that will hopefully and eventually curb the trafficking in smuggled birds. The former beginning to establish and maintain a national breeding registry of birds not commonly raised in captivity. The latter developing a state directory of small or large local operations providing domestically bred cage pet birds for retail sale.

**Urgent Concerns**

1. Possible over-restriction of the legitimate, responsible importer and possible weak prosecution of quarantine station operators guilty of questionable activities, poor sanitation, inhumane management of birds, violation of USDA regulations, and seriously deficient antibiotic treatment for psittacosis.

2. Deleterious effects from unsanitary conditions possible during 30-day quarantine period in USDA “isolettes.”

3. Continuing weaknesses in identification techniques for all birds and especially regarding smaller species.

4. Import station permits may not be granted in the most democratic, impartial basis so as to preclude monopolization and possible bird arbitrary price increases.

5. Lack of attention at quarantine stations to diseases of birds that do not affect poultry but can cause major losses to bird dealers and breeders.

6. Need for recognition and classification of aviculture as a significant, multimillion dollar, form of agricultural livestock production.
GENERAL RECOMMENDATIONS

The Subcommittee on Cage and Aviary Birds should continue its function and next year’s activity should emphasize: expanded and strengthened, instruction by veterinary colleges in avian medicine; and widespread development of state and other official directories of domestic bird breeding operations as positive rather than restrictive programs for aviculture.

The Committee recommends that USDA, APHIS, Veterinary Services, take corrective action concerning the several urgent quarantine facility concerns listed above.

Additionally, the Committee specifically recommends that: The present responsibility for psittacosis control in imported birds, now vested with the U.S. Public Health Service, has proved to be ineffectual and that authority for enforcement should now be given to USDA.

The Committee deliberated the problems and status of the bird import quarantine facilities. There are approximately 96 privately-owned quarantine stations with many pending applications. As a result of the discussion, a resolution was proposed requesting that the U.S. Animal Health Association urge the USDA to establish quarantine facilities for importing exotic birds as is done with all other animals and that no privately-owned stations be allowed. This proposal was approved by the Committee, and the resolution sent to the Resolutions Committee.

OTHER TOPICS

The Committee recommends that USAHA convey to USDA, SEA and APHIS the necessity to maintain adequate financial support for avian health and disease research and control programs in the 1981 budget. A copy of the Committee recommendation is to be sent to: National Turkey Federation; Southeastern Poultry and Egg Assn.; Pacific Egg and Poultry Association; National Broiler Council; Poultry and Egg Institute; American Association of Avian Pathologists; General Conference Committee; National Poultry Improvement Plan.

The subcommittee on extra support of area diagnostic laboratories to help with reagents and professional staff established in 1976 appears to be unnecessary at this time and the members present (Grumble, Rosenwald, and Bryant) recommended that it be inactivated. If the need should arise again, the subcommittee could then be reactivated.

The following subcommittees were formed:

AVIAN INFLUENZA: R. A. Bankowski, C. Beard, D. King, J. Pearson, I. Petersen, and B. S. Pomeroy, Chairman.

U.S. POULTRY HEALTH ADVISORY COMMITTEE TO APHIS: E. Bryant, H. Goldstein, R. Hogue, I. Peterson, B. Pomeroy, T. Ryan, and R. McCapes, Chairman.

MYCOPLASMOSIS: W. Dungan, K. Hand, D. Johnson, B. S. Pomeroy, R.
Yamamoto, I. Petersen, and E. T. Mallinson, Chairman.


CAGE AND AVIARY BIRDS: R. Cooper, G. J. Harrison, B. Levine, E. Mallinson, H. Nadler, J. Pearson, and A. C. Rissen, Jr. and R. E. Baer, Chairman.
Light and Hodes were early pioneers in rotavirus research in 1943 when they produced diarrhea in calves using a bacteria free fecal filtrate from humans which was later found to contain rotavirus particles. Although the problem of neonatal diarrhea was studied prior to that time, it was generally considered to have a bacterial etiology. In 1945, Reiman et al demonstrated diarrhea in human volunteers using a bacteria free fecal filtrate and in 1947, rotavirus was found in infant mice, yet it was not until 1969 that the reovirus-like agent (rotavirus) was isolated from calves. Since that time, rotavirus infections have been found in most species of domestic animals and birds as well as a large group of wild species. The disease has been known by such names as infantile enteritis, white scours, milk scours, traveler's diarrhea, Nebraska calf diarrhea, neonatal calf diarrhea, epizootic diarrhea of infant mice, and infantile gastroenteritis. The rotavirus has been called reovirus-like agent, O agent, SA-11, duovirus, orbivirus, orbivirus-like agent and in one report was considered an arbovirus.

The International Committee on the Taxonomy of Viruses has accepted the name rotavirus and placed it in the family Reoviridae. The rotaviruses from various species are morphologically indistinguishable. They are composed of a double layered capsid 70 nm in diameter with a hexagonal core of about 38 nm. It has been suggested that the capsomere arrangement resembles a spoked wheel hence the name rotavirus (rota-Latin = wheel). They all have a double stranded RNA genome which when electrophoresed on polyacrylamide gels is found to be composed of 11 or 12 segments. They are resistant to acid, ether, and chloroform and have a bouyant density in cesium chloride of 1.36 g/ml. Infectivity of bovine and murine rotavirus is destroyed at 50 C in the presence of 1 M MgCl2.

Bovine, simian, ovine, O agent, porcine and avian rotaviruses have been propagated in a number of cell lines. Human and murine rotaviruses have been propagated in cell cultures but these have been nonproductive infections. The difficulties in propagating rotaviruses
hamper the production of adequate antigen for large sero-epidemiologic studies.

Evidence exists that the antigens for immune electron microscopy, complement fixation and fluorescent antibody tests are group specific and exist on or in the inner capsid layer. The neutralizing and hemagglutinating antigens are in the outer capsid layer and are species or serotype specific. The cross antigenicity of the strains has allowed the use of simian or bovine rotaviruses in serologic studies.

PATHOGENESIS

After entering the gastrointestinal tract, the rotavirus infects the mature epithelial cells on the distal one half of the villi of the small intestine. This leads to a rapid sloughing of the cells, resulting in a decrease of the absorptive surface and diarrhea ensues. The replacement of the cells with virus resistant immature forms leads to a self-limiting infection, however, transient lactase intolerance often complicates the disease in neonates on a high lactose diet. Bacterial pathogens and other viral agents often invade the damaged epithelium destroying other epithelial cells in the crypts and cause a more severe or prolonged diarrhea. This condition may progress to intractable diarrhea, dehydration and death. The most common age for rotavirus to be a problem is from birth to weaning in calves, pigs, lambs, mice, and foals, from hatching to three weeks in turkeys, and from 6 months to 3 years of age in children. However, infections in adult cattle, humans and chickens have been well documented.

METHODS OF CONTROL

Control of rotavirus infection has been through sanitation, husbandry, and immunization — both active and passive.

Chemotherapy with Ribavirin, a synthetic nucleoside (1-B-D-ribofuranosyl-1,2,4, triazole-3-carbamoxide) has been tried in murine infections without success.

SANITATION AND DISINFECTION

Rotaviruses are very resistant to iodophore, chlorine, formalin, and phenol disinfectants. Four percent iodophore, three percent sodium hypochlorite, five percent Lysol, and ten percent formalin all failed to inactivate lamb rotaviruses in intestinal contents with a two hour exposure. The purified virus is only partially inactivated with these preparations. Since the virus is so widespread in nature and is resistant to disinfectants, these chemicals can only be expected to aid in control and cannot be relied upon as a means of eradicating the disease. A study in swine in which the premises were not cleaned and sterilized between litters has demonstrated consistent increase in rotavirus incidence so that after eight litters, all piglets were infected. We have observed that continuous re-use of brooding facilities by successive generations of turkey poults leads to an increasing of incidence of rotavirus diarrhea to
a point where the premises must be depopulated and sanitized.

ACTIVE IMMUNIZATION

The use of live virus vaccine in neonatal calves has been successful in some cases, however the use of monovalent rotavirus vaccine (Scourvax-Reo) which was introduced in 1973 has been discontinued in favor of a rotavirus-coronavirus vaccine combination (Scourvax II). The bovine rotavirus vaccine does not protect piglets from porcine rotavirus infection. Methods of evaluating the effectiveness of these two vaccines in calves have been a point of controversy, however, where an entire herd is vaccinated, rather than doing double blind studies, there is an apparent decrease in the incidence and severity of scours. The infection of epithelial cells with the attenuated rota- and corona vaccine viruses apparently makes the cells refractory to infection by virulent virus particles since resistance increases prior to the formation of antibodies. The combination vaccine has now been renamed Calf-Guard and is being used in the dams to give passive immunity to the calf. The lack of immunity in pigs vaccinated with the bovine rotavirus, the failure of one human serotype to protect form infection by a second serotype, and the failure of sera from one species to neutralize rotavirus from other species, all indicate the use of non-human rotavirus as a vaccine in humans will probably not prove successful.

PASSIVE IMMUNITY

The use of passive immunity to control neonatal diarrhea has been advocated by man for 50 years and practiced by nature for eons. Controlled studies on calves done by Theobald Smith in 1921 and 1922 helped to show colostrum was an effective preventive for calf scours. He felt at that time the etiologic agent of calf scours was Bacillus coli (E. Coli). The work of Wells and Snodgrass indicates the circulating rotavirus antibody is not effective in preventing rotavirus infection. However, they found that orally administered colostrum, serum and immunoglobulins effectively prevented infection in calves challenged with bovine rotavirus, and lambs challenged with ovine and human rotavirus. This use of exogenous passive immune protection may be practical in small or confined herds but is not applicable to large herds. In both large and small herds, it may be of value to vaccinate dams with both Calf-Guard and enterotoxigenic E. coli vaccine. This will provide a colostrum with high antibody levels to rotavirus, coronavirus, and enterotoxigenic E. coli.

Post-colostral milk from cows and ewes does not have adequate anti-rotavirus antibody to provide continuous protection. It may be necessary to employ an additional source of immunoglobulins for continuous prophylaxis. Ranchers have empirically used raw eggs for scours protection and treatment for many years. In our laboratory, passive protection of infant mice was achieved using immunoglobulins from the eggs of rotavirus hyperimmune chickens. Hens were vaccinated with
simian rotavirus and the eggs were collected after a high level of antirotavirus antibody was achieved in the hens' serum. The water soluble fraction was isolate from these eggs. When three day old mice were given this watersoluble fraction twice a day and challenged with murine rotavirus (greater than 10⁴ x ID) the incidence of rotavirus was 15%. A control group not given the WSF had a 90-100% attack rate with the same challenge dose. This demonstrates that the use of egg yolks or water soluble fraction from egg yolks may be a useful adjunct source of immunoglobulins for long term rotavirus prophylaxis.

There are few studies to date which evaluate the importance of rotavirus infection in human or animal morbidity and mortality. In one study,⁹⁰ bovine rotavirus was estimated to cause between 16.7 and 28.4 million dollars damage annually in the cattle industry. Bergeland and coworkers estimate losses of up to 50% of turkey poults in a flock.⁹ We have noted a tripling of the usual mortality in one house of 17,500 poults leading to excess loss of nearly 1000 four to fourteen day old poults.

It is clear that this group of viruses is quite ubiquitous and infects most domestic animals, many wild animals and man. As more extensive epidemiologic studies are conducted, the true impact of rotavirus infections will become apparent. Evidence available at this time certainly points out the need for establishing techniques to protect against rotavirus infection. It is apparent that both active and passive immune protection may be helpful in preventing rotavirus infection. As with any disease, spread by the fecal-oral route, the mainstay of disease control must be good sanitation, hygiene and husbandry.

REFERENCES


31. Rodger, S. M., Schagl, R. D., and Holmes, I. H.: Further biochemical characterization including detection of surface glycoproteins of human, calf and simian rotaviruses, J of...


USAHA, St. Louis, Mo.:59-64, 1973.


The meeting of the Committee was called to order at 1:40 p.m.,
Wednesday, October 31, 1979, with 21 members and guests present. The
subject for discussion was announced as contamination of feedstuff
(including water) by microbial and non-microbial environmental con-
taminates with potential for deleterious effects on animals and directly
or indirectly on humans.

Dr. Robert Singer presented case histories of inappropriate manage-
ment of waste substances resulting in environmental contamination, in
some instances leading to livestock deaths and possible human illness
(and death?). His talk, illustrated with slides, included a discussion of
arsenic contamination from rinsing a tank truck and allowing the waste
to simply run onto the ground resulting in toxicity in cattle. No human ill-
ness was known to have occurred in this instance, however, the same
truck next hauled a load of mayonnaise. Other specific sources of en-
vironmental contamination have included lead from used crank case oil,
old batteries, and in runoff from well-traveled roads. Lead toxicity has
been observed to result in blindness, lameness, and death in horses and
cattle and encephalitis in raccoons and foxes that has been clinically
mistaken for rabies.

The types of environmental contamination suggest the possibility of
water contamination in an area of livestone formations, such as Ken-
tucky, thus potentially setting the stage for livestock (and human?)
poisoning far removed from the actual site of contamination.

Dr. James Glosser of Montana presented an extensive review of the
recent problem of PCB contamination of animal feed involving 17 states.
The source of the contamination was a damaged transformer stored in
such a manner that leaking material became mixed with inedible waste in
a slaughter house. The result has been condemnation or “holding” at the
site of production of millions of dollars worth of animals and animal
products. No clinically recognized illness has been noted to date in the
involved animals nor in persons consuming meat, milk, or eggs from such
animals, however, the long-range implications of this contamination
episode are not clear.
Dr. R. L. Hosker presented a discussion of "Sulfonamide Residues in Swine," an abstract of his presentation follows.

SULFONAMIDE RESIDUES IN SWINE

Presentation to the Committee on Public Health and Environmental Quality

From 1974 to 1978, 10-15 percent of swine tissue samples collected at slaughter by U.S. Department of Agriculture - Food Safety and Quality Service were consistently found to be above the allowable level of sulfonamides (0.1 ppm), particularly sulfamethazine. In January 1978, members of the swine industry appealed to USDA officials contending that swine producers were accused of selling "violative" swine and penalized financially even through they had adhered to withdrawal recommendations and in some instances had never used sulfonamides.

In response, a program was developed by USDA agencies, FDA, the swine industry, the feed industry, and the drug industry. The goals were to assist violative producers by determining the cause on the individual farm and, hopefully, to reduce the incidence of violative swine at slaughter. A trial run was conducted in May 1978 by conducting an epidemiological investigation on 112 farms which had sold swine that had previously been tested at slaughter by FSQS. About three-fourths had marketed violative hogs and the remainder had been in compliance. From September 1978 through September 1979, each violative producer was contacted and offered a free epidemiologic investigation of his operation. During farm visits, appropriate finishing/withdrawal feed samples and components were collected and submitted for laboratory examination.

Management deficiencies were frequently found as were sulfas recycled in waste. However the striking finding was the frequency of contaminated feed. (Table 1). The contamination of feeds with sulfonamides cannot be condoned but the implications are alarming if a highly toxic chemical was to be involved rather than a sulfonamide. It can also partially explain why the PCB's and PBB's were so widely disseminated.

<table>
<thead>
<tr>
<th>Type of Feed</th>
<th>Samples Collected</th>
<th>Free of Sulfa</th>
<th>Contained Detectable Levels</th>
<th>Contained 1 PPM. and over</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill-Mixed</td>
<td>63</td>
<td>10 (16%)</td>
<td>53 (84%)</td>
<td>29 (46%)</td>
</tr>
<tr>
<td>Farm-Mixed</td>
<td>109</td>
<td>25 (23%)</td>
<td>84 (77%)</td>
<td>28 (26%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>172</td>
<td>35 (20%)</td>
<td>137 (80%)</td>
<td>57 (33%)</td>
</tr>
</tbody>
</table>
Slide shows available in each State through the State Federal Extension Service of SEA or Veterinary Services Area or District Veterinarian in Charge:

<table>
<thead>
<tr>
<th>TITLE</th>
<th>LENGTH</th>
<th>AVAILABILITY</th>
<th>PRODUCER</th>
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</thead>
<tbody>
<tr>
<td>Sulfonamide Carryover in Mixing and Feeding Equipment</td>
<td>20 min.</td>
<td>Now</td>
<td>Dr. Robert Wilcox, Kansas State University</td>
</tr>
<tr>
<td>Water Medication and Medication Delivery Devices</td>
<td>17 min.</td>
<td>Now</td>
<td>Dr. James McKean, Iowa State University</td>
</tr>
<tr>
<td>Sources of Sulfonamide Contamination, Their Identification and Correction</td>
<td>20 min.</td>
<td>Now</td>
<td>Dr. Richard Bevill, University of Illinois</td>
</tr>
<tr>
<td>Analytical Methods for the Detection of Sulfonamide in Swine Feeds and Plasma — Use in Epidemiological Studies</td>
<td>35 min.</td>
<td>Now</td>
<td>Dr. Richard Beville</td>
</tr>
<tr>
<td>Sulfonamide Residues in Swine Tissues — Some Problems and Solutions</td>
<td>12 min.</td>
<td>Now</td>
<td>Dr. W. E. Lloyd, Iowa State University</td>
</tr>
</tbody>
</table>

The committee called attention to its resolution of last year urging states to adopt appropriate regulations for animals exposed to environmental contamination.

Dr. W. R. Miller, member of AVMA Council on Public Health and Regulatory Medicine reported on the status of the AVMA resolution on the sale of raw milk (returned to Council by the House of Delegates). Dr. George Humphrey discussed the epidemiology of *Salmonella dublin* infections in cattle and humans.

The committee approved a resolution against the sale of raw milk or milk products for submission to the Committee on Resolutions.

The Committee respectfully submits its report to the Executive Committee for adoption.
ROLE OF NATIVE INTESTINAL MICROFLORA IN PROTECTION AGAINST PATHOGENS

G. H. Snoeyenbos
Department of Veterinary & Animal Sciences
University of Massachusetts
Amherst, MA 01003

INTRODUCTION
A substantial body of evidence has been developed which indicates that native bacteria play a significant role in protecting body surfaces against infection by some pathogens. The term competitive exclusion as suggested by Lloyd et al. will be used here to describe this mechanism. Although a great deal remains to be learned about this protective mechanism, a review of some of the available evidence should be useful at this point. Active investigation of this protective mechanism in domestic animals has been largely confined to chickens, but indirect evidence suggests that a comparable native protection mechanism occurs in a variety of birds and mammals. Although there is evidence that native microflora protect body surfaces against several pathogenic bacteria, this brief review will be largely confined to consideration of protection against salmonellae.

NATIVE INTESTINAL MICROFLORA

Mice
Bohnhoff et al. reported in 1954 that oral treatment of mice with a massive dose of streptomycin resulted in an increase in susceptibility to Salmonella typhimurium in the order of 100,000-fold. Subsequent work by the authors indicated that the streptomycin treatment substantially eliminated bacteroides species from the gut and that bacteroides produced volatile fatty acids which inhibited salmonella. It is of interest that attempts to re-colonize the gut with bacteroides species failed to completely restore the pre-existing level of resistance. Resistance was more fully restored by re-colonization with normally occurring microflora which were not protective by themselves.

Review of the rather extensive studies of the normal enteric microflora of the mouse, as well as extension of the work of Bohnhoff and Miller which demonstrated that normal mouse microflora also protects against such pathogens as Vibrio cholera and Shigella flexneri is beyond the scope of this brief paper.

Chickens
Nurmi and Rantala reported in 1973 that transfer of intestinal microflora from adult chickens to newly hatched chicks, which typically have a nearly sterile gut, quickly established a highly significant degree of protection against subsequent colonization by S. infantis. Later work in the same laboratory demonstrated that this protection was fully established within a few hours following oral dosage of the chick with a fecal suspension from adult chickens. It has been suggested that this
resistance was akin to the age resistance which commonly develops in the chick during the first few weeks of life. Highly variable degrees of protective activity have been found among microflora of different donor populations. Populations of chickens reared by the hen for consecutive generations have usually carried a highly protective microflora. It is apparent that efforts to circumvent transmission of pathogens between generations of chickens by rigid isolation of age groups has sometimes prevented transmission and gut colonization of native protective microflora.

Although there is substantial evidence of species specificity of native intestinal microflora, there appears to be some species crossover of the salmonella protective microflora. Intestinal microflora from the chicken and turkey are substantially if not completely mutually protective. Also, microflora from a mourning dove was reported to provide substantial protection to the chicken. Very limited work with intestinal microflora from mammalian species has not shown protection of the chick.

Most of the work with the chicken reported so far indicates that intestinal colonization by the broad range of paratyphoid salmonellae, including Arizona species, is inhibited by pre-existing colonization by the proper microflora. Rather recently published data demonstrates that the protective microflora can serve to hasten elimination of pre-existing infection. Unpublished work in Australia and in our laboratory has shown that colonization by some strains of *Escherichia coli* of the gut is inhibited significantly by native microflora. Use of nalidixic acid resistant strains of salmonella and of *E. coli* in particular offers great advantage in investigation. Although the significance of *E. coli* suppression is not clear, the findings suggest that the protective activity extends beyond exclusion of salmonella.

The interesting but limited evidence of improved growth rate in young chickens treated with protective microflora needs additional investigation.

**DISRUPTION OF PROTECTIVE MICROFLORA**

The initial work of Bohnhoff *et al.* using streptomycin to increase sensitivity of mice to salmonella was direct evidence that antibacterials could disrupt normal intestinal microflora and result in enhanced invasiveness by salmonella. Rantalä and Nurmi and Rantalä found that both furazolidone and tetracycline interfered severely with normal protective microflora. Smith and Tucker reported that oral administration of several antibacterials to salmonella-infected chicks resulted in excretion of larger numbers of salmonella for a longer period than unmedicated controls. Similarly, the duration of salmonella excretion in man has been reported to be increased by some antibiotic medication regimes. This enhancement of infection has been explained by disruption of normal enteric microflora.
A recent report indicates that stressor agents such as starvation, overheating, and chilling applied at the time of administering protective microflora decreases subsequent protection against salmonellae. These results may only reflect interference with gut colonization by the protective microflora.

MICROFLORA RESPONSIBLE FOR PROTECTION

Most of the investigators working with chickens have used intestinal contents, cecal contents or feces from donor birds, or anaerobic broth cultures made from the above materials as the source of protective microflora. Efforts to isolate the specifically effective bacteria have usually been disappointing. Soerjadi et al. isolated a strain of *Streptococcus faecalis* (Lancefield, Group D) which provided substantial protection to the chick. Rigby et al. isolated a Clostridial species which provided transient protection. We have not found significant protection from lactobacilli of avian origin as measured by subsequent fecal excretion of salmonellae.

Most investigators expect that more than one bacterium is essential for full protection of the chicken against salmonellae. There is no information to indicate whether the same or different microflora are protective against *E. coli* and salmonellae.

MECHANISM OF PROTECTION

The work of Bohnhoff et al. indicated that volatile fatty acids produced by the protective microflora in mice were of primary importance in protecting against invasion by salmonellae. Numerous other metabolites of the protective microflora may play a role in protecting the host against certain pathogens. There is extensive evidence that the native microflora form tenacious and persistent attachments to the intestinal mucosa of the normal animal. A continuous mat of microflora develops over the surface of the mucosa of at least parts of the gastrointestinal tract. Such mats of microflora appear to be protective in themselves as salmonellae are a pathogen which apparently must directly contact a particular mucosal surface in order to colonize and infect. In the chicken, the ceca are the major site to salmonellae colonization. The crop may also be heavily colonized but with less frequency. At both sites, colonizing salmonellae are firmly adherent to the mucosa. Major colonization by salmonellae does not occur on other areas of the gastrointestinal mucosa. It would appear that protection of the mucosa of the chicken ceca by native microflora is of primary importance. Protection of the crop is also important but relative significance is moderated by extensive destruction of detached salmonella in passage through the acid lumen of the proventriculus and gizzard.

In all normal mammals and birds extensively investigated, specific areas of the gastrointestinal tract are colonized by rather specific populations of microflora. Regions such as the ceca of the chicken contain
very complex populations including several hundreds of species. It is not at all clear how many members of this native microflora play a role in protection against salmonellae or whether different microflora are protective against different pathogens.

The relatively recent realization that cells produce a specific glycocalyx which functions in part by forming a tenacious attachment between dissimilar cells providing there is a good "fit" between the glycocalyxes offers a broadly applicable hypothesis for the mechanism of protection by normal microflora. A mat of microflora firmly attached by high specificity between the glycocalyxes of the host cell mucosa and the native microflora prevents contact, colonization, and infection by salmonellae. The reported therapeutic capabilities of protective microflora is explainable by displacement of salmonella colonies by protective microflora with greater mutual specificity of glycocalyx attachment.

Mucosal attachment appears to be the usual prerequisite for successful colonization of the gut by native microflora as well as by salmonella and some other pathogens. Capability of glycocalyx attachment or of attachment by pili can be expected to be of primary importance in competitive exclusion. Inhibition of pathogens by various metabolites of the protective microflora or by other means might be a highly important but sequentially secondary part of the defense mechanism.

SUMMARY

There is convincing evidence of the occurrence of a salmonella protective microflora in the gut of the native chicken and mouse. The finding that at least some strains of *E. coli* are largely contained in the chicken by such microflora may not be of practical significance in itself, but does suggest the possibility of activity against a range of enteric pathogens.

It seems clear that modern poultry husbandry practices designed to prevent transmission of pathogens between generations has also sometimes prevented transmission of protective microflora. Artificial transmission of a protective microflora to the newly hatched chick is an attractive approach to secure early and dependable protection against salmonellae. Similar artificial colonization of the gut might also have practical utility for re-establishing microflora which have been disrupted by antibacterial therapy or by disruptive stress. For experimental purposes we are using fecal material from an SPF chicken population with an intestinal microflora transferred from selected donor birds.

There is a high probability that microflora of comparable protective activity exists in domestic mammals; if so, the specifically protective microorganisms are likely to be quite host-specific. If such a microflora exists, there may not be dependable transmission between generations in some intensive management systems such as those used for veal production. Similarly, antibacterial therapy may seriously disrupt the protective mechanism.
Much more investigation is required to elucidate the significance of naturally occurring gastrointestinal microflora and the complex ecological relationships within these populations. Although protection against salmonella in the chicken by a fully functional microflora is imperfect, the protection is very substantial and appears to be a useful adjunct to more classical methods of prevention and control.

It would be surprising if native intestinal microflora comparable in protective activity to that demonstrated in the chicken and the mouse did not also occur in most mammals and birds. If such is the case, we should learn to utilize this naturally occurring mechanism to greatest advantage.

REFERENCES


Serotyping of salmonella and arizona cultures from animal disease cases and epidemiologically related sources is reported for October 1, 1977, through September 30, 1978. A total of 3,680 cultures were serotyped. The most frequently identified salmonella serotypes were Salmonella typhimurium, S. choleraesuis var. Kunzendorf, S. heidelberg, S. agona, and S. saint paul. The most frequently identified arizona serotype was 7a,7b:1,7,8. The most frequent sources of cultures in order of frequency were turkeys, swine, cattle, and chickens.

INTRODUCTION

Data for this report were accumulated at the National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, USDA, Ames, Iowa. Other laboratories contributing serotyping information were Paige Laboratory, University of Massachusetts, Amherst, Massachusetts, and the Animal Health Laboratories of the Wisconsin Department of Agriculture, Madison and Barron, Wisconsin.

The data, except for serotyping results, were provided by the many laboratories requesting serotyping services. As will be noted, some of the data are incomplete. However, we feel that good progress has been made toward improving the data, and we are continually moving in that direction.

The purpose of this report is to make the data available to epidemiologists and others who have a need for it. The data are presented in tables as in previous reports in order that comparisons can be easily made. The format for some of the tables has been modified to include additional information.

DISCUSSION

Salmonella and arizona cultures for serotyping were received from 44 states and territories during FY 1978 (Tables 1 and 2). This was an increase of 8 over the previous year.

The 3,680 cultures serotyped (Tables 3 and 4) were also more than for FY 1977. The increase was 81 cultures, which we consider insignificant. Considering that 8 additional states reported cultures, this may not represent any actual increase.

The total number of serotypes identified was 134 (107 salmonella-
Table 3 — and 27 arizona — Table 4). The 10 most common salmonella serotypes (Table 10) accounted for 58% of the salmonella cultures. At the other extreme, 27 salmonella serotypes were identified only one time. The data for arizonas was even more striking with one serotype accounting for 73% of the total and 15 serotypes being identified only once (Table 4).

Some of the serotypes that were noticeably more common than in FY 1977 included S. agona — from 173 to 210, S. albany — from 16 to 76, S. cubana — from 6 to 32, S. derby — from 56 to 80, S. heidelberg — from 188 to 234, S. kentucky — from 20 to 40, S. montevideo — from 59 to 91, and S. pullorum — from 36 to 68. A brief review of our records for the past 5 years indicated an upward trend for only one of these, S. agona. S agona was increasingly more common each year except FY 1977. Some of these serotype increases were largely in one state (S. albany in Georgia, S. cubana in California, S. derby in Iowa, S. kentucky in California, S. montevideo in Texas, and S. pullorum in Texas). Also, some increases were mostly from one species (S. albany from chickens, S. cubana from cattle, S. kentucky from cattle, S. montevideo from horses and S. pullorum from chickens).

For many years S. dublin in this country has been reported almost exclusively from cattle in the far western states. This year there were two isolations reported from Texas, one from Iowa, one from Louisiana and one from Puerto Rico. This may be an indication that S. dublin is spreading eastward. However, attempts to collect epidemiological data on some of the isolates by telephone were not successful, and we were unable to establish a source in any case.

The extent of the problem with S. dublin in the far west is not well defined. However, enough information is available to cause one to become concerned about the potential for the remainder of the country. In the western states reporting S. dublin isolations, S. dublin was reported 9 times more than S. typhimurium. In the national data, S. dublin was the second most common serotype from cattle and the eighth most common from all sources combined.
| SEROTYPE       | AL   | AZ   | AR   | CA   | CO   | CT   | DE   | DC   | GA   | ID   | IA   | KS   | LA   | MD   | MA   | RI   | SC   | VA   | WV   | OH   | IN   | MI   | WI   | MS   | MO   | IN   | SD   | ND   | NE   | OK   | TX   | CA   | NV   | UT   | AZ   | HI   | NE   | MD   | KY   | NC   | VA   | OR   | WA   | WY   | DC   | NH   | NJ   | NM   | TX   | FL   | GA   | CA   | IL   | IN   | KY   | NJ   | NY   | PA   | RI   | SC   | TN   | TX   | UT   | VA   | WV   | MS   | MO   | KS   | MN   | IA   | NE   | ND   | SD   | OK   | NM   | CO   | ID   | WA   | WY   | TITAN |  
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------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<table>
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<tr>
<th>TABLE 2. DISTRIBUTION OF SALMONELLA SEROTYPES BY STATE - FY78</th>
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<tbody>
<tr>
<td>STATE</td>
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Note: The table continues with similar entries for each state, summarizing the distribution of Salmonella serotypes across various states for the fiscal year 1978.
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| SEROTYPE | TURKEY | CHICKEN | PIGEON | QUICK | OTHER | AVIAN | CATTLE | SWINE | PIGEON | SHEEP | SNAKE | SNAKE | REPTILE | REPTILE | ANIMALS | REPTILE | BAVI- | MISC- | FEED | REAGENT | LAVI- | TOTAL |
|----------|--------|---------|--------|-------|-------|-------|--------|-------|--------|-------|-------|-------|--------|--------|---------|--------|-------|-------|--------|-------|-------|
| INVERNESS | 107 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| JAVA | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| JAVIANA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| JOHANNESBURG | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| KENTUCKY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| KETTLEBLS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| KREPELO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| LEXINGTON | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| LILLE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| LIVINGTONE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| LOCHTA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| LONDON | 4 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MADEIRA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MARINA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MANHATTAN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MARE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MEDE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MONTREUIL | 10 | 15 | 0 | 0 | 0 | 2 | 13 | 3 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MURCHHA | 9 | 3 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MUSESTER | 5 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| NEW-BRUNSWICK | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| NEWINGTON | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| NEWPORT | 5 | 5 | 0 | 0 | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| NIESTEDTEN | 0 | 7 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| OHO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| CRANE | 11 | 12 | 0 | 0 | 0 | 2 | 3 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| CRIC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| CIC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
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**TABLE 7.** CATTLE---MOST FREQUENTLY IDENTIFIED SEROTYPES IN FY78

**Notes:**
- Percent morbidity: Average highest and highest.
- Percent mortality: Average highest and highest.
- Highest: 100.
- Lowest: 0.

**Source:** Blackburn and Harrington
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Table 10. Salmonella Serotypes Identified Most Frequently During FY 1978 with Comparison Data for 5 Years (All Sources)

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(A) = var. Kunzendorf, (B) = var. Copenhagen

*Number of times the serotype was identified

**Rank beginning with the most common
THE STATUS OF SALMONELLA IN DRESSED POULTRY AND PORK PRODUCTS

Dr. Stanley S. Green, Staff Officer
Microbiology Division
United States Department of Agriculture
Food Safety and Quality Service, Washington, DC 20250

Good morning ladies and gentlemen. It is indeed a pleasure and honor to address this organization today and present information concerning the status of salmonellae in dressed poultry and raw pork products. This information is based on past and present United States Department of Agriculture, Food Safety and Quality Service in-plant surveys. Ralph W. Johnston, Acting Director of our Microbiology Division, was the scheduled speaker for this morning's session but was unable to attend because of recent developments in our Division. He asked me to express his apologies and present our survey findings to date. During the time I have with you today, I will be talking about a survey concerning Salmonella in freshly slaughtered young broilers that was recently completed. I will also briefly talk about the following: first, a current fresh pork sausage survey that is ongoing in 40 federally inspected establishments; second, a survey of chicken eviscerating plants as a corollary to a 1969 published report from our laboratories; third, preliminary information on Salmonella free turkeys sampled during September and October 1979; and lastly, the results of a 1979 cooked beef survey to determine the efficacy of cooking practices in eliminating salmonellae from cooked beef and roast beef, a problem that was most critical during the summer of 1977.

In a 1978 report entitled Recommendations for Reduction and Control of Salmonellosis, the USDA Advisory Committee on Salmonella recommended that benchmark studies be performed to determine the incidence of salmonellae in all phases of the meat and poultry food chain. These studies would provide a gauge by which to judge the effects of any Government or industry changes that are made to control salmonellae in the meat and poultry area. The recommended funds for these studies were not appropriated and the Microbiology Division of the Science Program Food Safety and Quality Service was asked to conduct some of the recommended benchmark studies.

The Microbiology Division had performed several studies on the incidence of salmonellae in meat and poultry over the past 15 years and we recommended, that rather than start new benchmark surveys, some of our older studies be reviewed and selected ones repeated. By repeating previous studies and stressing the strict adherence to past sampling procedures and analytical methodologies, we felt that more meaningful benchmark information would be obtained for the limited resources available. Further, an immediate approximate comparison could be observed and studied.
The FSQS Microbiology Division conducted two studies to determine
the incidence of Salmonella in whole, young ready-to-market chickens. The first survey was performed during late 1967 on whole bird samples submitted to our Beltsville Laboratory by USDA inspectors located in 15 poultry processing plants operating under Federal inspection. Results from the 1967 study showed an overall average Salmonella incidence of 28 percent in the birds. Individual plant results varied from a low of 7.5 percent to a high of approximately 74 percent.

This work was carried out for the purpose of determining the 1979 incidence of salmonellae in freshly slaughtered whole broilers and to compare these results to those gathered during the 1967 study.

Fifteen poultry processing plants under Federal inspection located in 11 states were selected for the 1967 study. Ten of the original 15 establishments were still operating during the 1979 study. Five new plants had to be chosen as replacements using the criteria that the selected plants be as close as possible in overall size, production volumes and location to the plants that were initially sampled in 1967.

Whole chickens collected for both studies were randomly selected at
the chill tank exit by USDA inspectors. Each bird was appropriately labeled and was placed in a plastic bag, frozen and shipped either to the Beltsville Laboratory for the 1967 study or to the FSQS Field Service Laboratories located in Athens, GA, or San Francisco, CA, for the 1979 study. Five hundred and ninety seven birds were submitted to Beltsville during the 13 week sampling period in 1967 and 601 birds were submitted to Athens and San Francisco during the 1979 study.

Sample analysis was initiated within 8 days of laboratory arrival. Each bird was completely thawed at refrigerator temperatures prior to testing. The individual whole thawed bird was removed from its shipping bag and placed into individual plastic bags. The body cavity was filled with a portion of 200 ml. tetrathionate (TT) broth containing 0.6 percent steamed tergitol. The remaining TT broth was poured over the bird's surface. The bag was sealed and shaken for 1 minute to ensure that the broth contacted all surfaces of the bird. Each bird's washings were then thoroughly drained into sterile, wide mouth containers, capped and incubated at 35°C for 18-24 hours.

Following incubation, the broth was streaked onto brilliant green sulfa agar plates and incubated at 35°C for 18-24 hours. Both typical and marginally suspicious colonies were then transferred to triple sugar iron agar and lysine iron agar for screening. Cultures identified biochemically as salmonellae were saved for future serological typing.

The map in Figure 1 shows the states that were involved in the 1967 and 1979 comparative surveys and their geographic distribution in relation to the five FSQS/Meat and Poultry Inspection Regions of the
country. The largest concentrations of establishments surveyed were found in the Southeast Region followed by the Southwest and the Northeast Regions respectively. These three areas had 13 of the 15 plants examined. They also correspond with areas processing the vast majority of broilers prepared annually in the U.S. The remaining two firms were located in the North Central and Western Regions.

Table 1 shows the results obtained after the 1967 study as compared to those obtained during the 1979 surveillance. In 1967 the lowest individual plant incidence of salmonellae was 7.5 percent while the highest was 73.7 percent, whereas in 1979 the lowest incidence was 2.5 percent and the highest was 87.5 percent. There was an overall increase in incidence of 8.3 percent in the 12 years between studies.

The data shown in Table 2 compare the results of the two broiler surveys as seen at the State level. Four establishments located in four States (MD, AL, AR, CA) had a net decrease in salmonellae incidences when compared to 1967. No change was evident in North Carolina, and increases in *Salmonella* incidence ranging from 7.2 to 62.5 percent were seen in eight plants located in six States.

Three geographic regions representing 6 establishments had increased *Salmonella* incidence levels when results of 1967 were compared to 1979 (Table 3). The Western Region illustrates the greatest decrease in *Salmonella* isolations while the North Central Region showed the greatest increase. These results, however, are based on only one establishment in each region as opposed to the results from the other regions in which each had three or more plants involved in the study.

After the 1967 survey results were summarized, all isolates serologically grouped as salmonellae were serotyped. The cultures identified and serologically grouped as salmonellae from the 1979 study are being serotyped and these results are not yet available. However, a breakdown and comparison of serogroups found in 1967 and 1979 has been done and is shown in Table 4. The increased number of Group B isolates is evident as are the decreases found in Group C. The other group differences do not appear noteworthy at this time but this may change once the serotypes from the 1979 study are determined and compared to those found in the earlier study.

**STUDY NUMBER TWO**

Turning now to other work, our first in-plant bacteriological survey of fresh pork sausage was conducted in 17 States during a 9 month period starting in September 1968. Forty four federally inspected plants were sampled at that time and analyses for salmonellae were performed on finished product prepared from whole hogs, slaughtered and eviscerated on the premises, chilled carcasses slaughtered and eviscerated on the premises, pork trimmings from chilled locally slaughtered animals, and frozen trimmings from more distant sources. Salmonellae were isolated from 28 percent of the 560 finished sausage samples collected. These

Again, as part of a recommendation from the USDA Salmonella Advisory Committee, the Microbiology Division is undertaking a comparative repeat study of that performed in 1968-1969. Of the 40 original federally inspected establishments surveyed, 27 were resampled and the substituted 13 plants were selected to match the original plants as to location, production volumes, and size. The methods followed were the same as previously used and published. Five samples submitted bimonthly by Federal inspectors at each establishment are now being sent to either the San Francisco, CA, St. Louis, MO, or Athens, GA, FSQS Field Service Laboratories for analysis over a 6-month period. The survey is 60 percent complete and at this time we are unable to make any comparisons regarding the incidence rate of salmonellae as published in the 1972 report to what is being found at this time.

STUDY NUMBER THREE

In 1967, an in-plant bacteriological survey of chicken eviscerating plants was performed by members of the FSQS Food Microbiology Branch, Beltsville, MD. (Surkiewicz, B. F., R. W. Johnston, A. B. Moran, and G. W. Krumm. 1969. A Bacteriological Survey of Chicken Eviscerating Plants. Food Technology 23:80-85.). This survey and the current 1979 in-plant repeat differed from study number one, previously described in that a) laboratory personnel visited individual establishments and collected samples, b) diverse in-plant sites were sampled, c) water and in-line samples were collected, d) general bacteriological enumeration as well as Salmonellae isolations were performed, and e) different analytical methodologies were employed. The incidence of salmonellae found in eviscerated chickens was 20.5 percent. In some plants a higher percentage of carcasses entering the chill tank were positive for salmonellae than at discharge. However, the total of positive birds at both chill tank entry and exit was the same.

A similar in-plant study of chicken slaughter plants located in the Mid-Atlantic States is currently underway. Analysis of the samples collected in these plants is still in progress and after evaluation, these results will be compared to the 1967 study to provide a benchmark on the incidences of Salmonellae in eviscerated chickens.

Concerning another aspect of our work, our Division was contacted by Staff members from the Veterinary Services Program in USDA’s Animal and Plant Health Inspection Service, and from the University of Minnesota regarding studies on the feasibility of developing Salmonella free turkeys. Results from the University study indicated that Salmonella free birds could be raised and we were asked to sample experimental and
control flocks as they went through the slaughter phase and the chill tanks.

Members of the Microbiology Division visited three turkey slaughter plants in Minnesota to collect whole bird rinse samples from the experimental and control flocks, as well as water and other environmental samples. All samples were frozen and shipped to the Beltsville Food Microbiology Laboratory and analyzed by lactose pre-enrichment and tetrathionate broth enrichment prior to streaking onto brilliant green sulfa and xylose lysine deoxycholate agar plates. These samples were collected during September and October 1979 and the Laboratory work is still ongoing.

Now, I would like to talk about a problem we had concerning roast beef. During the summer of 1977, over 200 cases of food borne salmonellosis were reported to have occurred by the Center for Disease Control. The implicated food product was precooked roast beef. In September 1977, the Department published an emergency regulation which stated that cooked beef and roast beef must be cooked to a minimum internal temperature of 145°F. As a result, no additional cases of salmonellosis were attributed to cooked or roast beef processed at 145°F. Several cases of salmonellosis were reported, however, and these illnesses implicated a firm whose beef was not cooked to the 145°F requirement. Instead, the beef was actually prepared with internal temperatures slightly in excess of 112°F.

While the 145°F cooking requirement was in effect, a joint Industry/USDA Task Force prepared a research protocol to study and develop alternative cooking methods for preparing "rare" roast beef. Results of the industry financed study were fruitful and a second regulation addressing alternative cooking procedures combining longer times and lower temperatures was published in July 1978. The Microbiology Division then undertook a survey of 28 federally inspected establishments preparing cooked beef and roast beef according to these alternative procedures to assure that there was industry compliance. Inspectors at the producing plants submitted 371 samples representing 840 individual beef rounds and 71 environmental samples (vacuum reservoir fluid). All samples directly related to beef rounds were negative for salmonellae when examined at our Field Service Laboratories in St. Louis, Athens, or San Francisco. We are pleased to report that salmonellae were not isolated from the samples and no cases of salmonellosis have been reported since the industry began using the alternate cooking procedures instituted by FSQS in 1978.

Comparisons between analytical results obtained in these studies and those of previous years are being evaluated by the Microbiology Division. All data will be submitted for statistical analysis to determine the degree of validity of the comparative studies. In the event that additional studies are deemed necessary, the information contained in these paired studies
Salmonella Incidence Studies in Whole Broilers — A Comparison of Results from Identical Surveys of 1967 and 1979

No Positive/No Tested 1979 1967

Microbiology Division, Food Safety and Quality Service United States Department of Agriculture
Table 1 - *Salmonellae* incidence in whole young chickens from 15 Federally inspected plants.

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>% Positive 1967</th>
<th>% Positive 1979</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 (6/40)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55 (22/40)</td>
</tr>
<tr>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20 (8/40)</td>
<td>34.1 (14/41)</td>
</tr>
<tr>
<td>3</td>
<td>7.5 (3/40)</td>
<td>7.5 (3/40)</td>
</tr>
<tr>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.7 (10/44)</td>
<td>2.5 (1/40)</td>
</tr>
<tr>
<td>5</td>
<td>26.2 (11/42)</td>
<td>77.5 (31/40)</td>
</tr>
<tr>
<td>6</td>
<td>17.5 (7/40)</td>
<td>50 (20/40)</td>
</tr>
<tr>
<td>7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15 (6/40)</td>
<td>15 (6/40)</td>
</tr>
<tr>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.8 (14/38)</td>
<td>7.5 (3/40)</td>
</tr>
<tr>
<td>9</td>
<td>55.3 (21/38)</td>
<td>62.5 (25/40)</td>
</tr>
<tr>
<td>10</td>
<td>73.7 (28/38)</td>
<td>7.5 (3/40)</td>
</tr>
<tr>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19 (8/42)</td>
<td>47.5 (19/40)</td>
</tr>
<tr>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.5 (13/40)</td>
<td>32.5 (13/40)</td>
</tr>
<tr>
<td>13</td>
<td>25 (9/36)</td>
<td>87.5 (35/40)</td>
</tr>
<tr>
<td>14</td>
<td>12.8 (5/39)</td>
<td>20 (8/40)</td>
</tr>
<tr>
<td>15</td>
<td>55 (22/40)</td>
<td>47.5 (19/40)</td>
</tr>
<tr>
<td>Total</td>
<td>28.6 (171/597)</td>
<td>36.9 (222/601)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Plant unavailable for 1979 survey. Replacement establishments selected were similar in size, production volumes, and location to that of original establishments sampled in 1967.

<sup>b</sup>Establishment under different corporate name in 1979.

<sup>c</sup>No. positive/total analyzed.
Table 2 - *Salmonellae* incidence in whole young chickens from 15 Federally inspected plants according to State.

<table>
<thead>
<tr>
<th>State</th>
<th>Plant</th>
<th>% Positive 1967</th>
<th>% Positive 1979</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>5</td>
<td>26.2 (11/42)a</td>
<td>77.5 (31/40)</td>
<td>+51.3</td>
</tr>
<tr>
<td>DE</td>
<td>9</td>
<td>55.3 (21/38)</td>
<td>62.5 (25/40)</td>
<td>+7.2</td>
</tr>
<tr>
<td>MD</td>
<td>10</td>
<td>73.7 (28/38)</td>
<td>7.5 (3/40)</td>
<td>-66.2</td>
</tr>
<tr>
<td>NC</td>
<td>3</td>
<td>7.5 (3/40)</td>
<td>7.5 (3/40)</td>
<td>NCb</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>32.5 (13/40)</td>
<td>32.5 (13/40)</td>
<td>NC</td>
</tr>
<tr>
<td>GA</td>
<td>6</td>
<td>17.5 (7/40)</td>
<td>50 (20/40)</td>
<td>+32.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15 (6/40)</td>
<td>55 (22/40)</td>
<td>+40</td>
</tr>
<tr>
<td>AL</td>
<td>15</td>
<td>55 (22/40)</td>
<td>47.5 (19/40)</td>
<td>-7.5</td>
</tr>
<tr>
<td>MS</td>
<td>14</td>
<td>12.8 (5/39)</td>
<td>20 (8/40)</td>
<td>+7.2</td>
</tr>
<tr>
<td>MN</td>
<td>13</td>
<td>25 (9/36)</td>
<td>87.5 (35/40)</td>
<td>+62.5</td>
</tr>
<tr>
<td>AR</td>
<td>7</td>
<td>15 (6/40)</td>
<td>15 (6/40)</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22.7 (10/44)</td>
<td>2.5 (1/40)</td>
<td>-20.2</td>
</tr>
<tr>
<td>TX</td>
<td>11</td>
<td>19 (8/42)</td>
<td>47.5 (19/40)</td>
<td>+28.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20 (8/40)</td>
<td>34.1 (14/41)</td>
<td>+14.1</td>
</tr>
<tr>
<td>CA</td>
<td>8</td>
<td>36.8 (14/38)</td>
<td>7.5 (3/40)</td>
<td>-29.3</td>
</tr>
</tbody>
</table>

*a* No. positive/total analyzed.

*b* No change.
Table 3 - *Salmonella* incidence in whole young chickens from 15 Federally inspected plants according to FSQS Region (geographic).

<table>
<thead>
<tr>
<th>Region</th>
<th>% Positive 1967</th>
<th>% Positive 1979</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>50.8 (60/118)(^a)</td>
<td>49.2 (59/120)</td>
<td>- 1.6</td>
</tr>
<tr>
<td>SE</td>
<td>23.4 (56/239)</td>
<td>35.4 (85/240)</td>
<td>+ 12</td>
</tr>
<tr>
<td>NC</td>
<td>25 (9/36)</td>
<td>87.5 (35/40)</td>
<td>+ 62.5</td>
</tr>
<tr>
<td>SW</td>
<td>19.3 (32/166)</td>
<td>24.8 (40/161)</td>
<td>+ 5.5</td>
</tr>
<tr>
<td>W</td>
<td>36.8 (14/38)</td>
<td>7.5 (3/40)</td>
<td>- 29.3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>28.6 (171/597)</strong></td>
<td><strong>36.9 (222/601)</strong></td>
<td>+ 8.3</td>
</tr>
</tbody>
</table>

\(^a\) No. positive/total analyzed

Table 4 - *Salmonella* incidence studies in whole broilers - Comparison by groups found in identical surveys of 1967 and 1979.

<table>
<thead>
<tr>
<th>Group</th>
<th>% 1967</th>
<th>% 1979</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>50.9</td>
<td>60.4</td>
</tr>
<tr>
<td>C(_1)</td>
<td>29.8</td>
<td>17.6</td>
</tr>
<tr>
<td>C(_2)</td>
<td>10.5</td>
<td>14.4</td>
</tr>
<tr>
<td>D</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>E (E(_1) - E(_4))</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td>G</td>
<td>-</td>
<td>1.4</td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE ON SALMONELLA

Chairman: Erskine V. Morse, West Lafayette, Ind.

Co-Chairman: G. H. Snoeyenbos, Amherst, Mass.


Meeting: Tuesday, 1:30 PM, Forum Room.

A total of twenty-five committee members and guests were in attendance. The following papers or presentations were given before the committee: Dr. L. Peterson (USDA-APHIS) discussed the current status of the National Poultry Improvement Plan (NPIP). Currently 22 states are U.S. Pullorum-Typhoid clean (chickens); three states are in the same category (turkeys), and seven additional states are working toward the goal. The objective is that all states become Pullorum-clean by 1990.

As recommended by the USDA, Secretary's Salmonella Task Force (1978 Report), feasibility studies were commenced in 1979. These studies were instituted in the states of Massachusetts, Minnesota and Georgia to ascertain whether poultry could be produced and maintained free of salmonellae. Dr. Pomeroy (Minn.) reported highly encouraging results in respect to maintaining parent turkey stock and their progeny substantially free of Salmonella. Dr. D. Johnson (Ga.) indicated preliminary and similar work directed toward broilers had started. Dr. Snoeyenbos (Mass.) commented on investigations which indicated that the current structure of the broiler industry precluded useful feasibility studies extending from foundation breeding stock to broiler stock.

Major restructuring of broiler production management appears essential to allow control of salmonellae. Future studies will be confined to evaluation at the parent stock and broiler production levels. Should the preceding studies prove to be promising, it is proposed that similar investigations be conducted with swine and cattle.

Dr. McCapes (Calif.) reviewed the historical aspects of the Dillon's Beach turkey Salmonella Project.

Dr. S. S. Green (USDA-FSQS) discussed the 1967 survey in comparison with preliminary 1979 data of the incidence of salmonellae contamination of processed poultry, pork sausage and precooked roast beef.

Dr. Eddie To (Monsanto Co., St. Louis, Mo.) reported on his experiments as follows:

Fresh poultry breasts were inoculated by dipping in a suspension containing either $10^3$ or $10^5$ cells of three strains of Salmonella per ml.
These breasts were then dipped in a solution of either 0, 2.5, 5.0 or 10.0% potassium sorbate for 1 minute. The 5.0 and 10.0% dip markedly reduced the growth rate of *Salmonella* at 10 and 22°C. when compared to controls, resulting in significantly lower *Salmonella* counts after 7 days of storage at these two temperatures. In two subsequent plant trials, in Georgia and Mississippi, whole broilers were removed from the final chill tank, and inoculated within 24 hours by dipping in a suspension containing 10⁸ cells of four strains of *Salmonella* per ml. Half of the birds were dipped in 5.0% potassium sorbate for 1 min. and the other half in water for 1 min. After 10 days’ storage at 3°C, significantly lower *Salmonella* counts were found in the sorbate treated broilers than on broilers dipped in water.

Dr. Bixler (FDA-Bur Vet Med) stated that as of 1976 existing policies of FDA will remain in effect unless it is clear that public health will be endangered. He further commented on the Canadian efforts to secure a Tripartate agreement among Canada, U.K. and the U.S. for reduction of salmonellae contamination in poultry and animal feeds.

Dr. Purchase (USDA-SEA-AR) reported on the funding of feasibility studies in poultry to support research at Minnesota and Massachusetts. Additional information was given relative to the regional USDA research at Athens, GA.

Dr. Harrington (USDA-NADC, Ames, Iowa) summarized the serologic typing results for the past year. The complete data appears as an appendix to this Committee Report.

It is apparent that the increased incidence of *S. dublin* is of major public health concern. In addition, this enteric pathogen causes abortion in cattle; it has also been demonstrated to be an asymptomatic, residual pathogen in the mammary gland of cattle.

The assembled Committee recommended that:

"Funding of studies to determine the feasibility of rearing poultry free of *Salmonella* under commercial conditions be expanded to include all species of food producing animals."

Dr. E. T. Mallinson (Pa.) submitted the following to be included with the committee report: Poultry transport crate sanitation study.

Recent reports from Dr. J. R. Pettit and others in Canada have given strong suggestion that:

1. Practical, reasonable improvements in broiler farms, broiler feed and hatchery sanitation and security have resulted in very significant reduction of salmonella contamination in 4,000 bird broiler test flocks up to marketing age (42 days).

2. This admirable situation was quickly reversed when the apparently clean broilers were transported to slaughter in dirty crates found contaminated with Salmonella at the rate of 14% positive. This crate
source of contamination spread to feathers, into plant and onto the final processed carcass.

Because some of our Pennsylvania broiler growers and their hatchery sources are already meeting, reasonable sanitation and security practices similar to those employed in the Canadian studies, we instituted a modest fact finding study with producers and allied indusrymen to see what the requirements would be for practical, industry accepted crate sanitation technology. We were also motivated by our long experience of finding the introduction of laryngotracheitis to be associated with the movement of dirty transport crates.

At this point, we have learned that the development of acceptable technology will most probably need monetary investments in equipment design and field trials by processing equipment manufacturers at least and possibly financial and technical support from government, academia and poultry made associations as well. Clearly, a breakthrough in crate sanitation technology is feasible and consistent with the history of the technology oriented poultry industry.

The results of the Pennsylvania study suggest that any crate sanitation system developed will need to meet the following criteria—not necessarily listed in order of importance:

1. Readily adaptable to existing plant designs and spacing problems.
2. Conservation of energy.
3. Speed — 1 crate every 7-8 seconds.
4. Non-damaging to crates and handlers.
5. Non-polluting to the environment.
MANAGEMENT FOR THE PREVENTION OF LIVESTOCK POISONING BY PLANTS

Lynn F. James, PhD
United States Department of Agriculture,
Science and Education Administration,
Agricultural Research, Poisonous Plant Research Laboratory,
1150 East 1400 North, Logan, Utah 84321.

The 48 contiguous states contain a total of 835 million acres of rangeland that produce enough forage to feed 18 million breeding cows (or their equivalent) yearlong. The forage on these lands is converted into food and fiber for human consumption by sheep, cattle, and wildlife. Additional ranges could be grazed and the forage-producing potentials of our current rangelands can be more than doubled. Rangeland then could produce forage for an equivalent of nearly 40 million breeding cows yearlong.

About 60% of the U.S. rangelands is in 11 Western States; 82% is in 17 Western States. This near billion acres produce more than one-half of the national production of livestock.

Beef is the top income producing agricultural commodity in 21 states and among the top 4 in 41 states. Sheep account for a smaller portion of the gross receipts but they are important and show promise of gaining in number.

Animal products contribute more than half of the total nutrients in the average U.S. diet, and two-thirds of the feed used in producing these animal products comes from substances that are undesirable or completely unusable for human consumption. About 75% of the total feed units consumed by beef cattle comes from forage of some kind.

What do such statistics have to do with poisonous plants? Simply this — not all of the vegetation produced on our ranges and pastures is wholesome forage. Some of it is poisonous.

Poisonous plants are one of the most important causes of economic loss to the livestock industry. The losses can take the form of deaths of livestock, abortions, decreased performance, weight losses, chronic illness, debilitation, photosensitization, and birth defects. In addition to these direct costs, land managers and livestock men must pay for problems associated with adapting ranges and pastures infested with plants poisonous to their grazing animals. These costs and problems include things such as fencing, decreased forage utilization, altered grazing programs, and in some instances, supplemental feeding programs and veterinary fees.

Few have attempted to quantify the economic losses to the livestock industry due to poisonous plants. Most authors who have written or spoken on this subject have been content to generalize (e.g., "yearly losses amount to many millions of dollars," or "poisonous plants exact a
costly toll,” or “poisonous plants are a major economic problem”). This is partly because notice is most easily given to more direct losses, as when large death tolls occur because certain plants such as halogeton, lupine, larkspur, and locoweed were grazed. Lesser death losses are usually ignored and many go unrecognized. Little if any thought is given to losses involved in decreased weight gains, increased management costs, loss of forage, altered grazing programs, etc. One estimate of the economic losses suffered by livestock in the 17 Western States was $107 million annually. The author of the figure admits to including only direct items such as death losses and abortions.

A survey in New Mexico a number of years ago on the effects of poisonous plants on livestock, cited as one of its main conclusions that livestock men do not report their poisonous plant problems because they can’t get help in preventing them. Thus, these losses have come to be considered as part of the cost of doing business in the livestock industry.

Obviously the effects of poisonous plants are many and varied. Therefore, so must be the methods that are applied in either researching the problems or in preventing losses due to poisonous plants. This demands a multi-disciplinary approach. Let us consider the various disciplines and what part each can play in solving the overall problem.

Veterinary medicine

This includes diagnosis of poisonous plant poisonings, treatment when possible (most of the time not), and assisting in designing management methods that can prevent poisonings. Poisonous plant problems should be handled as most veterinarians want to treat the infectious diseases; that is, via preventive medicine.

Chemistry

Identification of the toxic principles in poisonous plants is of great value in several ways. It not only aids in positively identifying poisonous plants but also in predicting a plant’s toxicity and devising methods for the prevention of intoxication.

Range management

Most poisonous plant problems occur under grazing conditions (range and pasture). This then is where the problem must be stopped.

Animal science

The management of the grazing animals themselves, can encourage or deter poisoning in many situations. This is a very brief treatment of an important idea that must be considered in thinking about the prevention of livestock poisoning by plants.

In specific terms, keep in mind that one of the goals of our research is to give veterinarians, ranchers, and land managers information regarding the effects of poisonous plants on livestock so they can make intelligent management decisions. We also want to extend an awareness of
poisonous plant problems. Treatment under most range and pasture grazing systems is impossible because few treatments are available, and the animals are dead when poisoning is detected. Therefore, prevention is of prime importance.

The following are examples of management programs that have been devised for the prevention of livestock poisoning by plants.

False hellebore causes a cyclopian-type malformation in lambs produced by ewes who grazed the plant on the 14th day of gestation. This incidence has varied from about 2% to 25% of a band of ewes. The occurrence was minimized by altering the grazing program so that the ewes did not graze the veratrum plants during the early part of gestation.

Certain species of lupine cause a skeletal malformation in calves. The lupine must be grazed by the cows between the 40th and 70th days of their gestation. The toxic alkaloid, anagyrine, is the compound responsible for the birth defect. Research further showed that this compound is high in the plant during its early stages of growth and in the seed. The malformation can be minimized by regulating the breeding season and/or the time when the cows are allowed to graze the lupine.

Halogeton, an annual that grows in the colder arid and semiarid regions of the West, has been responsible for the deaths of thousands of sheep. Sheep graze halogeton readily and usually without harm. However, when hungry sheep are allowed to graze in areas where quite a lot of halogeton grows, intoxication may occur. This can be avoided by not allowing sheep to become hungry, especially when being grazed in areas where halogeton is abundant.

Tall larkspur, *Delphinium barbeyi*, is a serious poisoning problem to the cattle men of the West. It has been shown that tall larkspur can be effectively and economically controlled by spraying it in two consecutive years with 2,4,5-T.

*Helium hoopesii*, sneezeweed, can be a serious poisoning problem to sheep grazing on the mountain ranges. The toxin in this plant has an accumulative effect. The impact of this toxin can be lessened on sheep that must graze it, by periodically removing the sheep for a few days from the areas where it grows. In effect, the sheep thereby experience alternating periods of intoxification and detoxification.

With a proper understanding of the etiology of plant poisoning, the toxin involved and its pattern of occurrence in the plant, and grazing programs, much can be done to minimize losses in livestock due to poisonous plants.

REFERENCES


REPORT OF THE COMMITTEE ON SHEEP AND GOATS

Chairman, F. James Schoenfeld, Salt Lake City, Utah.


There were 13 Committee Members present and 4 guests; a total of 17 were present.

The Committee met as requested by the President of USHA to consider the business of the Committee, and submits the following report:

Mr. Olin H. Timm was recognized for his contributions to the sheep industry and to the USAHA, being honored as the 1979 California Sheepman of the Year, as was reported in the *National Wool Grower*, September, 1979.

The Committee held open discussions on the several disease problems of sheep and goat diseases.

Dr. Guy Reynolds, Oregon State Extension Veterinarian, brought us up to date on the work done in Footrot research. Dr. Blain McGowan, School of Veterinary Medicine, University of California, updated the work with Footrot vaccine and related the progress as to the disassembly of a building, that it has to be a breakdown brick by brick. This disease and vaccine development involves very sophisticated biochemistry.

The discussion of Bluetongue was reported by several members. Dr. Hugh E. Metcalf, Veterinary Epidemiologist (Bluetongue) USDA, APHIS, Veterinary Services, Denver, Colorado, reports the following: — Starting in mid-April, 1979, a serious epizootic of Bluetongue in sheep and cattle has occurred in the State of Mississippi, extending into Alabama, Arkansas, Louisiana and possibly Tennessee. The epizootic has remained confined to the initial area during the summer and has not appeared to spread to other states. Many isolations of BT virus have been made from sheep, cattle and deer in the area and all those which have been typed have been serotype 17.

Dr. Michael M. Jochim, USDA-SEA-AR, Denver, Colorado, reported on the research on the development of monovalent modified live virus vaccine for Bluetongue: Research on the development of monovalent modified live virus vaccines for Bluetongue is continuing at the Arthropod-borne Animal Disease Research Laboratory (USDA-SER-AR, Denver, Colorado). Sheep inoculated with a bovine isolate of BTV serotype 17 that was passaged 23 times in cell culture, developed antibody to BTV and were refractory to challenge of immunity at 4 weeks after vaccination. All vaccinated sheep developed high viremia titers, but clinically only a slight febrile response was observed. *Culicoides variipennis* insects were fed on the blood of these vaccinated sheep; less
than 15% of the insects were infective 3 weeks later when they took a blood meal on susceptible host sheep. However, recipient sheep developed antibody to BTV, were clinically normal during the 4 weeks after infected insects were fed, and were refractory to challenge of immunity. These preliminary results are encouraging because they demonstrate that sheep are refractory to challenge after vaccination with a modified live BTV and that the vaccine virus does not appear to revert to the virulent form on passage through the vector.

Dr. T. Lynwood Barber, USDA-SEA, reported the progress of the advances made toward the development of a safe and effective inactivated virus vaccine for Bluetongue: Excellent progress is being made on studies toward the development of a safe and effective inactivated virus vaccine for Bluetongue. The research is being done at the USDA-SEA-AR Arthropod-borne Animal Diseases Research Laboratory at Denver and at the School of Veterinary Medicine, University of California, Davis, California. The research is being financially supported by USDA-SEA-AR, California Wool Growers Association, California Cattlemen's Association, Livestock Disease Research Laboratory and the Department of Food and Agriculture of the State of California. Scientists involved in the research include Drs. B. I. Osburn, T. L. Barber, Ja Rue Manning and Mr. Jeff Stott. A full status report on the experimental inactivated vaccine is being planned. Basic studies on the nature of the immunologic response of sheep to the vaccine are being continued. Likewise, Dr. Manning, at UCD/Davis, is testing additional chemicals as candidates for better inactivation of Bluetongue virus with better preservation of immunogenicity. An adjuvant is essential for the inactivated vaccine to be effective. Various adjuvants are being tested in order to find one that is easy to handle, inexpensive and effective.

Dr. J. L. Hourrigan, USDA, ADHIS, Veterinary Services, submitted the Scrapie Report (see Page 2-A following). He also reported on research work with Scrapies as to a project involving ovary transplant from infected sheep to non-infected sheep and then will examine the progeny for transmissability of the disease.

Dr. B. I. Osburn, University of California, Davis, reported on the Bluetongue Epidemiology Program in California: A cooperative study between the School of Veterinary Medicine, University of California, Bureau of Animal Health, Department of Food and Agriculture, State of California, and USDA/APHIS is underway to evaluate the epidemiology of Bluetongue virus infection in domestic and wild ruminants and insect vectors. Serological test results indicate that 30% of 5,281 samples from all species were positive for Bluetongue antibodies. In the different species, 36% of the sheep, 29% of the cattle, 20% of goats and 55% of wild ruminant species were positive. Of 44 virus isolations from cattle, 26 were serologically negative; most of the cattle virus isolates were from cattle that were not showing clinical evidence of disease. Viral isolates have also been made from antelope and goats. The predominant
serotypes are 11 and 17, followed by 13 and 10. The only licensed vaccines available are to serotype 10, and these provide little or no cross-protection to serotypes.
REPORT TO THE COMMITTEE ON DISEASES OF SHEEP AND GOATS

SCRAPIE

During FY 1979 scrapie was confirmed in four flocks in three States as follows:

A veterinary practitioner reported scrapie in a 39-month old Suffolk ewe in Tulsa, Oklahoma (Oklahoma No. 8). The ewe had been purchased when 3-months old, and was showing clinical signs 34 months later. The flock of six sheep was slaughtered.

Ohio outbreak No. 14 was found in Lucas County, Ohio, as a result of tracing exposed sheep imported from a Canadian flock. The Suffolk ewe was imported in June 1976 and died in April 1978 at 37-months of age. Brain tissue sent to the National Veterinary Services Laboratory (NVSL) was frozen in transit and, thus, not satisfactory for histological examination. The case was confirmed at NVSL when 19 of 20 mice were positive 375 to 433 days post-inoculation. The block of seven sheep was slaughtered. The source flock was slaughtered by Canadian authorities. The Canadian flock was responsible for spreading scrapie into six flocks: (1) Lucas County, Ohio, flock, (2) Hartford County, Connecticut, flock, and (3) four flocks in Canada.

Indiana outbreak No. 29 involved a Montgomery County flock. Two Hampshire ewes were confirmed at 39- and 60-months of age. A twin to one of the ewes had died a few months earlier after showing similar clinical signs. The flock of 31 sheep was slaughtered.

Indiana outbreak No. 30 in Vermillion County involved an unregistered Suffolk ewe 3½ years old. Another Suffolk ewe of the same age demonstrated clinical signs suggestive of scrapie, but brain tissue sent to NVSL had been frozen and were not suitable for histopathological studies. The two ewes, including the entire flock in Vermillion County, had been purchased a year earlier from another Indiana flock, considered the source of the outbreak. Both flocks, comprising 98 sheep, were slaughtered.

The amendment to 9 CFR, Part 54, effective September 15, 1978, which provides for appraisal of animals destroyed because of scrapie by qualified appraisers, and increased Federal compensation for animals destroyed of two-thirds the appraisal value up to $300 per head, will, we believe improve cooperation by the industry and the Scrapie Eradication Program will move forward.

SCRAPIE FIELD TRIAL, MISSION, TEXAS

The Scrapie Field Trial has been underway since November 1964. During this period scrapie has been confirmed by histopathological study or by mouse inoculation in 394 animals.

The work at Mission has confirmed that natural scrapie can be transmitted to previously unexposed nonbloodline sheep and goats placed on
infected premises with scrapie-affected sheep and goats. Scrapie has been transmitted naturally to Hampshire, Rambouillet, Suffolk, and Targhee sheep; and to Angora, Nubian, Angora X, Nubian X, Toggenburg, Nubian X, Toggenburg X, and mixed breed dairy goats.

Scrapie percentage in animals born at Mission and by breed was: Suffolks - 20, 34, and 39 percent; Rambouillet - 14 percent; Hampshire - 18 percent; Targhee - 19 percent; Angora goats - 26 percent; and dairy goats - 61 percent.

Goats, Suffolk, Rambouillet, and Targhee sheep developed scrapie if born on infected premises and removed from exposure at the following ages: goats - 6, 9, and 10 months; Suffolk - at birth, 4, 9, and 20 months; Rambouillet - at birth, 4, 9, and 20 months; and Targhees - at birth and 20 months.

This study demonstrates that young animals sold from infected flocks may harbor the scrapie virus and progress to clinical scrapie. Therefore, they must be destroyed if we are to eradicate this disease.

Sixty-one percent of dairy goat progeny born and reared in contact with scrapie goats and sheep developed scrapie. Of dairy goats born and reared in contact with scrapie goats, but not in contact with scrapie sheep, eight (82 percent of the progeny considered at risk) have developed scrapie.

Scrapie has not been directly linked to human disease. However, the evidence continues to draw closer the possibility of the human health hazard. Scrapie virus inoculated into Capuchin, Cynomolgus, Rhesus, Spider, or Squirrel monkey produces a disease with similar symptoms and brain damage as that produced by the viruses of transmissible mink encephalopathy (TME) and of the human diseases of kuru and Creutzfeldt-Jakob Disease (when inoculated into monkeys).

Studies on insect vectors revealed that the *Culicoides variipennis* are widely distributed in California: common breeding sites include lagoons on dairies, sewage disposal lagoons, irrigation ditches and along streams. A viral isolate has been made from *C. variipennis*. In other studies, the soft shell tick, *Ornithodonus Coriaceus* can obtain blood from vivemic animals, become infected with the Bluetongue virus, and the virus can be isolated from salivary glands as long as 90 to 100 or more days after ingesting the blood meal.

A report was made of an article in the *National Wool Grower* of October, 1979, entitled “Scrapie Virus — A Slow Agent of Death,” by Drs. J. R. Gorham and N. L. Gates.

Respiratory diseases in some areas of the U.S. were considered co-runners with Footrot and causes a great economic loss to the industry.

There is great concern about the production of safe and effective biologics in the controlling of certain diseases of sheep and goats. There was concern about the present Wampler Bill before the U.S. House and
its effect, if passed, upon the intrastate production of minor-species biologics.

Ram Epididymitis is a disease that needs a safe and effective vaccine produced to help in its control. It was pointed out that various surveys of the disease, by physical examination and a C.F. test on ewes and rams that the incidence was an average of 25% by palpation plus another 15% by the C.F. test, which gave an incidence of around 40%. The control of this disease is a rigid examining and culling program, plus a vaccination program with Brucella ovis. There is a need to rejuvenate more attention to this program.

Dr. Donald G. Waldhalm, University of Idaho, Veterinary Research Laboratory at Caldwell, reported that research is being conducted at the University to develop a vaccine against Enzootic abortion of ewes (EAE), using inactivated chlamydia that were grown on cell cultures, rather than in chicken embryos. Several lots of cell culture have been produced in the experiment. These are being combined with various adjuvants and are being tested in sheep. Both immune responses and degree of protection against abortion will be determined. It is anticipated that an economically feasible vaccine may result from these studies.

Dr. R. A. Robinson asked the Committee to review and update the "Justification for Veterinary Health Research for Sheep and Goats" and to return the suggestions that he might report back to the AVMA the feelings of this Committee.

It was brought to our attention the need for Liverfluke control in the sheep population. Dr. Thomas Snodgrass, F.D.A., said there was forthcoming an announcement of a new flukeicide which was soon to be available. Idaho reported that they were doing some fluke research. It is the feeling of the Committee that more attention should be given to the problem of Flukes and other parasitic diseases of sheep and goats.

The Committee reviewed the letter from Dr. Mulhern, Administrator, concerning Bluetongue. The Bluetongue discussion and the resolution submitted to the Resolutions Committee includes the feelings of this Committee.

The committee considered the following resolutions: Bluetongue, Footrot and Ram Epididymitis. The Committee approved these resolutions which are being sent to the Resolutions Committee.

The business of the Committee being completed, the meeting was adjourned.

F. James Schoenfeld
INTRODUCTION

The perpetuation of viral diseases requires a mechanism for the causative virus to survive in nature. The obligate nature of viral replication in living cells mandate a host-to-host transfer, an equilibrium of compensatory virus cell interaction, or a state of latency. One of the most perplexing, yet intriguing virus-host relationships observed in nature is the interactions between herpesviruses and the animals they infect. The research maze that surrounds this phenomenon is almost incomprehensible. Many theories are perpetrated to explain all or a portion of these well-known disease processes, and subsequent immunological relationships. Efforts to explain this mechanism have not abated since that so succinctly described by Du Castel in 1901 when he stated that "In our ignorance of the exact process of herpes, each medical generation created a theory adapted to the ideas and discoveries of the moment" (quoted by Nahmias and Dowdle 1968, p. 111). If the swine production and regulatory personnel respond effectively to the recent challenge of increased pseudorabies virus (PRV) infections in the U.S. swine population they must have new information concerning the epidemiology and biologic nature of the disease.

Adequate scientific information concerning chronic PRV infections and the spread of the disease in nature is lacking. Most of the scientific information concerning chronic herpesvirus infections in mammals has been gathered from research involving human herpes simplex virus-1 (HSV-1). It seems appropriate to review the nature of chronic herpesvirus infections in other mammals before concentrating upon swine pseudorabies virus infections.

Review of Literature of Chronic herpesvirus infections of mammals

The terminology describing persistent viral infections of host cells is not well-defined. Fenner (1968) used the term latency in a general sense, but Johnson (1970) used the term in a restricted sense to give the connotation of the covert nature of virus-cell relationships. Dulbecco's (1965) enunciation of the maintenance of equilibrium conditions of viral persistence in the cell depending upon the degree of cytocidal activity of the relationship brought some order to this controversy. His designation of a steady state of independent virus-cell interaction (IVCI), in which a
noncytocidal infection of all cells occurs and dependent virus-cell interaction (DVCI) where a minority of cells are cytocidally infected and synchronous replication of the nucleic acid of both the cell and virus occurs with a minute amount of spontaneous virus release are useful designations. Jack (1974) reviews the use and meaning of these terms and uses 4 human herpesviruses to illustrate the type of host cell-virus relationships that exist. He places all viral infections into 2 major divisions, acute and nonacute infections. Acute infections refer to the classical type described by Fenner (1968) and Johnson (1970) with primary interaction of the virus and host cell and viral replication with or without cell death. This infection is eliminated or progresses on to the nonacute state. The nonacute stage is subdivided into chronic and latent or covert infections. The chronic infection is divided into continuously detectable and discontinuously detectable. The slow infections are subdivided into slow-degenerative and slow-neoplastic. A salient feature of this scheme then becomes apparent in that, in true latency or covert infections, the virus must be completely undetectable in the interval following recovery from acute infections until exacerbation of the illness due to recrudescence of the virus, with recurrent illness that would progress through stages exhibiting features typical of the acute infection.

Jack (1974) suggests that human cytomegalovirus infections are predominantly chronic infections that may show both continuous and discontinuous excretion from the host, with the degree of detectability dependent upon the age of the host at the time of primary infection, or subsequent state of health, such as hormonal influences and intense chemotherapy with cytotoxic substances. He describes the host virus relationship of Varicella-Zoster Virus (VZV) as an example of the latent or covert state. The appearance of zoster (shingles) in adult life following childhood Varicella (chicken pox) virus infection usually represents a single attack. The zoster syndrome may also occur in children if provoked by disease or chemotherapy. Each person so affected gives a reliable history of varicella infection or chicken pox in childhood. The fact that zoster patients can transmit Varicella virus (and not vice versa), that no biological or serologic differences can be detected in VZV strains, and that there are long intervening periods of lack of virus detection indicating a true covert or latent infection. The recurrence of active lesions at unusual anatomical sites led to the speculation that the initiator of the second attack must be the sensory ganglion supplying the cutaneous area involved. This theory is further enhanced by the evidence of acute inflammatory process in the regional ganglion of infected areas in fatal zoster cases. Jack states that “it is tempting to view the mitotically inactive neurone as the site for the long-term persistence of “VZV” and if so, this would not necessitate a hypothesis of viral integration of host DNA. However, he further admits that if the virus is present in the satellite or glial cells that are capable of mitosis then an integrated state may be necessary to maintain it for long periods of time. Cytotoxic and
steroid therapy, neoplasia and other infections may activate VZV infections but unlike CMV there is no ensuing chronicity or generalized spread, which indicates that the pathogenic or immune mechanisms are quite different.

The theory that Epstein-Barr Virus (EBV) is truly a covert or latent infection gains support from the fact that partial integration of the genome in blastoid lymphocytes has been demonstrated. Further evidence to support this position is found in the fact that conditions that induce lymphoid hyperplasia such as leprosy, sarcoidosis, lupus erythematosus and Hodgkins disease also cause increased activity of endogenous EBV. The establishment of lymphoblastoid cell lines only from lymphocytes infected with EBV and its association with Burkitt's lymphoma and nasopharyngeal carcinoma indicates that this latent cell-virus relationship may also be carcinogenic.

The exact nature of the cell-virus relationships in nonacute HSV infections is humans is not known. Jack suggests that covert relationships analogous to the latency of VZV may exist in some individuals, whereas chronic infections with discontinuous excretion occurs in others. In a review of literature relating to the role of nervous tissue in maintenance of the chronic or latent nature of herpesvirus infections in man and experimental animals, Baringer (1975) concludes that the most significant HSV spread from the initial site of infection is through neuronal axon flow mechanisms, even though there is some evidence to indicate that contiguous cell-to-cell spread may occur along the supporting Schwann satellite cells of the nerve fibers and ganglions. A predominant number of reports by competent investigators seem to support the view that the neuron of the sensory ganglion innervating the site of the epithelial lesion recurrence provides the essential milieu for residence of the virus for prolonged periods. Baringer (1975) concedes that at present the scientific evidence is insufficient to determine the exact mechanism involved in the neuron-virus relationship and the pathophysiological alterations within the host that trigger renewed virus replication within the ganglion and subsequent epithelial lesions in areas that it innervates. Two basic theories to explain this immunologic phenomenon have gained a degree of prominence.

First, the theory proposed by Roizman (1965) is palatable because it agrees with the supposition that mammalian viral latency is comparable to the prophage state of lysogenic bacteria. This theory contends that viral replication is restricted in a static state within virogenic cells, but the potential for that replication is preserved by the incorporation of the viral genome within chromosomes of the neuron. The work of Yamamoto et al. (1977), showing a HSV directed peak of thymidine kinase activity 46 days longer than infectious virus could be detected, tends to support this theory.

The second theory, described in some detail by Ennis (1973), proposes that HSV exists in a dynamic state within low metabolic neurons of the
ganglion, in which the productive viral replication and related cytopathologic changes are effectively restricted by humoral and cellular host defense mechanisms. Recent investigations by Waltz et al. (1976) indicate that the number of chronically infected ganglia and subsequent recurrence may be directly related to the effectiveness of viral replication at the initial site of infection, and the ability of the host to respond immunologically to the infection.

Recrudescence of Herpesvirus Infections in Man

The precipitation of recurrent herpesvirus eruptions in man is known to be associated with a number of quite variant biophysical conditions. Conditions such as elevated temperature, exposure to ultraviolet light, strong winds, menstruation, emotional stress or physical trauma may evoke recurrent eruptions. The stress of pregnancy may potentiate primary HSV infections into generalized disseminated disease (Young et al. 1976). The common physiologic impact, if any, of these conditions is not known. Several methods of experimental induction of recurrent lesions have been successful. Good and Campbell (1948) reported that anaphylactic shock would induce recurrence of HSV in chronically infected mice. Other workers have shown that treatment with corticosteroids (Thygeson et al. 1953), epinephrine (Schmidt and Rasmussen 1960; Laibson and Kibrick 1966), and the induction of Arthus reaction may also cause the exacerbation of chronic HSV-1 infections in experimental animals (Anderson et al. 1961).

The exact mechanism involved in the variation of the host response to acute infections, the recovery from acute infections, the resistance to re-exposure to exogenous virus, and the development of lesions due to indigenous chronic or latent virus is not completely understood. Since recurrent lesions appear in patients with high serum antibody titers, most investigators believe that some other immune mechanism plays a predominant role. Recent investigations (Zisman et al. 1969; Mori et al. 1967) have shown that macrophages and thymus derived (T) lymphocytes are key factors in controlling chronic HSV infections in mice. An elegant series of experiments by Rager-Zisman and Allison (1976) using cyclophosphamide-treated-HSV infected mice has indicated that protection against this virus infection is predominantly T cell dependent. Their results also indicate that the macrophage plays an important role in the host's recovery process. Their studies seem to imply that humoral antibodies alone play no major role in the recovery of the host. However, they suggest that the data tends to support the theory that antibody-dependent cell-mediated cytotoxicity may well be operative in this specific incidence. Blank and Haines (1976) demonstrated that patients with recurrent HSV infections and high antibody titers could easily be super-infected in epithelial tissues at remote sites from recurrent lesions with both indigenous and exogenous virus. They seem to believe that humoral antibody plays little or no role in preventing recurrent or exogenous HSV infections.
Latent Herpesvirus Infections in Other Animals

The mouse cytomegalovirus is another example of a chronic herpesvirus infection with discontinuous detectable excretion (Medearis 1964) but it appears that a latent infection may exist in lymphoid tissue. Olding et al. (1975) demonstrated more specifically that the latent infection resided within the B lymphocyte population and that the virus could be recovered readily from lipopolysaccharide-induced blastogenesis of B lymphocytes from chronically infected mice.

The infection of guinea pigs with a guinea pig herpesvirus leads to a chronic infection from which virus can be isolated from the blood and somatic tissue for periods up to 24 months following infection. Pregnant, chronically infected animals can transmit chronic infections to fetuses (Lam and Hsiung 1971).

The infection of rabbits by Herpesvirus cuniculi is considered to be both discontinuously detectable and latent since virus could be detected in some animals as long as 100 days after infection and primary kidney cell cultures failed to yield infectious virus but contained viral antigen that could be detected by immunofluorescence.

The nature of the immune mechanisms responsible for the recovery of cattle from acute IBRV infections, resistance to re-infection and the recrudescence of chronic or latent infection has not until recently been the subject of critical, refined scientific evaluations. The role of environmentally-induced physiologic stress in IBRV infections has been investigated (Bowes et al. 1970; Baczynski et al. 1975; Mihaljovic et al. 1973), but careful cause-related scientific analysis is difficult. The role of shipping stress, hormonal imbalances during calving (Snowden 1965), and nutritional deficiencies (Crane 1965) have also been suspect. One of the most intriguing theories is based upon the fact that certain groups of viruses may suppress the cell-mediated immune (CMI) response of acutely infected animals or depress CMI in chronically infected animals thereby allowing recurrent disease to develop. Myxoviruses have been shown to affect the CMI response in other animals. The recent report (Mensik et al. 1976) of reactivation of IBRV infection in young calves by experimental PI-3 virus infection tends to support this hypothesis.

The report by Sheffy and Davies (1972) of the corticosteroid (CS) reactivation of IBRV from both naturally and experimentally infected cattle sparked a flurry of scientific activity. They reported that 5 of 6 bulls that had humoral anti-IBRV antibodies for a period of 4 years without obvious clinical disease shed IBRV in nasal secretions within 3-5 days post treatment with dexamethasone. Nineteen pregnant Holstein heifers 11 of which had been vaccinated with commercial vaccine, were exposed to virulent IBRV and treated with dexamethasone 3-9 months later for a period of 6 or 7 days and slaughtered 24 hours after the last treatment. IBRV was recovered readily from nasal secretions and
respiratory tract of treated animals, but much less frequently from somatic tissues such as nervous tissue, adrenals and reproductive tract. Virus was also recovered from the upper respiratory tract and ovarian tissues of 1 of 9 untreated animals. Sheffy and Rodman (1973) reported corticosteroid-induced recrudescence of commercial modified live IBRV vaccine given 2½ months previously by the intravenous, intrapreputial and intranasal routes. They indicated that even though commercial inactivated IBRV vaccine injected intramuscularly evoked humoral antibody development, it failed to protect against acute infection or the establishment of chronic infections that could be exacerbated with corticosteroids. Davies and Duncan (1974) reported the use of dexamethasone and ACTH to induce recrudescence of IBRV from cattle experimentally infected by intranasal and intravaginal inoculation. In these experiments virus could be isolated easily from respiratory and genital tissue 2-5 days post treatment. Darcel and Doward (1975) demonstrated that IBRV vaccine strain could be recovered from leukocytes and vaginal secretions of CS treated cattle six weeks after intramuscular injection of the modified live virus vaccine. CS injections have been used to determine that a chronic IBRV infection existed in naturally exposed animals (Bottcher and Mahler 1970; Huck et al. 1973; Gibbs et al. 1975). In each instance the synthetic hormone treatment was used as a tool for determining the presence of virus in animals that were suspected to be chronically infected because they had antibody in their serums.

Davies and Carmichael (1973) studied the effect of dexamethasone and ACTH upon the cell-mediated immune response during primary and recurrent infections. They suggested that suppression of cell-mediated immunity, as measured by lymphocyte transformation in whole blood cultures, occurred during CS induced recrudescence but that adrenocorticotropic hormone (ACTH) and trigeminal neurectomy induced recrudescence without a concomitant immunosuppression. Rouse and co-workers have recently published a series of papers (Rouse and Babiuk 1974a, 1974b, 1975a, 1975b; Rouse et al. 1976; Babiuk and Rouse 1975; Babiuk et al. 1975) concerning their investigations into the immune mechanisms of acute and chronic IBR infections. Their conclusion is that almost all well-known immune mechanisms play a role in the recovery from, the maintenance of, and the recurrence of IBRV infections.

Recent investigations (Smith 1977) have shown that chronic IBR virus infections both from vaccine and field strains could be recrudesced with corticosteroid in virtually all previously infected animals and that altered T lymphocyte response occurred in contrast to failure of recrudescence when B lymphocyte and neutrophil function was altered with cyclophosphamide.

**MATERIALS AND METHODS**

Recent investigations in our laboratory has been focused upon the role of wildlife and the chronic infected swine upon the epidemiology of the
disease. Standard laboratory procedures used in virus isolation, and serological tests such as microtechnique of serum-virus neutralization (SVN), microimmuno-diffusion-in-agar-gel tests (MIDT), radioimmunoassay (RIA), and the enzyme-linked immunosorbent assay (ELISA) are routine and involve only slight modifications of published procedure. We frequently utilize the palpebral skin test (Smith and Mengling 1976) as a diagnostic and research tool.

RESULTS

Attempts to cause recrudescence or recurrent disease in previously PRV infected or vaccinated swine have been uniformly unsuccessful. Corticosteroid therapy and immunosuppressive cobalt gama irradiation exposure failed to cause shedding of the virus in respiratory secretions. We placed 10 (125 #) previously infected and/or vaccinated swine on daily massive intravenous doses of dexamethasone (20 mg) and flumethasone (5.0 mg). Nasal and throat swabs were taken daily from post treatment day 1 through 8 (PTD 1-8) and tested for virus shedding. On PTD-8 the animals were sacrificed and tissue samples from various body organs, including brain, lymph nodes, tonsils, and spleen were tested for presence of virus. No PRV was isolated from nasal swabs or tissues. Hematologic parameters, however, indicated that even such massive doses, as these used, failed to cause evidence of immunosuppression.

Two (2-2½ month) old pigs 60 days after natural acute PRV infection were exposed to a cobalt 60 gamma irradiation source of 14R/min for 21 minutes for a total whole body dose of approximately 300 R. Hematalogic studies showed severe lymphopenia and neutropenia but no PRV virus was isolated from throat and nasal swabs or from tissue of 1 pig necropsied 5 days post treatment.

Studies to determine the potential of wildlife to serve as a reservoir of PRV infection have been conducted. Wild mammals and birds were trapped on or adjacent to farms where PRV had been diagnosed in swine. We have failed to incriminate any species of animal as reservoirs of infection. In all outbreaks careful evaluation of circumstantial evidence indicates that the outbreak was related to importation of infected breeding stock, however PRV antibody has been found in sera of feral cats and oppossums trapped at random from general farming areas (see table 1). Others have discussed the potential of rats (Shope, 1935) and raccoons (Kanitz, 1977) as potential reservoirs. The preponderance of evidence at the present time seems to indicate that wildlife play a minor role, if any, in the epidemiology of PRV infections of swine.

The reliability of various PRV diagnostic tests have been evaluated in our laboratory. We have found that the microimmunodiffusion test (MIDT) (Gutekunst, D. E. et al. 1978), the radioimmunoassay (RIA), the palpebral skin test (Smith 1976) (PST) (see table 2), and the ELISA (Saunders and Clinard 1976) test (below) are dependable methods for PRV diagnosis when compared to the standard serum-virus neutralization...
(SN) test. We have further modified the MIDT test by using a microhematocrit capillary tube for collecting blood from an ear vein prick, which can be done at the same time as the subcutaneous palpebral injection or skin test is conducted. The use of the hematocrit centrifuge and the capillary tube to place the plasma in the agarose pattern reduces the time and expense of the test. By utilizing the same antigen as used in PST we have two simple tests that can be conducted simultaneously and results known within 24 hours of the initial contact with the pig.

Many investigations have criticized the PST because of the potential of creating hypersensitivity reactions and antibody development in swine. Recently we conducted a series of experiments using miniature pigs to determine the immunologic consequence of frequent PST application. In pigs injected with various amounts of effective PST antigen we caused the development of serum neutralizing antibodies when injected on a weekly and biweekly basis. However DTH sensitivity only developed in those pigs that had routine amounts or twice the routine amount of PST antigen injected on a weekly basis. Those animals injected biweekly with 0.1 ml of the PST antigen did not develop DTH sensitivity even though antibody titers did develop (table 3). Further testing must be conducted to determine the frequency and amount of testing that can be done without causing false positives. The same immunologic principles, however, apply to other skin tests such as tuberculosis and histoplasmosis that are being used routinely in veterinary and human medicine.

DISCUSSION

Indisputable scientific evidence to prove the exact nature of PRV infection of swine is lacking. Preliminary evidence may indicate that a true latency exist with the PRV genome integrated into nervous tissue, however, epidemiological facts tend to support the idea that a low level of sporadic virus secretion from chronically infected swine tissues is probable. In reality there is no need to exclude either possibility. The application of sophisticated techniques may answer the question in the future. In the mean time, we should make maximum use of the diagnostic and research tools at hand.

Should eradication of PRV on a national level become a reality, as prudence suggest, then a simple, inexpensive field test of PRV infection in swine would be mandatory. I believe the present technology brought about by recent research efforts has provided us with such a technique. The combination of the palpebral skin test and the microimmunodiffusion in agar-gel using the capillary hematocrit tube modification is a simple inexpensive technique that would provide a high degree of dependability. It is simple enough that the practicing Veterinarian or regulatory personnel could conduct both tests in field situations and diagnose PRV with a high degree of accuracy. The use of both tests simultaneously would provide a highly desirable level of confidence in the test.
Table 1. Microimmunodiffusion test (MIDT) for Pseudorabies virus antibody in Tennessee Wildlife sera.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Number sera tested</th>
<th>Number positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opossums</td>
<td>354</td>
<td>2</td>
</tr>
<tr>
<td>Cats (Feral-domestic)</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>European Wild hogs</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Black bears</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Norway rats</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Skunks</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>533</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Comparison of Microimmunodiffusion (MIDT), radioimmunoassay (RIA), serum-virus neutralization (SN), and the palpebral skin test (PST) for pseudorabies diagnosis in infected swine.

<table>
<thead>
<tr>
<th>number tested</th>
<th>SN titer</th>
<th>MIDT</th>
<th>RIA</th>
<th>PST</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1:4</td>
<td>3/21</td>
<td>4/1</td>
<td>4/1</td>
</tr>
<tr>
<td>5</td>
<td>1:8</td>
<td>5/0</td>
<td>5/0</td>
<td>4/1</td>
</tr>
<tr>
<td>11</td>
<td>1:16</td>
<td>11/0</td>
<td>11/0</td>
<td>10/1</td>
</tr>
<tr>
<td>3</td>
<td>1:32</td>
<td>3/0</td>
<td>3/0</td>
<td>3/0</td>
</tr>
<tr>
<td>1</td>
<td>1:64</td>
<td>1/0</td>
<td>1/0</td>
<td>1/0</td>
</tr>
<tr>
<td>1</td>
<td>1:128</td>
<td>1/0</td>
<td>0/1</td>
<td>1/0</td>
</tr>
<tr>
<td>Totals</td>
<td>27</td>
<td>26/1</td>
<td>24/3</td>
<td>25/2</td>
</tr>
<tr>
<td>% Positive</td>
<td>96</td>
<td>89</td>
<td>93</td>
<td>89</td>
</tr>
</tbody>
</table>

1. number positive over number negative
2. 6 of 10 piglets in this sows litter had died of classical clinical signs of PRV, and others had CNS signs of PRV at the time serum was drawn.
### Table 3. Development of Pseudorabies virus antibody and delayed hypersensitivity in miniature swine following repeated subcutaneous palpebral injections of inactivated pseudorabies virus antigen.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>cell control Ag</td>
<td>- - - - - -</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>II</td>
<td>PRV Ag 0.2 ml</td>
<td>- 6 12 12 16</td>
</tr>
<tr>
<td></td>
<td>Biweekly</td>
<td>- 24 64 64 96</td>
</tr>
<tr>
<td>III</td>
<td>PRV Ag 0.2 ml</td>
<td>- 8 96 128 142 128</td>
</tr>
<tr>
<td></td>
<td>Weekly</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>IV</td>
<td>PRV Ag 0.4 ml</td>
<td>- 4 8 8 16 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 4 64 128 192 128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2+</td>
</tr>
</tbody>
</table>

Days Post Initial Injection

1. each group represents an average response in 2 miniature swine
2. upper entry is average serum-virus neutralization antibody reciprocal dilution titer
3. lower entry in Enzyme-linked immunosorbent assay reciprocal dilution titer
4. degree of reaction to skin test antigen

### REFERENCES


Rager-Zisman, B., and A. C. Allison. 1976. Mechanism of immunologic resistance to herpes


LATENCY IN PSEUDORABIES VACCINATED PIGS

R. A. Crandell, R. E. Mock, and G. M. Mesfin

INTRODUCTION

Latency is a characteristic of the herpes viruses and is probably the most intriguing and troublesome feature of pseudorabies (PR) virus infections in swine. Several weeks after an initial infection in swine, the virus disappears from the nasopharyngeal region and enters into a quiescent stage referred to as a latent state. Recrudescence with virus excretion is believed to occur after certain stimuli or conditions. This phenomenon of latency with reactivation of PR virus has been recognized primarily by epizootiological and clinical evidence but was recently demonstrated experimentally.\(^1\)

The persistence of PR infections in swine herds has long been recognized as a problem for the effective control of the disease. The use of vaccines during the past decade has been ineffective in controlling the spread of virus in Central and Eastern Europe.\(^2\) Although attenuated vaccines have reportedly reduced economic losses, the virus continues to circulate in the infected swine herd. The recent approval and use of PR vaccines in the United States has stimulated our interest in the possible occurrence of latent or persistent infections with virulent virus in vaccinated. Neither the induction of permanent infection in swine with live vaccine virus nor the establishment of a latent infection with field virus in vaccinated swine has been demonstrated.

REVIEW

The mechanism for the persistence of PR virus in an infected environment has been suggested to be either a successive transfer of virus from one animal to another or the establishment of a latent infection.\(^3\) In support of the successive transfer hypothesis, Sabo\(^4\) demonstrated that virulent virus persisted for 18 days in the tonsillar and oropharyngeal mucosa of orally infected pigs. Virus persisted for 20 days in pigs challenged with the virulent virus after immunization with the BUK strain. With these observations, he postulated that there was enough time to infect a number of susceptible animals to maintain circulation of the virus for an unlimited period of time. Because of the persistence of virus in the nasopharyngeal region of vaccinated animals, he concluded that the immunization with live attenuated virus would probably not prevent the circulation of virulent virus and would not eliminate it from a population of pigs.
Several studies have demonstrated that PR virus can persist in clinically healthy pigs in the presence of specific neutralizing antibody. Kojnok\textsuperscript{1} tested a herd and found 3.4\% of the animals to be asymptomatic carriers of the virus. Although these animals were identified as clinically normal, PR virus was recovered from either the nasal cavity or the pharyngeal region or both. Virulent PR virus was shown to persist in a vaccinated herd for 10 months and in a nonvaccinated herd for an 11-month period.\textsuperscript{5} The virus was recovered more consistently from the weanling pigs than the suckling pigs or sows even though all 3 groups had a high proportion of seropositive animals. The percentage of virus-positive pigs in an infected environment as a result of recrudescence from a latent state is unknown.

Although latent infections were demonstrated in hydrocortisone-treated and untreated pigs by culturing tissue explants, virus excretion did not occur from the nasopharyngeal mucosa.\textsuperscript{1} Six-week-old weanling pigs were inoculated orally with virulent virus. Two regimens of hydrocortisone treatment were: 1) 2 pigs each received 5 daily doses of 100 mg IV beginning on postinfection (PI) day 153; 2) 2 pigs each received 5 daily doses IV of 200 mg beginning on PI day 167. The pigs were necropsied 3-4 days after drug treatment. Virus was released from explants derived from both treated and untreated pigs and was demonstrated in the Gasserian ganglia, nasal mucosa, lymph nodes, and tonsils. Neutralizing antibody titers remained fairly high throughout the experiment.

**EXPERIMENTAL**

Twelve PR seronegative weanling pigs were vaccinated with 2 intramuscular (IM) inoculations of modified-live pseudorabies virus vaccine (PR Vac, Norden Laboratories) 2 weeks apart. Three weeks later 6 vaccinates were challenged by intranasal inoculation with 2 x 10\textsuperscript{4} tissue culture infective doses of a field virus. The challenged animals showed a mild clinical response but completely recovered with PR neutralizing antibody titers ranging from 512 to 2048. Approximately 90 days later, each of a pair of these vaccinated-challenged pigs received 100 mg dexamethasone IM for either 4 days, 5 days, or 6 days. The 6 unchallenged vaccinates were treated similarly. Nasal swabs were collected daily for virus isolation during the period of drug treatment. The day after the last dexamethasone injection, each pair of animals was killed and necropsied. Brain, liver, lung, adrenal, spleen, tonsil, and trigeminal ganglia were processed for virus isolation studies.

Pseudorabies virus was isolated from 5 of the 6 vaccinated-challenged animals which were later treated with dexamethasone. The virus was recovered from the tonsillar swabs of 3 animals, the tonsillar tissues of 2 animals and from the lung of 1 animal. Two pigs held their head in a tilted position on the 5th dexamethasone treatment day. Microscopically, a mild to moderate nonsupportive encephalitis was seen in the midbrain and brainstem in 5 of the 6 animals.
Virus was not recovered nor was there clinical signs in the vaccinated nonchallenged group of pigs.

The recovered virus was shown to be virulent by pig inoculation studies. The virus was differentiated from the vaccine virus by its ability to cause paralysis and death in susceptible pigs.

**DISCUSSION**

The results clearly show that virulent PR virus was recovered from vaccinated-challenged pigs after they were treated with dexamethasone, whereas virus was not isolated from vaccinated nonchallenged pigs. The fact that only the pigs in the vaccinated and challenged group yielded virus after dexamethasone is evidence that a latent infection was established.

The validity of the virus isolations was shown by reisolation of virus from duplicate preparations of the same tissues.

Although the vaccinated animals withstood a severe virus challenge, they were infected in spite of the neutralizing antibody. Various investigators have associated the presence of antibody with the disappearance of virus in the nasopharyngeal region. In the present study, challenge virus was not recovered beyond 5 days post challenge. However, after the administration of dexamethasone, virus was isolated from the tonsils in the presence of high levels of antibody.

Latent infection with infectious bovine rhinotracheitis (IBR) virus was established in calves vaccinated with an inactivated IBR vaccine. As demonstrated in this study, that infection was also induced in calves with antibody. That virus was reactivated with synthetic corticosteroid and ACTH treatment 2 to 3 months after intranasal exposure to virulent virus. In another study a feline viral rhinotracheitis (FVR) virus carrier state was established in FVR-vaccinated cats. A combination of dexamethasone trimethylacetate and prednisolone administered intramuscularly was used to induce the virus shedding.

Sabo stated that immunization of pigs with live attenuated virus would probably not stop the circulation of virulent PR virus in an endemic herd. Our results support that opinion. Although no attempt was made to demonstrate virus shedding in this study, the data emphasizes the importance of latent infections in vaccinated pigs as a potential source of virulent virus.

**REFERENCES**


TEST AND REMOVAL PROCEDURES VS VACCINATION FOR
CONTROL AND ERADICATION OF PSEUDORABIES IN MISSOURI

David G. Thawley, B.V.Sc., Ph.D., James C. Wright, D.V.M., M.S.
Robert F. Solorzano, Ph.D.
Department of Veterinary Microbiology, College of Veterinary Medicine
University of Missouri, Columbia, Mo.

Swine and Poultry Staff
USDA-APHIS Veterinary Services, Hyattsville, MD.

SUMMARY

Within the swine industry of the United States, concern over an increase of pseudorabies has prompted studies to evaluate the effectiveness of various control measures which may be employed in a nationwide or statewide eradication program.

This report is a summary of a study to evaluate the effectiveness of a "test and removal" program verses control by vaccination.

Of 10 farms employing "test and removal" procedures, 8 remained free of pseudorabies virus (PRV) seropositives after the initial herd serum-neutralization test. All herds remained "clear" following a second test 30 days later. On each farm when vaccination was employed the PRV serological profile became confused. Serological test results alone could not be used as a monitor of virus movement. In fact, evidence of continued virus circulation post-vaccination existed on each of four farms where vaccination was performed. This would indicate that vaccination does not prevent continued virus circulation in infected herds.

Due to apparent effectiveness of a "test and removal" program, its favorable cost-benefit comparison and problems encountered with vaccination regimes, we feel a "test and removal" means of control should be investigated further as a practice for widespread control.

INTRODUCTION

Concern over an increase of pseudorabies within many states has prompted further study of the epidemiology of this disease and to develop means of control. Since 1974 a marked increase in the reported cases of pseudorabies has occurred. The number of laboratory confirmed cases increased from 125 in 1974, 225 in 1975, 714 in 1976, to 1256 in 1977 (Schnurrenberger, 1978). The number of reported outbreaks in 1978 held relatively constant with the 1977 figure.

Initial sentiment among producers and researchers was for an eradication program without use of vaccine (Crandell, 1975). However,
early in 1977, producer demand changed from control by eradication to control and containment with the use of vaccine.

As recently as 1977, little was known of the prevalence of pseudorabies or its cost to the swine industry in the United States. The disease was increasing rapidly. Straggling financial losses were being experienced by some producers. In 1977, 7,271 sera from slaughter hogs were tested at the national veterinary services laboratory [NVSL]. Of these, 3.53% were pseudorabies positive (Schnurrenberger, 1978).

In the past 12 months as a major problem in many states has resulted from widespread use of swine testing programs. Many herds now being identified as seropositives have no known history of clinical pseudorabies. This is particularly true in Missouri. Identification of these herds has recently caused losses associated with reduced marketability of stock, particularly for seedstock producers. Therefore, there is not only a need to evaluate means of “cleaning up” known infected herds but also to identify effective means of converting seropositive herds to seronegative.

With this background, in late 1977 studies were initiated to determine the feasibility of a control program by attempting to rid herds of pseudorabies seropositive animals. This report covers results from some of these studies performed during the first 18 months.

**MATERIALS AND METHODS**

Fourteen herds containing seropositive breeding stock were selected and placed on one of two control programs.

**Test and Removal of all PRV seropositive stock**

This program is described in Figure 1. An initial PRV serum neutralization test was performed on all breeding stock. All PRV seropositives were removed from the premises within 21 days of the test. The herd was retested approximately 30 days after their removal. Additional seropositive animals were removed. Once a farm had one “clear” test, the herd was retested at approximately 3 month intervals. At all times breeding stock were separated by a minimum of double fencing from weaned growing stock.

**Vaccination of either the breeding herd or offspring**

Vaccination regimes employed on each of 4 vaccinated herds varied with each “on farm” situation. Programs for herds #11 and #12 employed vaccination of the breeding animals only. Vaccine was administered approximately one month before each breeding.

In herd #13 vaccination was initially performed on both breeding and offspring herds. The entire breeding herd was vaccinated approximately 2 weeks prior to farrowing. Pigs were vaccinated at 10 weeks of age. Subsequent vaccination was only performed in the breeding herd 1 month prior to breeding.
In herd #14 vaccination was performed on all breeding stock 1 month prior to breeding. All pigs were vaccinated at 4 weeks of age.

With all vaccination regimes a modified live PRV vaccine* was given by intramuscularly into the hind leg. Following vaccination periodic PRV serological testing was performed to monitor PRV titers in both adults and offspring. In all herds breeding stock were separated from grazing stock following weaning.

Serum samples for testing were stored at \(-20^\circ\text{C}\). Neutralization tests were performed using the piggy back microtiter transfer technique (Catalano et al, 1969). A constant virus-diluted serum method was used with a virus challenge of 100 TCID\(_{50}\) (Hill et al., 1977). A final serum dilution of \(1:256\) was used.

RESULTS AND DISCUSSION

Test and Removal Program

To date, of the 10 swine operations which have employed a test and removal program, all are free of pseudorabies seropositive stock. A total of 709 sows and gilts were initially tested on the farms. Distribution of pseudorabies titers on each of these farms is in Table 1.

After removal of PRV seropositive stock from each premise a period of approximately 30 days elapsed before the second testing. At the second test only on two farms were seropositive stock found. On farm #1, one sow had seroconverted to 1:8 and on farm #9 two sows seroconverted to 1:16 and 1:32 respectively.

At the 150 day test no seroconversions were identified on any of the farms. To date all have completed the 275 and 365 day tests. No new seroconversions have been identified.

From the above data we have sound evidence that "clean-up" of herds containing PRV positive stock most likely can be rapidly achieved by a test and removal program. More severe measures of control such as depopulation were not necessary to achieve these ends.

Vaccination Programs

The sero-epidemiologic data from 4 farms which used vaccination programs are presented below. Because of the unique on-farm situation of each operation, and the need to examine individual data on these farms it is necessary to present the data for each farm separately.

Farm #11

At the time a vaccination program was initiated, this farm was experiencing a mild clinical episode of neonatal mortality and repeat breeding problems. From 100 sows and breeding gilts, 27 were psuedorabies sero-tested before vaccination. Distribution of PRV titers from this test together with that from a repeat test 30 days following

*Norden Laboratories, Lincoln, Nebraska.
vaccination is in Table 2. These sows were then bred and permitted to farrow. One or two offspring from each of the 27 monitored sows were identified and PRV serotested at 3, 8, 10, and 15 weeks of age. Distribution of these titers is in Table 3. From this data it would appear that only 8% of the piglets with passively transferred PRV titers at 3 weeks of age became seronegative by 10 weeks. A number of titers still persisted until 10 weeks of age. All were PRV seronegative by 15 weeks. Approximately 9 months after the original sow testing those of the initial 27 still present on the farm were retested. Data from this test is in Table 3. Most of these sows still possessed moderate pseudorabies titers. However, it could not be determined whether these titers were due to the original challenge, vaccination, or from a field infection in the interval between swine vaccination. We therefore tested the young breeding gilts in the herd which had been raised since the original vaccination program, and were therefore unvaccinated.

Thirty of these gilts were tested. Distribution of their pseudorabies neutralizing titers is in Table 2. The majority of these gilts (83%) possessed pseudorabies neutralizing titers. This would indicate that pseudorabies virus had circulated on this farm since the vaccination program was initiated. This fact would indicate that although the vaccination program may have improved the clinical disease status on the premises, vaccination did not prevent the continued circulation of the virus.

Herd #12

After pseudorabies virus was isolated from dead neonatal piglets in this herd a vaccination program was initiated. PRV titer distribution of a sample of 39 sows at the time of vaccination, 30 days later, and 9 months later are in Table 4. It was impossible for us to determine whether virus circulation had continued within the herd after vaccination or whether the herd was free of virus. One piglet was selected from each tested sow. Its titer was monitored for 15 weeks. Distribution of titers at 3, 8, 10 and 15 weeks are in Table 5. Good passive immunity was initially transferred to the offspring. By eight weeks 58% of the piglets had negative PRV titers. At 10 weeks 86% had negative PRV titers. At 10 weeks of age all 39 pigs had sero-converted to negative.

Nine months after the original sow test each was rebled. PRV titer distribution from this test is in Table 4. At this time we also PRV tested 25 breeding gilts. These had been purchased PRV seronegative since the time of the initial test. They had not been vaccinated. Titer distribution of these gilts is in Table 4. Sixty-eight percent possessed pseudorabies titers. This would indicate continued virus circulation after the initial vaccination program.

Herd #13

A vaccination program was initiated on this farm following substantial livestock losses due to pseudorabies. The entire sow herd (36 sows) was
pseudorabies tested during the outbreak in March 1978. Each was vaccinated at this time. Distribution of PRV titers from this test and a second test 30 days later is in Table 6. Between pre- and post-vaccination tests 5 sows died. Another 24 had either produced litters of dead piglets or those which died within the first 24 hours of life.

By July 1978, only 8 of the original 36 sows remained on the premises. These were again pseudorabies tested. All were seropositive with titers ranging from 1:32 to 1:128. Five were retested in October 1978 [6 months later]. Only 1 had an appreciable drop in titer.

Forty-seven pigs from the sows above were psuedorabies tested and vaccinated in July 1978 [approximately 10 weeks of age]. Titer distribution from this test is in Table 6. Thirty-six of these pigs were retested in October 1978. Only 4 [11%] were pseudorabies seropositive at 22 weeks of age and 14 [38%] had converted from seropositive to seronegative. Prevaccination titers in these animals were probably from passively transferred antibodies. Effectiveness of the vaccination program in the pigs could be seriously questioned. In December 1978, the pigs were again retested. Twenty-nine of 35 [82%] were pseudorabies seronegative while 4 possessed titers of 1:2, one at 1:4, and 1 at 1:8. This indicates that pseudorabies virus was likely circulating within this herd. It could be easily conceived that the few low titers present were due to vaccination carried out at ten weeks of age.

However, in December 1978, we also sero-tested the 8 remaining sows. Titers of these sows remained virtually constant from the original test at the time of the outbreak until December 1978. Therefore, we had no indication that virus had been recently circulating within this herd. Implications were that restocking of this herd with PRV negative stock could be safely carried out. However, in October 1978 and again in December, each animal was tonsil swabbed and a virus culture performed. Pseudorabies virus was isolated in October from 2 of the 9 adult sows. During the December test 3 of the 8 adult sows tested positive. Pseudorabies virus was still present in this herd. Vaccination had not resulted in its eradication.

Farm #14

On this farm a 30% baby pig loss was experienced during January 1978. Pseudorabies virus was isolated from dead pigs. Forty-five of 120 sows were psuedorabies tested 2 months following the outbreak. All 45 were PRV seropositive. Distribution of titers is in Table 7. Adult vaccination would not assist on this farm. Most titers were in excess of that which we would expect to achieve by vaccination. However, a vaccination of all pigs at 4 weeks of age was recommended and performed. Seventeen sows were retested 6 months later. All 17 were still pseudorabies seropositive. Two of the 17 sows had titers of a fourfold decrease. The titer of one sow increased from 1:16 to 1:64 [fourfold increase].

Sixty-five piglets were tested at 3 weeks of age. Distribution of PRV
titers are in Table 8. The piglets were vaccinated at 4 weeks of age. Fifty-one of these were retested at weaning [8 weeks]. Twenty-three [45%] had a twofold or greater decrease in titer indicating a lack of response to vaccination.

Eighteen of the 51 were retested at 4 to 6 months after weaning. Six had a twofold or greater increase in titer from the previous test. Five had also converted from a seronegative to titers of 1:64, 1:32, 1:16, 1:8 and 1:4 respectively. Distribution of these titers is in Table 7. One of these animals had a titer of 1:4 and 2 had 1:16. Pseudorabies virus was isolated from a tonsil swab taken from one of these gilts at this time.

GENERAL DISCUSSION

From results to date 10 herds on a “test removal” program have become free of pseudorabies seropositive animals. However, successful outcome of the “test and removal” program contrasts with a confusing epidemiologic picture found in vaccinated herds. Some evidence of continued virus circulation after vaccination exists in each of four herds. Vaccination stopped clinical losses in each of these herds. However, it seems that vaccination must be continued on the long term to avoid new outbreaks of clinical disease. Use of vaccine has considerably confused pseudorabies serologic profiles within these herds. This makes it difficult to monitor virus movement within these herds.

Poor response to vaccination of pigs from immune sows is of interest. Even at 10 weeks of age very little antibody response was observed in these pigs. Many vaccinates did not possess detectable PRV titers at vaccination. Reasons for this are not known. More research is needed to study both humoral and cellular immunoepidemiology of this disease. In the meantime use of vaccine in pigs from immune sows should be given careful consideration.

Farms were quickly “cleared” of PRV seropositives by a “test and removal” plan. With problems encountered in vaccination regimes, we feel test and removal should be investigated further as a promising practice in widespread control programs.

With PRV prevalence data, we can only hypothesize a cost benefit analysis of each of two control approaches. If we assume the following:

a. 3.5% of U.S. swine farms carry PRV positive stock. Fifty percent of breeding stock are PRV positive in these positive herds.

b. On an average two tests are required to clear a herd of PRV positive stock.

c. The interval between tests is approximately 45 days until a herd has one clear test. Once free of PRV seropositives, a farm is tested semi-annually.

d. The average cost of PRV testing is $6.00 per head per test.

e. Cost of vaccination is performed at each breeding. Each pig is
vaccinated at 6 weeks of age.

f. Sow vaccination is performed at each breeding. Each pig is vaccinated at 6 weeks of age.

g. Each sow produces on average 15 pigs per year.

Over the initial 5 years for 100 herds with an average of 100 sows per herd approximate costs of a test and slaughter program would be:

### 3.5 Infected Herds

**Year 1**

- 1,400 PRV tests @ $6.00* each = $8,400
- Replacement costs for 175 positive sows @ $50.00** each = 8,750

**Years 2-5**

- 2,800 test at $6.00 each = 16,800

Subtotal = $33,950

### 96.5 Uninfected Herds

**Years 1-5**

- 96,500 test at $6.00* = 579,000

Total cost of 5 year “test and removal” program = 612,950

* Cost of sample collection, serum neutralization testing and reporting.
** Cost differential between sale price of seropositive sow and purchase price of replacement.

Estimate costs of a five year PRV vaccination program for 100 farms with 100 sows/herd:

**Years 1-5**

- Sow vaccination — 100 sows per herd @ $1.00/head = $100.00
- Offspring vaccination — 15 offspring/sow/year @ $1.00 each = 750.00

Total 5 year cost of vaccination program = $850.00

In conclusion, over the initial 5 years a widespread vaccination program would have direct costs approximately 37% greater than those generated by a test and removal program. Additional benefits associated with a “test and removal” program include the likelihood that continued annual vaccination costs would be avoided. In the long term, nationwide eradication of this disease may be achieved. However, the cost of a vaccination program would be reduced considerably if only sow vaccination is performed. It is our opinion that while current prevalence of pseudorabies within the United States is low compared with that in Europe we should be giving strong consideration to initiation of a test and removal program with an ultimate goal of nationwide eradication.
Table 1

Distribution of PRV Titers by Farm Prior to Removal of Seropositive Swine

<table>
<thead>
<tr>
<th>PRV Titer</th>
<th>neg.</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
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<th>1:32</th>
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</tr>
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<td>1</td>
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</tr>
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<td>-</td>
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<td>6</td>
<td>6</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>152</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>63</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

528 18 14 16 19 36 41 37
Table 2

PRV Titer Distributions From 27 Sows and Unvaccinated gilts on Farm #11

<table>
<thead>
<tr>
<th>PRV Titer</th>
<th>Before Vaccination</th>
<th>30 days post Vaccination</th>
<th>9 month post Vaccination (10/19/78)</th>
<th>Unvaccinated gilts (11/21/78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg.</td>
<td>13 (48%)</td>
<td>2 (7%)</td>
<td>6 (23%)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>1:2</td>
<td>1 (4%)</td>
<td>6 (22%)</td>
<td>5 (19%)</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>1:4</td>
<td>3 (11%)</td>
<td>2 (7%)</td>
<td>5 (19%)</td>
<td>8 (26%)</td>
</tr>
<tr>
<td>1:8</td>
<td>3 (11%)</td>
<td>7 (25%)</td>
<td>4 (15%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>1:16</td>
<td>4 (15%)</td>
<td>5 (18%)</td>
<td>6 (23%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>1:32</td>
<td>2 (7%)</td>
<td>5 (18%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:64</td>
<td>1 (4%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:128+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Total tested 27 27 26 30

*Unvaccinated gilts tested 9 months after vaccination of all other breeding stock on the farm.
Table 3

PRV Titer Distribution in the Offspring of Herd #11 at 3, 8, 10, and 15 Weeks of Age

<table>
<thead>
<tr>
<th>Titer</th>
<th>3 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg.</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>1:2</td>
<td>5</td>
<td>14</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>1:4</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>1:8</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>1:16</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:32</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:64</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:128</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 4

PRV Titer Distribution of 39 Sows and a Sample of Unvaccinated Gilts on Farm #12

<table>
<thead>
<tr>
<th>PRV titer</th>
<th>Prevaccination test</th>
<th>30 days post vaccination</th>
<th>9 month post vaccination</th>
<th>Unvaccinated*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12/7/78</td>
</tr>
<tr>
<td>Neg.</td>
<td>23</td>
<td>4</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>1:2</td>
<td>1</td>
<td>14</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>1:4</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>1:8</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>1:16</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>1:32</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1:64</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1:128</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total # tested</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>25</td>
</tr>
</tbody>
</table>

*Unvaccinated gilts tested 9 months after vaccination of all other breeding stock on the farm.
Table 5

PRV Titer Distribution in a Sample of Offspring in Herd #12 at 3, 8, 10 and 15 Weeks of Age

<table>
<thead>
<tr>
<th>PRV titer</th>
<th>3 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg.</td>
<td>8</td>
<td>23</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>1:2</td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>1:4</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>1:8</td>
<td>6</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:16</td>
<td>13</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:32</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:64</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:128+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total tested</strong></td>
<td><strong>39</strong></td>
<td><strong>39</strong></td>
<td><strong>39</strong></td>
<td><strong>39</strong></td>
</tr>
</tbody>
</table>
Table 6

PRV Titer Distribution of Sows and Pigs of Farm #13

<table>
<thead>
<tr>
<th>PRV Titers</th>
<th>Prevaccination 4/22/78</th>
<th>30 days post vaccination 5/24/78</th>
<th>Age 10 weeks 7/12/78</th>
<th>Age 22 weeks 10/6/78</th>
<th>Age 31 weeks 12/11/78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg.</td>
<td>0 -</td>
<td>0 -</td>
<td>22 (47%)</td>
<td>32 (89%)</td>
<td>29 (83%)</td>
</tr>
<tr>
<td>1:2</td>
<td>0 -</td>
<td>0 -</td>
<td>14 (30%)</td>
<td>3 (8%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>1:4</td>
<td>0 -</td>
<td>0 -</td>
<td>4 (8%)</td>
<td>-</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>1:8</td>
<td>1 (3%)</td>
<td>0 -</td>
<td>2 (4%)</td>
<td>-</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>1:16</td>
<td>6 (17%)</td>
<td>0 -</td>
<td>3 (6%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:32</td>
<td>8 (22%)</td>
<td>2 (6%)</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
<td>-</td>
</tr>
<tr>
<td>1:64</td>
<td>16 (44%)</td>
<td>14 (45%)</td>
<td>1 (2%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:128</td>
<td>5 (14%)</td>
<td>13 (42%)</td>
<td>1 (2%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:256+</td>
<td>0 (0%)</td>
<td>2 (6%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>31</td>
<td>47</td>
<td>36</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 7

PRV Titer Distribution of Sows
and Selected Pigs on Farm #14

<table>
<thead>
<tr>
<th>Sow Herd</th>
<th>3 weeks Pre-Vaccination</th>
<th>8 weeks Post-Vacc. (weaning)</th>
<th>4-6 months Post-Vacc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer</td>
<td>4/6/78</td>
<td>10/19/78,</td>
<td></td>
</tr>
<tr>
<td>Neg.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>-</td>
<td>1 (6%)</td>
<td>15 (29%)</td>
</tr>
<tr>
<td>1:4</td>
<td>4 (9%)</td>
<td>7 (41%)</td>
<td>27 (53%)</td>
</tr>
<tr>
<td>1:8</td>
<td>10 (22%)</td>
<td>1 (6%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>1:16</td>
<td>9 (20%)</td>
<td>10 (15%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>1:32</td>
<td>17 (38%)</td>
<td>4 (23%)</td>
<td></td>
</tr>
<tr>
<td>1:64</td>
<td>3 (7%)</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>1:128</td>
<td>2 (4%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1:256+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

15 (29%)  5 (28%)
7 (41%)  3 (17%)
1 (6%)  3 (6%)
1 (6%)  1 (6%)
1 (6%)  1 (6%)
1 (6%)  1 (6%)
51  18
Figure 1

Test and Removal Program

Initial Test

PRV -ves

If herd test +ve

Removal within 21 days of test

PRV -ves

30 days following removal

PRV -ves

Herd -ve

3 month test

Herd -ve

3 month retest

PRV +ves

Removal within 21 days of test

PRV -ves
REFERENCES


REPORT OF THE COMMITTEE
ON TRANSMISSIBLE DISEASES OF SWINE

Chairman: Lowell W. Hinchman, Indianapolis, Ind.

Co-Chairman: Robert F. Behlow, Raleigh, N.C.


The Committee on Transmissible Diseases of Swine met on October 30, 1979, at 1:30 P.M. (16 members present)

Dr. A. R. Smith presented a paper on epererythrozoonosis discussing symptoms and diagnostic procedures. The use of an indirect hemagglutination procedure as an adjunctive serological test was described and it indicated that reactions in dilutions over 1:40 were useful as a herd screening procedure. Females in the breeding herd are the best subjects for this procedure and animals under 10 weeks of age are not reliable test subjects.

Dr. D. L. Harris gave a status report on Swine Dysentery and indicated that asymptomatic swine could shed Treponema hydysenteriae for 70 days after exposure. A microagglutination test has been utilized in clearing 4 out of 5 herds quarantined for this disease in Iowa. It was recommended that swine dysentery research be directed toward:

1. Establishing the true incidence of the disease
2. Determining the economic impact on the industry if currently used drugs are withdrawn from the market
3. Further development of serological detection procedures.

The subcommittee on Pseudorabies met on October 29, 1979, at 1:30 P.M. and its report will be published in the proceedings. Dr. John Kluge discussed the subjects offered at that time and indicated that two research groups have shown that vaccination will not prevent a persistent or latent infection. It was also noted that a latent infection had been known to persist for 13 months.

Concern relative to the absence of a prescribed program for eradication was indicated by members of the swine industry as well as available information relative to the economic impact of the disease at this time. As a result of this discussion, a resolution was constructed and approved by the Transmissible Swine Disease Committee and will be submitted to the Resolution Committee. The resolution is designed to encompass four areas of concern:
1. Additional funding of pseudorabies programs

2. The immediate creation of an ad hoc committee by APHIS representing scientists and all aspects of the swine industry to conduct a feasibility and cost study

3. USDA funding for pseudorabies support continuation

4. Proper dissemination of information relative to the advantages and disadvantages of vaccine usage in pseudorabies programs.

Dr. Howard Hill discussed parvoviruses stating that there was a widespread occurrence in swine. It was also noted that killed vaccines have been used successfully in some herds, however, there are no federally licensed vaccines available at this time. It is therefore apparent that there is a need for a product to be produced that can be transported interstate.

Dr. Robert Glock discussed proliferative enteritis and indicated widespread occurrence of the disease. It evinces a low morbidity and is not considered to be a systemic condition. Early treatment is indicated, however, it was cautioned that a subtherapeutic drug level usage would not be productive.

Dr. C. A. Mebus gave an update on African Swine Fever and indicated that continued surveillance is being maintained and containment of the virus had been adequate.

1979 PSEUDORABIES SUBCOMMITTEE REPORT

Chairman: J. P. Kluge

The subcommittee met from 1:30 - 5:00 p.m. October 29, 1979, with approximately 50 guests and the following members in attendance: D. P. Gustafson, M. H. Lang, W. C. Stewart, H. T. Hill, J. A. Downard, C. L. Kanitz, R. E. Hall, D. G. Thawley, C. J. Mare, R. D. Glock, and L. W. Hinchman.

The informational program consisted of the following seven presentations:


Field results to date with four methods of eliminating PRV from swine herds were summarized. Vaccination is preventing clinical signs in many herds but some herds continue to experience reduction in litter size and 10 - 20% of pigs have a 3 - 4 week retardation of growth. In one herd that has maintained vaccination of sows and pigs the virus continues to cycle as evidenced by a positive PRV isolation rate of 19% at slaughter. In FY 1980 selected field trial herds will continue to be monitored. Studies on cell mediated immunity and infection of swine embryos will continue to be supported. A pilot PR eradication project involving four town-
ships in Michigan will continue and possibly a second project started in another state. Support will also continue for epidemiologists at Purdue and Iowa State University.

2. Epidemiologic monitoring following vaccination and test and removal means of pseudorabies control in swine herds. D. G. Thawley and J. C. Wright, University of Missouri.

Success was reported in lowering PR incidence, under Missouri conditions, by test and removal of positive animals and vaccination. There have been problems in some herds in that vaccinated pigs do not seroconvert. In general, vaccination has caused the following problems:

A. Virus continues to circulate in herds.
B. Seroconversion from vaccination has complicated regulatory decision making.
C. Vaccination has created a false sense of security for procedures.


Epidemiology of disease in Iowa and research in Europe on persistent PVR infection was reviewed. Work at ISU has shown that PRV could be recovered by tissue fragment and co-culture techniques 3 weeks - 13 months post inoculation. PRV was recovered from nasal cavity of a sow on the 3rd post farrowing day, seventeen months after inoculation. The sow had an SN titer of 1:32.


PRV-DNA was demonstrated in 58% of vaccinated pigs two months after challenge by nucleic acid hybridization studies. 30% of the field samples checked from problem herds were also positive. These animals were considered as potential shedders.

5. Progress report on studies of the susceptibility of fertilized swine ova to pseudorabies virus. D. P. Gustafson et al, Purdue University.

On the basis of preliminary results it appears that PRV was spread to sows from PRV inoculated embryos, however, trials are being repeated at this time for confirmation. Similar results to those at ISU have been obtained using a modified co-culture technique to demonstrate persistent PRV infection in pigs. An indirect HA test utilizing tanned sheep erythrocytes is being developed. Toxic serum does not interfere with the test and results are available in two hours.


Pigs were vaccinated with two doses of or a combination of commercially available MLV and/or killed PRV vaccines. Pigs were
challenged with virulent PRV. Three and four months after challenge treatment of the pigs with a combination of ACTH and dexamethasone resulted in PRV recrudescence in a high percentage of the vaccinated pigs.


Pigs were vaccinated with two doses of PR Vac (Norden Laboratories). Three weeks later pigs were challenged by intranasal inoculation of field PRV. Three months later dexamethasone was administered to the pigs for 4-6 days. Field PRV was recovered from tonsillar swabs or tissues from 5 of the 6 vaccinated-challenged animals that were later treated with dexamethasone.

Discussion followed on the presentations.

Dr. Lang proposed a change in Federal regulations concerning progeny testing of offspring from pseudorabies controlled herds. Various modifications were considered and no action was taken.

Mr. E. Juhl reported the following regulation was recently passed by the National Association of Swine Registries - "Realizing that the purebred seedstock segment of the swine industry is highly important, and that the disease pseudorabies is of great concern to all swine producers, we believe it is time for a united effort of all seedstock producers for a uniform set of regulations for all segments of the industry."

A resolution was prepared concerning research and program funding, creation of an ad hoc committee by the USDA to conduct a fiscal and feasibility study of an eradication program and the dissemination of information relative to advantages and disadvantages of vaccine use. This resolution will be presented to the Transmissable Disease Committee.
MYCOBACTERIUM AVIUM SEROTYPE 4 INFECTION IN SWINE

Charles O. Thoen, D.V.M., Ph.D. and William J. Owen, D.V.M.
Department of Veterinary Microbiology and Preventive Medicine
College of Veterinary Medicine, Iowa State University
Ames, Iowa 50011

and

Elmer M. Himes, D.V.M.
National Veterinary Services Laboratories
Veterinary Services
Animal Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa 50010

SUMMARY

Six swine experimentally inoculated with Mycobacterium avium serotype 4 had microscopic granulomas in the cervical or mesenteric lymph nodes at necropsy 80 days later. Tuberculin tests and enzyme linked immunosorbent assay (ELISA) responses were evaluated at 10 weeks after the pigs were inoculated. Positive responses were observed on both tests in inoculated pigs, whereas results in noninoculated pigs were negative. Positive ELISA reactions were obtained on sera from 2 pigs exposed to pigs given 50 mg M. avium serotype 4 orally; suspicious tuberculin skin test responses were observed in each of the pigs at 10 weeks. A comparison of ELISA reactions and tuberculin skin test responses was made in swine naturally infected with M. avium serotype 4.

INTRODUCTION

Mycobacterial lymphadenitis in swine in the United States is most commonly caused by Mycobacterium avium serotypes 1 and 2, the organisms responsible for tuberculosis in chickens. More recently a new dimension of swine tuberculosis has been recognized in large confinement herds in various geographical regions of the country. Mycobacterium avium serotypes 4 and 8 have been associated with these epizootics. The importance of these disease outbreaks in swine has been emphasized by the dramatic economic losses resulting from condemnations and necessary processing of carcasses with evidence of extensive disease. Similar infections have been reported in swine in other countries.

No definitive information is available on the source(s), mode(s) of transmission or on pathogenicity of M. avium serotype 4 in pigs. However, some reports suggest that the use of sawdust in farrowing stalls or growing pens has been associated with disease in slaughter swine from which serotypes of M. avium other than types 1 and 2 have been isolated. The isolation of these organisms from soil, water and sawdust and the importance of insects in the transmission of these
infections in swine has been investigated.\textsuperscript{1,3,6,8,10,12,14,15,17,18,20,21,28,29}

The objectives of the present investigations were (1) to obtain information on the occurrence of lesions in tissues of swine given \textit{M. avium} serotype 4 orally and intravenously (2) to determine if pigs exposed to swine given \textit{M. avium} serotype 4 become tuberculin positive (3) to compare the tuberculin test results in pigs experimentally and naturally exposed to \textit{M. avium} serotype 4 and (4) to evaluate an enzyme-linked immunosorbent assay (ELISA) for detecting antibodies in swine infected with serotype 4.

**MATERIALS AND METHODS**

**Microorganisms** — \textit{Mycobacterium avium} serotype 4 isolated from tissues of a pig in Iowa with granulomatous lesions in the mesenteric lymph nodes was used in the inoculums. The inoculums were prepared from a 10-day subculture of \textit{M. avium} serotype 4 in Dubos' liquid medium with oleic acid albumin complex. The cells were harvested by centrifugation at 1000 x g for 20 minutes, weighed and suspended in sterile Butterfield buffer (pH 7.2). The viability of the cell suspension was determined by subculture on appropriate mediums; purity of the inoculums was determined from smear specimens stained with carbol fuchsin and gram's stain. The killed cell suspension used for ELISA was prepared from cells autoclaved at 123°C for 20 minutes.

**Animals** — Ten specific pathogen free pigs (10 weeks of age, weighing 18-22 Kg) were distributed among 5 groups (Table 1). In groups I and II the inoculum was deposited in the caudal area of the pharynx using a syringe. The group III pigs were inoculated intravenously with 5.0 mg of the inoculum. Group IV pigs were exposed to the Group II pigs by pen contact starting on day 1.

**Tuberculin Skin Tests** — Preinoculation tuberculin skin tests were made in 1 pig in each group (Table 1), by injecting 0.1 ml of \textit{M. avium} PPD intradermally in the dorsal surface of the left ear and 0.1 ml of avian old tuberculin (OT) in the right ear. Tuberculin tests were conducted on all pigs at 10 weeks post inoculation; skin test sites were observed at 48 hours for the presence of erythema, induration and necrosis.

**Enzyme-linked Immunosorbent Assay** — Serum samples were collected for ELISA from the experimentally inoculated pigs at 10 weeks after inoculation. Serums from 11 pigs naturally exposed to \textit{M. avium} serotype 4 were collected at 8 weeks of age. Serums from 12 tuberculin-positive pigs were collected at slaughter.

ELISA was conducted using heat-killed cells of \textit{M. avium} serotype 4 and \textit{M. avium} purified protein derivatives by a procedure described previously.\textsuperscript{48} The reactions were stopped at 15 minutes using .05 ml of 0.1 M hydrofluoric acid. The color developed by the reaction was graded from 1 to 4 for intensity of green color. No color change (0) was considered negative.
Microscopic Examinations — Tissue sections were made and stained with hematoxylin and eosin; replicate sections were stained with auramine-O.\textsuperscript{33}

Mycobacteriologic Examinations — Cervical and mesenteric lymph nodes were collected and placed in separate jars containing a saturated solution of sodium borate.\textsuperscript{37} The tissues were processed using 2\% NaOH and inoculated onto culture mediums as described previously.\textsuperscript{30,31} The inoculated mediums were incubated at 37°C and observed each week for growth. Acid fast isolates were identified by biochemical tests and by serological procedures.\textsuperscript{19,32,37}

RESULTS

Tuberculin skin test responses observed at 10 weeks after inoculation are tabulated (Table 2). Group I and II given 5 and 50 mg of \textit{M. avium} serotype 4 respectively and group III pigs given 5 mg of cells intravenously were positive on tuberculin-skin tests. Necrosis was apparent at the avian O.T. injection site in 2 animals; no necrosis was observed at the PPD injection sites. Suspicious tuberculin skin test responses were observed to both avian PPD and O.T. in each of the 2 pigs exposed to the Group II pigs.

The results of ELISA on sera of swine in each of the 5 groups of animals are shown in Table 3. Positive ELISA reactions were observed at serum dilutions of 1:40 for each of the pigs experimentally inoculated with \textit{M. avium} serotype 4 and in 2 pigs exposed by pen contact to the Group II animals. Either \textit{M. avium} serotype 4 cells or \textit{M. avium} PPD were suitable antigens. No ELISA reactions were observed in sera of noninfected controls.

Microscopic granulomas were observed in the cervical or mesenteric lymph nodes of each of the pigs inoculated orally with 5 mg or 50 mg and in 2 pigs inoculated intravenously with 50 mg of \textit{M. avium} or serotype 4 at 80 days post inoculation. The lesions were characterized by a dense connective tissue capsule which surrounded accumulations of macrophages and granulocytes (Figure 1). A central area of caseation necrosis was present. Numerous acid-fast bacilli were observed in separate sections stained with auramine-O.

Microscopic examination of sections of liver of pig 228 revealed the presence of focal granulomas characterized by Langhans'-type giant cells (Figure 2). Some caseation necrosis was present. Acid-fast bacilli were present in appropriately stained sections.

Microscopic granulomas were observed in the cervical lymph node of pig 232 exposed to Group II pigs. Acid-fast bacilli were observed in a replicate section. No lesions were observed in either of the noninfected controls.

\textit{Mycobacterium avium} was isolated from cervical or mesenteric lymph nodes of each of the 6 pigs inoculated experimentally and the 2 pigs
exposed to the Group II pigs. Serologic studies revealed that isolates shared antigens with *M. avium* serotype 4 and serotype 8.

The results of the tuberculin skin tests on pigs from tuberculin-positive sows farrowed on contaminated sawdust, straw or wood shavings are shown in Table 4. No positive tuberculin responses were observed in any of the pigs at 26 and 57 days. Eight of 11 pigs maintained on contaminated sawdust were positive on tuberculin tests conducted at 112 days. One of 7 pigs maintained on straw was positive and 3 of 9 pigs kept on wood shavings had positive tuberculin skin test responses at 112 days. ELISA tests conducted on serum collected from each of the 11 pigs maintained on contaminated sawdust are tabulated (Table 5). Positive reactions were observed at 8 weeks in 7 of the 11 pigs at serum dilutions of 1:10; 4 pigs were positive at serum dilutions of 1:20.

The ELISA responses on 12 tuberculin-positive slaughter pigs and 2 tuberculin-negative pigs are shown in Table 6. Positive reactions were observed in the 12 tuberculin-positive animals using heat-killed cell antigen at a serum dilution of 1:20 following 30 minutes incubation. No ELISA reactions were observed in the noninfected controls.

**DISCUSSION**

*Mycobacterium avium* serotype 4 has been isolated from swine originating in different geographical areas of the United States. The economic importance of serotype 4 infections in large confinement herds is emphasized by the finding that 5 to 30% of the pigs in the herd may have tuberculous lesions at slaughter. Federal regulations require that carcasses with lesions at two or more primary sites must be processed at 76.6°C for 30 minutes thereby greatly reducing the value and limiting its use to canned products.

ELISA have recently been evaluated for detecting mycobacterial antibody in the serum of swine and chickens infected with *M. avium*. The practical value of ELISA as a routine test will depend on the reliability of detecting swine infected with all the reported *M. avium* complex serotypes. There is a need to validate the association of delayed type tuberculin skin tests and *in vitro* ELISA in market swine. An advantage of the ELISA is that it provides a rapid test which requires only one handling of animals for the collection of serum.

Congenital infections caused by *M. avium* have been reported in swine. However, convincing evidence for pig to pig transmission is not available. There is no definitive information on the properties of mycobacteria responsible for virulence.

Tuberculin skin tests have been used in the diagnosis of tuberculosis in swine; however the reliability of these tests in the diagnosis of diseased animals is not established. Either *M. avium* PPD or Avian O.T. provided satisfactory delayed type responses in this study in experimentally infected pigs. Tuberculins for use in testing swine, as in
cattle, should be standardized under conditions in which they will be used. It should be emphasized that only suspicious reactions were observed in the 2 pigs exposed by pen contact to experimentally infected animals; however, ELISA reactions were positive on each animal.

Figure 1.
Section of cervical lymph node obtained from pig 227 at necropsy 80 days after oral inoculation of 50 mg of *Mycobacterium avium* serotype 4. Tubercle with fibrous connective tissue encapsulating a central area of lymphocytes and granulocytes. Caseation necrosis is present. Hematoxylin and eosin x 100. *M. avium* serotype 4 was isolated on culture.
Section of the liver obtained from Pig 228 at necropsy 80 days after oral inoculation of 50 mg of *Mycobacterium avium* serotype 4. Foci of accumulations of epithelioid cells with Langhans'-type giant cells are present. Hematoxylin and eosin x 250. Acid-fast bacilli were observed on separate section stained with auramine-0.

**Table 1.** EXPERIMENTAL INFECTIONS OF SWINE WITH *M. AVIUM* SEROTYPE 4. *

<table>
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<tr>
<th>GROUP</th>
<th>PIG NO.</th>
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<th>INOCULUM</th>
<th>ROUTE</th>
<th>SKIN TEST</th>
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<td>I</td>
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</tr>
<tr>
<td></td>
<td>226</td>
<td>Sero. 4</td>
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<td>Pharynx</td>
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</tr>
<tr>
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<td>M. avium</td>
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<tr>
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<td>Pharynx</td>
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<td>229</td>
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<tr>
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* Shared antigenic determinants with *M. avium* serotype 8.
Table 2. TUBERCULIN SKIN TESTS MADE AT 10 WEEKS IN PIGS INOCULATED WITH M. AVIUM SEROTYPE 4.

<table>
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*Millimeters Swelling

KEY: N = NECROSIS
NR = NO REACTION OBSERVED

Table 3. RESULTS OF ENZYME-LINKED IMMUNOSORBENT ASSAY ON SWINE INOCULATED WITH M. AVIUM SEROTYPE 4.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PIG NO.</th>
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<th>M. AVIUM CELLS*</th>
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*PREPARED FROM CULTURE OF M. AVIUM SEROTYPE 4 WHICH SHARED ANTIGENS WITH SEROTYPE 8.
### Table 4. TUBERCULIN SKIN TEST RESPONSES IN PIGS* FROM TUBERCULIN - POSITIVE SOWS FARROWED ON CONTAMINATED SAWDUST, STRAW AND WOOD SHAVINGS.

<table>
<thead>
<tr>
<th>DATE OF TUBERCULIN TEST</th>
<th>11 PIGS ON SAWDUST</th>
<th>7 PIGS ON STRAW</th>
<th>9 PIGS ON WOOD SHAVINGS</th>
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<tr>
<td>2-1-79</td>
<td>11 Neg.</td>
<td>7 Neg.</td>
<td>9 Neg.</td>
</tr>
<tr>
<td>3-1-79</td>
<td>11 Neg.</td>
<td>7 Neg.</td>
<td>9 Neg.</td>
</tr>
<tr>
<td>4-24-79</td>
<td>8 Pos.</td>
<td>1 Pos.</td>
<td>3 Pos.</td>
</tr>
<tr>
<td></td>
<td>3 Neg.</td>
<td>6 Neg</td>
<td>6 Neg</td>
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</table>

* Born on 1/5/79

### Table 5.

RESULTS OF ENZYME-LINKED IMMUNOSORBENT ASSAY ON SERUM OF 11 PIGS FROM A TUBERCULIN POSITIVE SOW.

<table>
<thead>
<tr>
<th>PIG NO.</th>
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*M. AVIUM SEROTYPE 4 WAS USED AS THE ANTIGEN*
Table 6. Comparison of enzyme immunoassay reactions in serums of 12 tuberculin-positive swine and 2 tuberculin negative swine using heat-killed cells of *M. avium* and purified protein derivative tuberculin of *M. avium*.

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Heat killed cells of <em>M. avium</em> Serotype 4</th>
<th>Purified Protein Derivative Tuberculin of <em>M. avium</em></th>
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<tr>
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REFERENCES


20. Szabo, I, Tuboly, S, Szeky, A: Swine lymphadenitis due to Mycobacterium avium and...


EFFECTIVENESS OF KILLED VIRULENT CELL VACCINE AGAINST PARATUBERCULOSIS

A. B. Larsen, DVM and R. S. Merkal, PhD

The National Animal Disease Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, P.O. Box 70, Ames, IA 50010.

SUMMARY

The efficacy of a killed vaccine prepared from virulent Mycobacterium paratuberculosis bacilli was studied for 5 years in a herd of cattle infected with the disease. During the study, the cattle were kept under observation for signs of disease and tested with allergens to determine the effect of the vaccine on tuberculin hypersensitivity. Fecal specimens were cultured periodically, and tissues were obtained at time of slaughter. Adverse effects from the vaccine were not observed except for the persistent nodules that developed at the vaccination site. Although the vaccine reduced losses from the disease and hypersensitivity resulting from it did not confuse the tuberculin test, it does not appear to have any advantage over a vaccine prepared from killed avirulent cells of M paratuberculosis.

A killed bacterial vaccine prepared from a laboratory-adapted, avirulent (nonmycobactin-requiring) stock culture of Mycobacterium paratuberculosis effectively reduced losses from paratuberculosis (Johne's disease) in cattle when used in a field trial.¹

Results in the literature had shown that a killed bacterial vaccine against tuberculosis prepared from a virulent culture of Mycobacterium tuberculosis contained antigenic components that a bacterin prepared from an avirulent culture of M tuberculosis did not.³ Therefore, we postulated that killed bacterins against mycobacterial diseases prepared from virulent cultures might be more effective than those prepared from avirulent cultures.

The purpose of the present study was to determine whether a killed bacterial vaccine prepared from virulent culture of M paratuberculosis would be a better immunizing agent than a vaccine prepared from an avirulent stock culture that had been tested previously.¹

MATERIALS AND METHODS

Experimental Cattle

Calves used in this study were from a herd consisting of 35 adults. This herd had experienced severe losses from paratuberculosis. The owner agreed to notify us when each heifer calf was born and to sell ex-

The authors thank Dr. Terry Jackson (NADC) for making the histopathological examinations.

No endorsements are implied herein.
The animals used in this study were limited to heifer calves born during the study. The calves were divided into 3 groups by a randomization sequence. The procedure provided approximately equal numbers of calves in each group. Calves in group 1 were given the vaccine prepared from avirulent cells, calves in group 2 were given the vaccine prepared from virulent cells, and calves in group 3 were unvaccinated controls. All calves were identified by a tattoo in the ear, and the principals were vaccinated before they were 30 days old. The vaccine preparations were killed by heating 5 minutes at 121°C. Each dose of vaccine consisted of 25 mg (dry wt) of *M. paratuberculosis* in 0.5 ml of mineral oil administered subcutaneously in the brisket. Calves were exposed by natural contact with infected adults in the herd.

**Bacterins**

Avirulent cell bacterin: This product was prepared by culturing a stock culture of *M. paratuberculosis* on Dorset and Henley medium for 2 weeks. At that time, the bacterial mass was harvested and incorporated into mineral oil as previously described.¹

Virulent cell bacterin: A strain of *M. paratuberculosis* recently isolated from a cow was transferred to egg yolk medium containing 2 mg of mycobactin per 1 and incubated for 10 weeks at 38°C in Roux flasks. The resulting growth was washed off the medium with saline. The bacterial mass was removed from the saline and incorporated into mineral oil by the procedure used in preparing the avirulent cell bacterin. When experimental animals were 2½ years old and annually thereafter, each animal was tested with a comparative tuberculin test in the cervical region and with a caudal fold tuberculin test. The procedure for these tests was that outlined by the U.S. Department of Agriculture.

**Postmortem Examination**

The owner sent experimental cattle to slaughter when they were no longer profitable. Each animal was identified, and Meat Inspection personnel at the slaughter plant obtained the terminal part of the ileum and attached mesenteric lymph nodes from each animal. These specimens were frozen and sent to us. The owner gave us the reason for slaughter. If he stated that clinical Johne’s disease was the reason and this reason was confirmed in the laboratory, the animal was recorded as a confirmed clinical case. If the reason for slaughter was not clinical Johne’s disease and the laboratory examination was positive for *M. paratuberculosis*, the animal was recorded as infected. If results of laboratory examination were negative, the animal was recorded as not infected. Laboratory examination consisted of: (1) culturing the tissues for the presence of *M. paratuberculosis* and (2) histopathologic examination of tissue sections for the presence of *M. paratuberculosis* and lesions compatible with paratuberculosis.
RESULTS

Data have been obtained from 26 experimental cattle remaining in the herd and from 17 that have been sent to slaughter. Adverse effects from the vaccine were not observed except for the nodules that generally developed at the vaccination site. When present, these nodules varied in size up to 18 cm in diameter. The average diameter of the nodules that resulted from the avirulent cell vaccine was 9.1 cm, and the average size of the nodules from the virulent cell vaccine was 9.8 cm.

Tuberculin Tests

The results of the caudal fold tuberculin test are as follows: (1) Of 20 tests of cattle (each tested one or more times) receiving the virulent cell vaccine, 9 showed small reactions classed as p1 and 1 showed a reaction classed as p2; (2) of 26 tests of cattle receiving the avirulent cell vaccine, 5 showed reactions classed as p1, 1 showed a reaction classed as p2; and 1 showed a large reaction classed as p3; and (3) of 20 controls, 1 showed a reaction classed as p1, the other 19 were negative.

The results of comparative cervical tests with purified protein derivative (PPD) tuberculins prepared from *Mycobacterium avium* and *Mycobacterium bovis* were plotted on cervical test scattergrams similar to those used by the U.S. Department of Agriculture (VS form 6-22D) to determine whether an animal showing a caudal fold response is most likely negative, suspicious, or positive for bovine tuberculosis (Fig 1).

The average reaction for 22 tests on cattle given the avirulent cell vaccine was 12 mm for avium PPD, and 3.7 mm for bovine PPD. The average reaction for 21 tests on cattle given the virulent cell vaccine was 11.5 mm for avium PPD, and 4.1 mm for bovine PPD. The controls had either no reactions or very small reactions to the test products.

Postmortem Examination

Postmortem data have been obtained from 17 cattle. Nine of these were nonvaccinated controls. Four of these showed signs of disease; these 4 and 2 others were found to be infected on postmortem examination. Six cattle were virulent cell vaccinates and all were negative on postmortem examination. (Feces of 1 virulent cell vaccinate still living has been found to contain *M paratuberculosis* bacilli.) Of two avirulent cell vaccinates, neither showed signs of disease, but one was infected.

These data were analyzed statistically. The virulent cell vaccine protected significantly (<0.05) against paratuberculosis when compared with the controls. The protective ability of the virulent cell vaccine could not be compared with that of the avirulent cell vaccine because too few avirulent cell vaccinates were slaughtered.

DISCUSSION

Although the number of slaughtered cattle is not large, the virulent cell vaccine was found to protect significantly against the disease.
Results in a previous study showed that the avirulent cell vaccine also protects significantly against the disease. Because both vaccines protect at about the same level, and because avirulent cell vaccine is much easier to prepare than virulent cell vaccine, the avirulent cell vaccine is the one that we will continue to use in our experimental vaccination studies.

Figure 1
Comparative cervical test results from 2 vaccines, 1 prepared from avirulent cells and 1 from virulent cells. Increased skin thickness due to *Mycobacterium avium* purified protein.
derivative (PPD) is plotted on the vertical axis and increased thickness due to *Mycobacterium bovis* PPD is plotted on the horizontal axis. These two values are then represented for each animal by a single dot on the scattergram. The classification of each animal is according to the zone into which the results are plotted.

**REFERENCES**


REPORT OF THE SUBCOMMITTEE ON THE REEVALUATION OF PROCEDURES FOR TRAINING OF ACCREDITED VETERINARIANS IN CONDUCTING THE TUBERCULIN TEST AND TUBERCULIN TEST TRAINING IN VETERINARY COLLEGES

A. R. McLaughlin, D.V.M.
C. E. Boyd, D.V.M.
P. L. Smith, D.V.M.

<table>
<thead>
<tr>
<th>State</th>
<th>Accredited veterinarians in contacts with accredited veterinarians</th>
<th>(B) Before accreditation</th>
<th>(C) By whom</th>
<th>Submission to regulatory agency responsible for TB testing on accredited veterinarian</th>
<th>(G) By whom</th>
<th>TP test or C-C test</th>
<th>C-C test on suspect or negative cases</th>
<th>C-C test on positive cases</th>
<th>Records of test results</th>
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Legend:
- Yes: Procedure is followed.
- No: Procedure is not followed.

485
### ACCREDITED VETERINARIAN TRAINING

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*Appeared as "SELDOM" on questionnaire
### TUBERCULOSIS TEST TRAINING

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Statistically it appears that bovine tuberculosis in the United States is on the wane. There were 17 tuberculous herds discovered nationwide in fiscal year 1979. Of this number, only nine affected herds were found in the continental United States during this period of time. This compares to a total of 27 affected herds found last year and 29 affected herds found the previous year.

One cannot help but speculate on whether this is an actual decline in the incidence of bovine tuberculosis or whether the commitment to finding the disease has faltered the past 3 years. Only time will give us the final answer to this question.

With the addition of Arizona and Vermont to the list of states which have reached accredited-free state status this list now numbers 16. In addition, eight states have not found any bovine tuberculosis in the past 5 or more years (Figure 1).

It is interesting to note that in the past two years, two accredited-free states have discovered tuberculosis in the state shortly after reaching accredited-free state status. Extensive epidemiology in each case revealed no extension of infection from the primary herds.

It is significant that neither of these states relaxed their vigilance against bovine tuberculosis after they were accredited-free. Each accredited-free state must guard against the tendency to be complacent about the disease simply because a piece of paper has declared the state "free of bovine tuberculosis."

As previously stated, only nine tuberculous herds were found in fiscal year 1979 in the eight contiguous states (Figure 2). Ohio and Texas found two affected herds in each state and Arizona, Oklahoma, Iowa, Indiana and Pennsylvania found one herd in each state. Tuberculosis was found in only seven of the Continental States during the year. In addition, one herd was discovered in Hawaii and seven herds in the Commonwealth of Puerto Rico.

The program continues to be largely dependent upon traceback of tuberculous slaughter cattle for the detection of affected herds (Figure 3). There were 10 herds found by this means in fiscal year 1979 with an additional 5 herds located by tracing sales from the herds originally found through the tracing of *M. bovis* infected slaughter cattle. One herd was found as a result of milk ordinance testing and one herd on import testing for interstate movement.
Epidemiologic tracing accounted for 15 of the 17 herds found to be infected with *M. bovis* in fiscal year 1979 (Figure 4). Six of the seven affected herds found in Puerto Rico during the period were the result of epidemiologic tracing carried out by a special work force in the Commonwealth. Where special emphasis is placed upon good epidemiology, as in work forces, good results are obtained.

For the past several years, special emphasis has been placed upon the depopulation of tuberculosis affected herds. Much of the program progress toward eradication in recent years can be attributed to the elimination of foci of infection through the depopulation of such herds. In fiscal year 1979, 13 of the 17 affected herds found were depopulated for a depopulation rate of 76 percent (Figure 5). This rate is another milestone in the eradication of bovine tuberculosis because it represents the highest depopulation rate ever attained by the program.

It is significant that every affected herd found in the continental United States was depopulated (Figure 6). Only the one herd found in Hawaii and 3 of the herds found in Puerto Rico were not depopulated. It is reported that the owner of the herd in Hawaii is voluntarily depopulating the herd over a period of time without indemnity. Hopefully, therefore, this herd will also be slaughtered and will remove another potential for spread of the disease.

There were 1,741 regular-kill investigations conducted this fiscal year. A large percentage (96%) of these cases were not laboratory confirmed as tuberculosis and were administratively closed without a field investigation (Figure 7). Sixty-two cases were laboratory confirmed to be suggestive or compatible with a diagnosis of tuberculosis and were investigated in the field.

In 34 of these cases, no individual animal identification was provided by slaughter inspection personnel (Figure 8). Only three of these investigations (9 percent) were successful in locating the source herd of the tuberculous regular-kill animal. Thirty-one (91 percent) were unsuccessful. On the other hand, where individual animal identification was provided, 22 of 28 cases investigated were successful (79 percent). It is readily apparent that the investigation success rate is directly tied to individual animal identification.

Figure 9 shows in more detail the results of the investigation of the 62 cases of tuberculosis which were field investigated in fiscal year 1979. The success rate has dropped from 52.3 percent in fiscal year 1978 to 40.3 percent this fiscal year. This is primarily because of the increase in unsuccessful feedlot cases from 19 in fiscal year 1978 to 33 in fiscal year 1979. One area of concern, however, is the four *M. bovis* cases that were successfully traced back to the source herd only to find that herd negative to a tuberculin test. One cannot help but wonder if the source herd were indeed found in these cases.

In fiscal year 1978, 2,228 submission were made of thoracic granulomas
by slaughter inspectors. In fiscal year 1979, 1,699 such submissions were made (Figure 10). This decrease of 529 submissions (24 percent) is cause for grave concern in the program because the backbone of the program is slaughter surveillance. A concerted effort is needed by all program personnel both State and Federal to increase the submission rate.

A study of figure 11 shows that only 88 of the Federally inspected slaughter establishments slaughtering over 20,000 cows annually submitted any thoracic granulomas in fiscal year 1979. This is down from 117 establishments submitting in fiscal year 1978 (down 25%). It must also be pointed out, however, that the total number of establishments slaughtering over 20,000 cows annually dropped from 145 establishments in FY 1978 to 115 establishments in FY 1979 (down 21 percent).

Figures 12 and 13 summarize the results of comparative-cervical (c-c) tuberculin testing for the period July 1, 1978, to June 30, 1979, and for the past 5 years. It is interesting to note that the total number of c-c tests performed has dropped markedly the past 3 years from 8,804 tests in 1976-1977 to 4,712 tests in 1978-1979. This is a reduction of 47 percent in 3 years and probably reflects fewer numbers of caudal fold test suspects as a result of the greater specificity of the purified protein derivative tuberculin now in use in this country.

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**Tuberculosis Eradication**

**Bovine Tuberculosis Area Status**

**September 30, 1979**

![Map of United States showing area status]

- Accredited Free States (16) plus Virgin Islands
- Modified Accredited Areas (34) plus Puerto Rico
- No M. Bovis for Over 5 Years (9)

*Figure 1*
Tuberculosis Eradication

17 Tuberculous Herds by Location

FY 1979

Figure 2

Tuberculosis Eradication

Methods of Locating 17 Tuberculous Herds Initially Detected during FY-79

- Tracing Exposed Cattle from Affected Herds (5)
- Traceback of Regular Kill Slaughter Animals (10)
- Import Testing (1)
- Milk Ordinance Testing (1)

Figure 3
Tuberculosis Eradication

Detecting Herds with TB Infection: 1969 through 1979

Figure 4

Tuberculosis Eradication

Herd Found vs. Herds Depopulated
FY 1969-79

Figure 5
Tuberculosis Eradication
Proportion of Tuberculous Herds Depopulated
FY 1979

Figure 6

Tuberculosis Eradication
Tuberculosis Traceback Investigations (Regular Kill)
FY 1979 (Cases Closed)

Figure 7
Tuberculosis Eradication

62 Tuberculous Cases (Regular Kill) Animals Identified and Unidentified

FY 1979

34 with no identification

28 with identification

91%

9%

21%

79%

Unsuccessful
Successful

Figure 8

Tuberculosis Eradication

62 High Risk 6-35 Cases Closed

FY 1979

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Figure 9
Tuberculosis Eradication
**Number of 6-35's Submitted FY 79**

- Number of Adult Animals
- Number of Adults with Identification
- Number of ID Devices Submitted

**Figure 10**

Tuberculosis Eradication
**Granulomas Submitted**

From 115 Federal Establishments Slaughtering Over 20,000 Cows
(July 1, 1978 through June 30, 1979)

Veterinary Services Laboratories, Ames, Iowa

None: 27 Establishments
1-9 Cases: 71 Establishments
10-108 Cases: 17 Establishments

**Figure 11**
### Table: Comparative-Cervical Retest Results 7-1-78 to 6-30-79

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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Neg.</td>
<td>3291</td>
<td>93.0</td>
<td>832</td>
<td>90.9</td>
<td>219</td>
<td>89.8</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>Sus.</td>
<td>194</td>
<td>5.4</td>
<td>80</td>
<td>8.7</td>
<td>15</td>
<td>6.1</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Rea.</td>
<td>59</td>
<td>1.6</td>
<td>4</td>
<td>.4</td>
<td>10</td>
<td>4.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3544</td>
<td>100.0</td>
<td>916</td>
<td>100.0</td>
<td>244</td>
<td>100.0</td>
<td>8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 12

### Table: Summary of Comparative-Cervical Tests Conducted

<table>
<thead>
<tr>
<th>Reporting Period</th>
<th>C-C Test Within 10 Days</th>
<th>C-C Test after 60 Days</th>
<th>Second C-C Test</th>
<th>Third C-C Test</th>
<th>Total Animals C-C Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>FY 1975</td>
<td>2,828</td>
<td>66</td>
<td>1,481</td>
<td>34</td>
<td>78</td>
</tr>
<tr>
<td>FY 1976</td>
<td>5,220</td>
<td>75</td>
<td>1,720</td>
<td>25</td>
<td>236</td>
</tr>
<tr>
<td>7-1-76 to 6-30-77</td>
<td>6,914</td>
<td>82.5</td>
<td>1,468</td>
<td>17.5</td>
<td>422</td>
</tr>
<tr>
<td>7-1-77 to 6-30-78</td>
<td>4,957</td>
<td>77.6</td>
<td>1,427</td>
<td>22.4</td>
<td>264</td>
</tr>
<tr>
<td>7-1-78 to 6-30-79</td>
<td>3,544</td>
<td>79.5</td>
<td>916</td>
<td>20.5</td>
<td>244</td>
</tr>
</tbody>
</table>

Figure 13
INCIDENCE OF SWINE TUBERCULOSIS AT SLAUGHTER FOR 1977 AND 1978

Gerald R. Snyder, D.V.M.

Tuberculosis Incidence Per 1000 Swine Slaughtered Under Federal Inspection
January 1, 1977 — December 31, 1977

U.S. Incidence 7.31

Tuberculosis Incidence per 1000 Swine Slaughtered under Federal Inspection in 1978

U.S. Incidence 8.015

*No Swine Slaughtered
REPORT OF THE SUBCOMMITTEE ON TUBERCULOSIS
ACCREDITED HERDS OF GOATS

Paul L. Spencer, D.V.M.

The following action has been taken by this Committee during the past two years:

1. A survey was made of approximately 30 states felt to have a nominal dairy goat population. 21 states presently have laws or regulations providing for tuberculosis accredited goat herds, although 2 of these states have no individual herds in the state at the present time. The number of herds in the 18 states remaining ranged from a low of 1 to a high of 221 (an average of 23 herds per state). With only one exception, the States with accredited herds follow the Uniform Rules and Methods for bovine tuberculosis accredited herds for their accredited goat herds. The one state exception has slightly more stringent requirements.

2. Two veterinarians, nationally known as authorities on disease of goats, one in Illinois and one in Pennsylvania, have been contacted for their opinions. Both are in agreement that the incidence of tuberculosis in goats in the United States is very low, or possibly non-existent and that there are many other diseases of goats posing greater problems to the dairy goat industry. Both support development of an accredited herd plan for goats. Neither person has any reason to doubt the accuracy of the intradermal tuberculin test in goats. Both veterinarians remarked on the serious problem of Johne's Disease in goats but had never observed any cross-reactions when they had tested goats that were later proved to have Johne's Disease.

3. A check of literature reveals confirmation of tuberculosis in goats in the United States in the 1930's. One of the veterinarians mentioned above had personal experience with the disease in goats about 20 years ago.

4. Several regulatory veterinarians with expertise in bovine tuberculosis eradication were interviewed, plus three regional tuberculosis epidemiologists. None expressed any doubts as to the 0.1 ml PPD tuberculin test being reliable in goats.

5. The subcommittee recommends that the U.S.A.H.A. Committee on Tuberculosis and Johne's Disease recommend the development of provisions for an Accredited Herd Plan for dairy goats. Same to be considered for inclusion in the Uniform Methods and Rules - Bovine Tuberculosis Eradication at the 1979 meeting. A suggested format is attached.

Part : Accredited Herd Plan for Dairy Goats:

1. Animals to be tested — Testing of herds for accreditation or
reaccreditation shall include all goats over 6 months of age. All natural additions shall be individually identified and recorded on the test report as members of the herd at the time of the annual test.

2. Additions — Herd additions must originate directly from one of the following:
   a. Accredited herd
   b. Herd in an Accredited Free State
   c. Herd in a Modified Accredited Area that has passed a herd test of all animals over 6 months of age within 12 months, and the individual animals for addition were negative to the tuberculin test conducted within 60 days.
   d. Herd in a Modified Accredited Area not meeting requirement of (a), (b), or (c) of this paragraph, individual animals for addition must pass a negative test within 60 days prior to entering the premises of the accredited herd and must be kept in isolation from all members of the herd until negative to a test conducted after 60 days of date of entry.

   Animals added under (b), (c), and (d) shall not receive accredited herd status for sale purposes until they have been members of the herd at least 60 days and are included in a herd retest.

3. Accreditation and reaccreditation — To qualify for accredited herd status, the herd must pass at least two consecutive annual tuberculin tests with no evidence of bovine tuberculosis disclosed. All animals must be bona fide members of the herd. Qualified herds may be issued a certificate by the local State and Federal officials. The accreditation period will be 12 months (365 days) from the anniversary date and not 12 months from the date of the reaccreditation test. To qualify for accreditation the herd must pass an annual test within a period of 10 to 14 months of the anniversary date.
REPORT OF THE COMMITTEE ON TUBERCULOSIS
AND JOHNE'S DISEASE

Chairman: John M. Dick, Harrisburg, Pennsylvania
Co-Chairman: Paul L. Spencer, Springfield, Illinois


The Committee met on the afternoon of October 30 & 31, 1979.

Sixteen committee members and fourteen guests were in attendance for all or a part of the meeting.

Committee members were introduced. Many changes in committee membership since last year were noted by the chairman.

The chairman appointed two subcommittees as follows:
1. Johne's Disease: Drs. Flint (Chairman), D. Smith, McLaughlin, Hoffsis, and Merkal.
2. Swine Mycobacteriosis: Drs. Frye (Chairman), Snyder, Thoen, Hughes, and Mr. Lichtman.

The two subcommittees were instructed to review papers and data presented on their assigned subjects and submit recommendations for the consideration of the full committee on Wednesday. These subcommittees will continue to function throughout the year, to develop guidelines for the control of Johne's disease and swine Mycobacteriosis.

Special reports were presented and discussed as follows:
1. Johne's Disease in Two Large Dairy Herds and Goat Herds in California, by Dr. George B. E. West.
3. Johne's Disease Program in Oregon, by Dr. Dean H. Smith.
6. Incidence of Swine Tuberculosis at Slaughter, by Dr. Gerald R. Snyder.
7. U.S.D.A. Time-Temperature Study for the Destruction of M. avium and M. bovis Organisms in Meat Products. Project by Dr. Richard
Merkal, presented by Dr. Gerald R. Snyder. This project is in the final stages of completion.

8. Epidemiology and Pathogenesis of Swine Mycobacteriosis in Georgia, by Dr. John R. Cole, Jr.


In executive session, the following subcommittee reports were presented and motions passed for acceptance by the Executive Committee:

1. Establishment of Tuberculosis-Free Accredited Herds of Goats, presented by Dr. Paul L. Spencer. Motion for acceptance of subcommittee report by Dr. McLaughlin, seconded by Dr. Flint. Following discussion, the subcommittee report was approved. Establishment of Tuberculosis Free Accredited Herds of goats will follow the UM&R requirements for cattle.

2. Reevaluation of Procedures for Training of Accredited Veterinarians in Conducting the Tuberculin Test and Tuberculin Test Training in Veterinary Colleges, presented by Dr. A.R. McLaughlin. Motion by Dr. Nadler, seconded by Dr. Stadler. The committee recommends that Veterinary Services Tuberculosis Epidemiologists conduct tuberculosis seminars at veterinary colleges for the instruction of students prior to their graduation and accreditation. This instruction shall include the use of sensitized cattle. Following discussion, the motion was adopted. Motion by Dr. McLaughlin, seconded by Dr. Stadler.

The committee also recommends a continuing education program for accredited veterinarians to discuss the tuberculosis program, procedures and involvement. This program could be conducted in conjunction with the continuing education requirements recommended by the Brucellosis Committee. Following discussion, motion was adopted.

3. Johne's Disease, presented by Dr. J.G. Flint. Motion by Dr. LaBranche, seconded by Dr. Carey, to accept the following subcommittee report.

SUBCOMMITTEE REPORT

This subcommittee met on Wednesday, October 31, from 9:00 am to 11:00 am and outlined the following procedures relative to Johne's disease.

a. Quarantines are counter-productive and cause disease not to be reported.
b. The Committee encourages USDA approval of a vaccine manufactured by licensed establishments to be made available to the states under strict control and supervision of the state animal health official. This vaccine shall be used only in herds in which the disease has been confirmed by laboratory diagnosis.

c. Results of the survey conducted by AABP were given by Dr. Hoffsis at the 1979 AVMA Meeting and will be published in the AVMA convention issue. This survey shows that the disease is widespread except in a few western states.

d. The subcommittee suggests a survey of slaughter cattle be conducted to determine the incidence of the disease, probably using ileo-caecal nodes for the histopathological study. Funding of such a survey remains to be determined. Reliable figures could probably be obtained by conducting a survey in about three large plants at different geographical locations.

e. The subcommittee encourages continuing research to develop a better test.

Motion adopted to accept this subcommittee report.

4. *Swine Mycobacteriosis*, presented by Dr. G. H. Frye. Motion by Dr. McLaughlin, seconded by Dr. Carey, to accept the following subcommittee report.

The subcommittee recommends consideration of the following subjects that were reviewed.

a. **Packer Compensation and Providing Incentive for Producers to Reduce or Eliminate Swine Tuberculosis in His Herd.**

The committee feels that full unqualified compensation, either directly to owners or indirectly via packers, provide little incentive to reduce mycobacteriosis in a herd.

There are two possible methods for providing compensation while still maintaining incentive to eliminate the disease.

(1) Compensation should be tied to the initiation of control measures in the herd, or

(2) Provide only partial compensation for losses sustained from tuberculosis. Owners who continue to share the monetary losses from disease will have more of an interest in reducing that loss.

b. **Indemnity**

Indemnification to owners for losses due to mycobacteriosis should be provided only in conjunction with an active program to reduce the disease in a state or area.

Indemnity without such a program would be an unending transfer of taxpayers' money to producers without causing any
impact on the problem itself.

c. **Field Studies.**

Organized field studies are essential for greater understanding of swine tuberculosis under farm conditions. These will develop information on sources of infection of serotypes other than 1 or 2; on modes of transmission; on suitable procedures for disinfecting infected premises; on the effectiveness of various control measures and other epidemiological information.

These studies should be supported by definitive bacteriologic examinations.

This information could be used to design future control or eradication activities.

d. **Research.**

Investigations are needed that will provide a better understanding of the pathogenesis of *M. avium* complex infections in swine.

The development of diagnostic tests that will provide rapid detection of infected animals should be emphasized.

e. **Adoption of Time and Temperature Changes for Handling Passed for Cooking Carcasses**

Time and temperatures for processing lesioned carcasses as described by Merkal et. al. in the *Journal of Applied and Environmental Microbiology*, November 1979, should be adopted.

f. **Identification.**

Proper identification is essential for several programs to control or eradicate diseases in swine. This committee shall continue to support identification which will permit rapid and accurate traceback of lesioned swine to herds of origin.

g. It is recommended that the Tuberculosis Committee, through the USAHA, should make its recommendations relative to swine tuberculosis in the above-mentioned areas known to disease control officials for their guidance when considering the disease in swine.

5. **Proposed changes in the Uniform Methods and Rules**, presented by Dr. R. W. Bennett. Following prolonged discussion, motion by Dr. Stadler, seconded by Dr. D. Smith, to accept the proposed changes as amended by the committee was adopted.

These proposed changes in the UM&R are as follows:

**PART II: OFFICIAL TEST REQUIREMENTS**

B. **Restriction of Personnel to Apply Tuberculin Tests.**

Tuberculin tests shall be applied by a veterinarian employed in a full-
time capacity by the state or the USDA, or by an accredited veterinarian. Technicians employed by state or Federal governments approved by said governments may conduct routine screening tuberculin tests when directly supervised by state or Federal veterinarians.

E. *Comparative-Cervical Test.*

The official test for retesting suspects. It shall be applied only by a full-time state or Federal regulatory veterinarian.

G. *Tuberculin Test Interpretation.*

The following are guidelines for classification of cattle tested.

1. **Routine Screening Tuberculin Tests** — All responding animals shall be quarantined to the premises until retested by the comparative-cervical test.

2. **Official Caudal Fold Tuberculin Test** — All responses shall be recorded and the animal classified as a suspect and quarantined for retest as provided in Part III, Section B, unless, in the professional judgment of the testing veterinarian, the reactor classification is indicated.

3. **Comparative Cervical Test** — Responses shall be reported and plotted on the scattergram.

4. **Single Cervical Test** — All animals with a response shall be classified as a reactor. Responses shall be recorded in millimeters.

You will note that the deviator classification has been deleted from the UM&Rs. There will only be negative, suspect, and reactor classifications.

**PART III: DISPOSITION OF TUBERCULIN RESPONSE CATTLE**

A. **Disposition of Reactors**

1. Reactors must remain on the premises where disclosed until a state or Federal permit for movement has been obtained. Movement for immediate slaughter must be direct to a slaughtering establishment, where approved state or Federal inspection is maintained, within 15 days of classification or the animals must be destroyed under the direct supervision of a regulatory veterinarian to assure that the carcass is either cooked or condemned.

B. **Disposition of Suspects**

Suspects to the caudal tuberculin test shall be quarantined to the premises where found until: (There follows three dispositions as in the present UM&R).

The present subcommittee shall continue to review and recommend changes in the UM&R, as outlined by the Committee during discussion, for adoption at the next USAHA session.

Motion adopted to accept this subcommittee report.
Motion by Dr. Carey, seconded by Dr. McLaughlin, to include recommended changes relative to accredited herds in goats in the UM&R changes. Motion adopted.

The chairman reviewed motions made by the committee in 1978 and accepted by the Executive Committee and commented on progress, or lack of progress, made during the last year.

Meeting adjourned.
IMPLICATION OF WHITE-TAILED DEER IN THE
BOOPHILUS ANNULATUS TICK ERADICATION PROGRAM

J.H. Gray, Regional Epidemiologist
United States Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, Austin, Texas

R.L. Payne, Research Coordinator
Southeastern Cooperative Wildlife Disease Study
Department of Parasitology, College of Veterinary Medicine
University of Georgia, Athens, Georgia

G. O. Schubert, Chief Staff Veterinarian
United States Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, Hyattsville, Maryland

W.H. Garnett, Veterinary Medical Officer
Texas-United States Fever Tick Program
United States Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, Laredo, Texas

INTRODUCTION

Cattle fever ticks (*Boophilus annulatus*) first were described from white-tailed deer (*Odocoileus virginianus*) in east Florida during 1821. Some authorities consider these arthropods to be indigenous to North America and suggest white-tailed deer and American bison (*Bison bison*) as original hosts. Other authorities believe that *B. annulatus* was introduced through the importation of cattle. Regardless of origin, *B. annulatus* was shown to be the vector of southern cattle fever (bovine piroplasmosis) in 1889. Eradication of this decimating disease subsequently was based on elimination of the one-host arthropod vector. Two possible methods of fever tick eradication were considered, i.e., (1) pasture vacation, and (2) systematic livestock dipping in an appropriate acaricide. Livestock dipping ultimately was chosen as the most effective approach.

A national fever tick eradication program involving the systematic dipping of cattle was initiated in the United States in 1906. Eradication was completed in 1943 except for several areas which subsequently were recognized in Florida. Portions of the Texas/Mexico border later became reinfested resulting from the introduction of Mexican cattle. Sporadic concern has been expressed regarding the involvement of white-tailed deer throughout the history of the tick eradication program, and *B. annulatus*
have been collected from deer in Texas, Florida, and California.\textsuperscript{3,7,8} The status of white-tailed deer as reservoirs of fever ticks, however, has been a matter of major controversy. Opinions regarding the significance of deer in perpetuation of fever ticks include both extremes, i.e., (1) deer are of no consequence, and (2) deer alone are responsible for fever tick infestations among livestock. The latter concept resulted in the questionable depopulation of white-tailed deer from some areas of Florida during 1937–39.\textsuperscript{19}

The area presently infested with \textit{B. annulatus} is located in Webb County, Texas, approximately 60 miles northwest of Laredo along the Rio Grande River. According to Hourrigan, \textit{B. annulatus} first was collected from deer in this area during 1933,\textsuperscript{7} with occasional recoveries of fever ticks from deer to the present time.\textsuperscript{4} Recent attempts to eradicate \textit{B. annulatus} within the problem area have been limited to a policy of pasture vacation for a predetermined period depending on the season of the year, which results in the starvation of larval ticks, provided all hosts have been removed. The pasture vacation method of fever tick eradication was first described by Curtice in 1889,\textsuperscript{7} but this method was not feasible early in the eradication program because of the vast land areas infested with fever ticks. The pasture vacation method, however, has proven ineffective in eradicating \textit{B. annulatus} from the current problem area. Recent livestock dipping in this area has been restricted to meeting Texas Animal Health Commission/United States Department of Agriculture minimum requirements for the movement of cattle.

In order to more closely define the involvement of white-tailed deer in the fever tick eradication program, USDA, APHIS, VS, initiated a comprehensive wildlife surveillance program during the spring of 1979. The primary objectives of initial fever tick surveillance among wildlife populations were as follows: (1) to determine the extent to which \textit{B. annulatus} infest white-tailed deer within the problem area, and (2) to determine the geographic distribution of deer infested with \textit{Boophilus}.

\textbf{MATERIALS AND METHODS}

The area presently infested with \textit{B. annulatus} (Fig. 1) includes portions of the Apache, Petty, and Briscoe Chupadero ranches in Webb County. The zone is comprised of approximately 116,000 acres and is divided into pastures and traps ranging from 200 to 6,000 acres. Deer population estimates by pasture were obtained through aerial census prior to surveillance activities.

Representative samples of deer were collected from each pasture within the problem area under a permit from the Texas Parks and Wildlife Department. Deer were shot in the head or neck with high velocity center fire rifles equipped with telescopic sights. Whenever possible, a 5 percent sample of deer within each pasture was examined, with a minimum sample of five deer on areas of 1,000 acres or more. Sampling was initiated on April 10, 1979, and continued through June 20, 1979. Sampling of deer
within pastures began along the northern boundary of the eradication zone and proceeded southerly towards the Rio Grande River.

Immediately after collection of each animal, APHIS, VS Tick inspectors examined the carcass, collected and counted all ticks, and placed them in vials of alcohol with glycerine. The only exceptions were two heavily infested animals where the numbers of ticks were estimated. Ticks were delivered for identification to the Dimmit County Tick Office, USDA, Carrizo Springs, Texas. Samples were forwarded to the Texas-United States Tick Laboratory, Austin, Texas, for identification confirmation, and subsequently received by the National Veterinary Services Laboratories (NVSL), Ames, Iowa. Field dressed carcasses of most deer collected were refrigerated at 38°F and donated to the Rio Grande Childrens Home, Mission, Texas.

RESULTS

During surveillance activities, 297 deer (7.2 percent of the estimated total population) were examined from 52 pastures and traps (Table 1). Twenty-nine deer from 10 pastures were infested with *B. annulatus* (Table 2). Infestation among deer sampled from these 10 pastures ranged from 6.3 to 100 percent. Numbers of fever ticks observed on infested deer varied from 1 to 25, with an average of 6 per infested animal. Fever ticks recovered from deer included both nymphal and adult stages. Fever ticks were recovered from deer in 6 pastures supporting infested cattle, from deer in 2 pastures supporting non-infested cattle, and from deer in 2 pastures where cattle were absent. Deer were not infested in 10 pastures supporting infested cattle.

DISCUSSION

Prior to intensive white-tailed deer fever tick surveillance, Veterinary Services personnel had determined that cattle were infested with *B. annulatus* in 16 pastures (Table 3), and occurrence of fever ticks among white-tailed deer closely followed the known infested areas. Two broad geographic areas of infestation among deer and cattle were evident (Fig. 1). Cattle were infested in 6 of the 10 pastures in which *B. annulatus* were recovered from deer. Recovery of fever ticks from deer in 4 pastures previously not known to have been infested enabled Veterinary Services to revise the status of these pastures.

Previous studies have shown that tropical fever tick (*B. microplus*) can be perpetuated on white-tailed deer for 20 years in the absence of livestock. In the present study, deer were shown to support *B. annulatus* within vacated pastures. Deer also were implicated in the transportation of ticks from infested to noninfested pastures due to current land management practices (root plowing) within the problem area. The system of root plowing has a pronounced effect on deer population distribution because of the vast areas completely depleted of vegetation, which initiates deer movement into adjacent pastures. Pasture vacation therefore
is not an effective eradication measure in the area of concern.

Although white-tailed deer are of significance both as maintenance and transport hosts for *B. annulatus* within the current problem area, previous studies have shown that the tropical fever tick (*B. microplus*) can be eradicated by the systematic dipping of cattle where deer and cattle cohabit the same areas. Considering the involvement of white-tailed deer and their probable relationship to the ineffectiveness of the pasture vacation approach, implementation of a systematic cattle dipping program for eradication of *B. annulatus* is warranted.

1. The pasture vacation system of fever tick control should be approached with extreme caution where white-tailed deer and cattle cohabit the same area.

2. Successful fever tick eradication measures should include the stocking of cattle in all *B. annulatus* infested (cattle and/or deer) pastures at normal rates. Non-infested pastures in close proximity to areas of known infestation also must be included.

3. An area systematic cattle dipping program should be initiated, with dipping scheduled at prescribed intervals to be assured that cycling of *B. annulatus* is prohibited.

4. Spraying of horses used to work cattle should be conducted at regular intervals to prevent accidental spread of fever ticks.

5. Fever tick surveillance among white-tailed deer should continue at regular intervals during and after establishment of an area systematic cattle dipping program.

6. Wildlife surveillance activities should be expanded to include other salient species.

ACKNOWLEDGMENTS

For invaluable cooperation, appreciation is expressed to the following members of the Texas Parks and Wildlife Department, Austin, Texas: Ted L. Clark, Director of Wildlife; Chester L. Burdett, Director of Law Enforcement; Charles K. Winkler, Big Game Program Director; Donald J. Caudle, Law Enforcement Regional Director; and Michael D. Hobson, Wildlife Biologist. Dr. E. S. Cox, Mr. R. J. Ciskey, Dr. Wilson O. Boaz and the USDA Tick Force Staff of the Dimmit and Webb County Tick Offices provided vital assistance and expertise. Also, this study would not have been possible without the full cooperation of the respective landowners involved, i.e., Bill Blocker, Apache Ranch; Dolph Briscoe, Chupadero Ranch; and O S. Petty, Jr., Petty Ranch.
Figure 1
### TABLE 1
APHIS/SCWDS Fever Tick Surveillance by Pasture
White-tailed Deer
Webb County, Texas (4/10/79 - 6/20/79)

#### APACHE RANCH

<table>
<thead>
<tr>
<th>Pasture</th>
<th>Acreage</th>
<th>Acres/Deer</th>
<th>Total Deer</th>
<th>Deer Sampled</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apache</td>
<td>6,000</td>
<td>30</td>
<td>200</td>
<td>9</td>
<td>Neg.</td>
</tr>
<tr>
<td>Camino*</td>
<td>2,000</td>
<td>30</td>
<td>66</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Espada</td>
<td>4,000</td>
<td>30</td>
<td>132</td>
<td>7</td>
<td>Neg.</td>
</tr>
<tr>
<td>Gato</td>
<td>1,800</td>
<td>30</td>
<td>60</td>
<td>1</td>
<td>Neg.</td>
</tr>
<tr>
<td>Little Tee Pee</td>
<td>2,000</td>
<td>30</td>
<td>66</td>
<td>4</td>
<td>Neg.</td>
</tr>
<tr>
<td>Tee Pee</td>
<td>3,920</td>
<td>30</td>
<td>129</td>
<td>6</td>
<td>Neg.</td>
</tr>
<tr>
<td><strong>Totals:</strong></td>
<td>19,720</td>
<td>-</td>
<td><strong>633</strong></td>
<td><strong>32 (4.9%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

#### PETTY RANCH

<table>
<thead>
<tr>
<th>Pasture</th>
<th>Acreage</th>
<th>Acres/Deer</th>
<th>Total Deer</th>
<th>Deer Sampled</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cougar</td>
<td>1,800</td>
<td>31</td>
<td>58</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>East Rincon</td>
<td>2,500</td>
<td>35</td>
<td>71</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Moca</td>
<td>2,500</td>
<td>23</td>
<td>109</td>
<td>4</td>
<td>Neg.</td>
</tr>
<tr>
<td>West Coma</td>
<td>2,500</td>
<td>27</td>
<td>93</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>West Rincon</td>
<td>2,500</td>
<td>30</td>
<td>83</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td><strong>Totals:</strong></td>
<td>11,800</td>
<td>-</td>
<td><strong>414</strong></td>
<td><strong>24 (5.8%)</strong></td>
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</tr>
</tbody>
</table>

#### BRISCOE RANCH

<table>
<thead>
<tr>
<th>Pasture</th>
<th>Acreage</th>
<th>Acres/Deer</th>
<th>Total Deer</th>
<th>Deer Sampled</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arroyo</td>
<td>2,000</td>
<td>38</td>
<td>53</td>
<td>5</td>
<td>Pos.</td>
</tr>
<tr>
<td>Augra</td>
<td>1,550</td>
<td>71</td>
<td>22</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Bigford Trap*</td>
<td>900</td>
<td>15</td>
<td>60</td>
<td>6</td>
<td>Pos.</td>
</tr>
<tr>
<td>Bull Trap*</td>
<td>980</td>
<td>50</td>
<td>20</td>
<td>5</td>
<td>Pos.</td>
</tr>
<tr>
<td>Cabeza*</td>
<td>1,300</td>
<td>26</td>
<td>52</td>
<td>5</td>
<td>Pos.</td>
</tr>
<tr>
<td>Chupadero</td>
<td>1,700</td>
<td>30</td>
<td>57</td>
<td>4</td>
<td>Neg.</td>
</tr>
<tr>
<td>Chupadero Vat</td>
<td>600</td>
<td>24</td>
<td>25</td>
<td>2</td>
<td>Neg.</td>
</tr>
<tr>
<td>Cow Trap</td>
<td>615</td>
<td>40</td>
<td>16</td>
<td>6</td>
<td>Neg.</td>
</tr>
<tr>
<td>Diablo</td>
<td>5,000</td>
<td>26</td>
<td>193</td>
<td>11</td>
<td>Pos.</td>
</tr>
<tr>
<td>Dillon*</td>
<td>3,022</td>
<td>29</td>
<td>104</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>East Loza</td>
<td>3,000</td>
<td>62</td>
<td>49</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>1500 Acre Trap</td>
<td>1,500</td>
<td>16</td>
<td>94</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Horse Trap (N.)</td>
<td>800</td>
<td>30</td>
<td>27</td>
<td>2</td>
<td>Neg.</td>
</tr>
<tr>
<td>Horse Trap (S.)</td>
<td>200</td>
<td>36</td>
<td>6</td>
<td>4</td>
<td>Neg.</td>
</tr>
<tr>
<td>Leona</td>
<td>1,595</td>
<td>1,100</td>
<td>2</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Llano Trap</td>
<td>1,500</td>
<td>24</td>
<td>63</td>
<td>5</td>
<td>Pos.</td>
</tr>
<tr>
<td>Lower Guajolote*</td>
<td>2,219</td>
<td>36</td>
<td>62</td>
<td>5</td>
<td>Neg.</td>
</tr>
</tbody>
</table>
TABLE 1 - Continued

BRISCOE RANCH - Continued

<table>
<thead>
<tr>
<th>Pasture</th>
<th>Acreage</th>
<th>Acres/Deer</th>
<th>Total Deer</th>
<th>Deer Sampled</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Torodilla</td>
<td>1,950</td>
<td>36</td>
<td>53</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Middle Guajolote¹</td>
<td>3,075</td>
<td>14</td>
<td>220</td>
<td>14</td>
<td>Pos.</td>
</tr>
<tr>
<td>Middle Torodilla</td>
<td>2,072</td>
<td>80</td>
<td>26</td>
<td>6</td>
<td>Neg.</td>
</tr>
<tr>
<td>N. Lower Jardin*</td>
<td>4,200</td>
<td>13</td>
<td>324</td>
<td>16</td>
<td>Pos.</td>
</tr>
<tr>
<td>N. Loza</td>
<td>2,600</td>
<td>24</td>
<td>109</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Novia*</td>
<td>1,365</td>
<td>218</td>
<td>7</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Number 6 Trap</td>
<td>500</td>
<td>24</td>
<td>21</td>
<td>2</td>
<td>Neg.</td>
</tr>
<tr>
<td>Ojito</td>
<td>2,000</td>
<td>17</td>
<td>118</td>
<td>6</td>
<td>Neg.</td>
</tr>
<tr>
<td>Ojito Trap</td>
<td>700</td>
<td>17</td>
<td>41</td>
<td>2</td>
<td>Neg.</td>
</tr>
<tr>
<td>Pila*</td>
<td>1,300</td>
<td>73</td>
<td>18</td>
<td>5</td>
<td>Pos.</td>
</tr>
<tr>
<td>Quatalvo*</td>
<td>2,000</td>
<td>19</td>
<td>105</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>San Ambrosia</td>
<td>4,600</td>
<td>30</td>
<td>153</td>
<td>8</td>
<td>Neg.</td>
</tr>
<tr>
<td>San Lorenzo</td>
<td>1,664</td>
<td>153</td>
<td>11</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>San Lorenzo Trap</td>
<td>1,200</td>
<td>153</td>
<td>8</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>S. Lower Jardin*</td>
<td>2,500</td>
<td>13</td>
<td>193</td>
<td>10</td>
<td>Neg.</td>
</tr>
<tr>
<td>S. Loza*</td>
<td>5,500</td>
<td>96</td>
<td>58</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Toro</td>
<td>2,500</td>
<td>24</td>
<td>104</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Torodillo*</td>
<td>1,200</td>
<td>99</td>
<td>12</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>Upper Jardin*</td>
<td>3,200</td>
<td>15</td>
<td>214</td>
<td>12</td>
<td>Neg.</td>
</tr>
<tr>
<td>Upper Torodilla</td>
<td>1,935</td>
<td>53</td>
<td>37</td>
<td>6</td>
<td>Neg.</td>
</tr>
<tr>
<td>West East Loza</td>
<td>3,000</td>
<td>62</td>
<td>49</td>
<td>5</td>
<td>Neg.</td>
</tr>
<tr>
<td>West Loza</td>
<td>4,000</td>
<td>24</td>
<td>167</td>
<td>8</td>
<td>Neg.</td>
</tr>
<tr>
<td>Windmill*</td>
<td>2,640</td>
<td>33</td>
<td>80</td>
<td>9</td>
<td>Pos.</td>
</tr>
<tr>
<td>Worm Trap</td>
<td>200</td>
<td>24</td>
<td>9</td>
<td>2</td>
<td>Neg.</td>
</tr>
<tr>
<td>Totals:</td>
<td>84,382</td>
<td>3,042</td>
<td>241</td>
<td>(7.9%)</td>
<td>~</td>
</tr>
</tbody>
</table>

¹Middle Guajolote Pasture totally root plowed since aerial census.

*Fever tick infested pastures-cattle.

Summary: Total pastures/traps=52:297 samples taken
Tick infested pastures-cattle=16
Tick infested pastures-deer=10
Tick infested pastures-cattle/deer=6
### TABLE 2
Summary of APHIS/SCWDS Fever Tick Surveillance
White-tailed Deer - *Boophilus* Infested Pastures
Webb County, Texas (4/10/79 - 6/20/79)

<table>
<thead>
<tr>
<th>BRISCOE RANCH</th>
<th>Deer Sampled</th>
<th>Deer Positive</th>
<th>Pasture Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arroyo</td>
<td>5</td>
<td>3</td>
<td>cattle absent-not infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vacated 10/14/77</td>
</tr>
<tr>
<td>Bigford Trap*</td>
<td>6</td>
<td>2</td>
<td>cattle present-infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>quarantined 10/9/78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vacated 10/9/78 - 3/2/79</td>
</tr>
<tr>
<td>Bull Trap*</td>
<td>5</td>
<td>2</td>
<td>cattle present-infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>quarantined 9/20/78</td>
</tr>
<tr>
<td>Cabeza*</td>
<td>5</td>
<td>5</td>
<td>cattle present-infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>quarantined 9/29/78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vacated 12/9/78 - 1/22/79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dipped 3/2/79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cattle infested</td>
</tr>
<tr>
<td>Diablo</td>
<td>11</td>
<td>5</td>
<td>cattle present-not infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cattle inspected 5/10/79</td>
</tr>
<tr>
<td>Llano Trap</td>
<td>5</td>
<td>1</td>
<td>cattle absent-not infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vacated 1/6/77</td>
</tr>
<tr>
<td>Middle Guajolote</td>
<td>14</td>
<td>2</td>
<td>cattle present-not infested</td>
</tr>
<tr>
<td>N. Lower Jardin*</td>
<td>16</td>
<td>1</td>
<td>cattle present-infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>quarantined 2/21/79</td>
</tr>
<tr>
<td>Pila*</td>
<td>5</td>
<td>1</td>
<td>cattle present-infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>quarantined 9/21/78</td>
</tr>
<tr>
<td>Windmill*</td>
<td>9</td>
<td>7</td>
<td>cattle present-infested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>quarantined 1/25/79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dipped 5/2/79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ticks present 225/426</td>
</tr>
</tbody>
</table>

*Totals: 81 29 29

*Fever tick infested pastures-cattle.
TABLE 3
APHIS/SCWDS Fever Tick Surveillance
Cattle - Boophilus Infested Pastures
Webb County, Texas

<table>
<thead>
<tr>
<th>Pasture</th>
<th>Pasture Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camino</td>
<td>cattle absent 1/15/79</td>
</tr>
<tr>
<td></td>
<td>vacated 1/31/79</td>
</tr>
<tr>
<td>Tee Pee</td>
<td>cattle present 5/25/79</td>
</tr>
<tr>
<td>BRISCOE RANCH</td>
<td></td>
</tr>
<tr>
<td>Pasture</td>
<td>Pasture Status</td>
</tr>
<tr>
<td>Bigford Trap*</td>
<td>cattle present 10/9/78 - vacated 10/9/78 - 3/2/79</td>
</tr>
<tr>
<td>Bull Trap*</td>
<td>cattle present 9/20/78</td>
</tr>
<tr>
<td>Cabeza*</td>
<td>cattle present 9/20/78</td>
</tr>
<tr>
<td></td>
<td>vacated 12/9/78 - 1/22/79</td>
</tr>
<tr>
<td></td>
<td>dipped 3/2/79 - cattle infested</td>
</tr>
<tr>
<td>Dillon</td>
<td>cattle present 9/19/78</td>
</tr>
<tr>
<td>Lower Guajolote</td>
<td>cattle present 8/8/78 - reduced 10/31/78</td>
</tr>
<tr>
<td>N. Lower Jardin*</td>
<td>cattle present 2/21/79</td>
</tr>
<tr>
<td>Novia</td>
<td>cattle present 9/28/78</td>
</tr>
<tr>
<td>Pila*</td>
<td>cattle present 9/21/78</td>
</tr>
<tr>
<td>Quatralvo</td>
<td>cattle present 2/15/79</td>
</tr>
<tr>
<td>S. Lower Jardin</td>
<td>cattle present 2/21/79</td>
</tr>
<tr>
<td></td>
<td>became opened to N. Lower Jardin</td>
</tr>
<tr>
<td>S. Loza</td>
<td>cattle present 12/3/78</td>
</tr>
<tr>
<td>Torodillo</td>
<td>cattle absent 10/24/78 - vacated 11/2/78</td>
</tr>
<tr>
<td>Upper Jardin</td>
<td>cattle present 3/1/79</td>
</tr>
<tr>
<td>indmill*</td>
<td>cattle present 1/25/79</td>
</tr>
<tr>
<td></td>
<td>dipped 5/2/79 - 225 of 426 infested</td>
</tr>
</tbody>
</table>

*Fever tick infested pastures-deer.
REFERENCES


REPORT OF THE COMMITTEE ON WILDLIFE DISEASES

Chairman: F. A. Hayes, Athens, GA


On October 29, 1979, the Committee on Wildlife Diseases convened for the sixth time since its predecessor committee was established in 1975. The first item on this year's agenda was to review the committee report of 1978 and herein consider the status of each salient item presented during the previous meeting. Summary statements with a synopsis of action to date are cited as follows:

OLD BUSINESS:

A. Memorandum of Understanding between the Animal and Plant Health Inspection Service (APHIS) of USDA and the Fish and Wildlife Service (FWS) of USDI.

This memorandum of understanding has been finalized and signed by authorized officials of FWS and APHIS. Both federal agencies are commended by this committee for the foresight shown in this agreement.

Recommended Action: None required. Objectives accomplished.

B. Proposed Memorandum of Understanding and a Cooperative Agreement between APHIS and State Fish and Wildlife Agencies.

In the event of an animal disease emergency, e.g., introduction of foot-and-mouth disease, rinderpest, African swine fever, exotic Newcastle disease, etc., a majority of wildlife species is not covered by the aforementioned memorandum between FWS and APHIS. In the 1978 report from this committee, it was suggested that APHIS consider a similar memorandum of understanding with individual state wildlife agencies. Since then, APHIS has concurred with this need and for that of a cooperative agreement.

The Committee on Wildlife Diseases agrees in principle with this need, in addition to which a need for a cooperative agreement with APHIS is recognized. The latter is necessary in order to provide reimbursement to state wildlife agencies for services, supplies, and equipment rendered during a national animal disease emergency as declared by the Secretary of Agriculture.

Recommended Action: That this committee endorse the need for these two documents and suggest that APHIS personnel work with
the Fish and Wildlife Health Committee of the International Association of Fish and Wildlife Agencies (IAFWA) in developing a satisfactory model memorandum of understanding and cooperative agreement to be submitted to the Executive Committee of IAFWA during the March 1980 meeting at Miami Beach, Florida.

C. **Compensation for relocation of wildlife in the event of depopulation as an essential measure for preventing spread of a dangerous communicable disease.**

The pending need for provisions suggested by this summary statement was thoroughly reviewed in the 1978 report from this committee. Although progress has been made in this regard, objectiveness to date have not been realized.

Recommended Action: That APHIS continue pursuing whatever measures necessary for empowering the Secretary of Agriculture with standby authority to borrow from the Commodity Credit Corporation to compensate State Fish and Wildlife Agencies for relocation of wildlife following necessary depopulation in the event of a national animal disease emergency.

D. **African swine fever in the Western Hemisphere with potential involvement of wildlife of North America.**

A resolution was adopted at the 82nd Annual Convention of USAHA in support of all governmental agencies and biologically sound legislation directed toward preventing spread of African swine fever (ASF) in the Western Hemisphere.

Recommended Action: That this committee commend Emergency Programs of Veterinary Services, APHIS, USDA, for accomplishments to date on the Dominican Republic and further support all biologically and sociologically sound measures now in effect to eradicate ASF from the Island of Hispaniola.

This résumé of salient "old business" of the Committee on Wildlife Diseases has been presented as a possible remedy to previous shortcomings through which some committee reports have not been translated into the action visualized by the committee during its deliberations. It is recommended that hereafter this become standard policy of the Committee on Wildlife Diseases.

**NEW BUSINESS:**

A. **Wildlife involvement in the Boophilus tick eradication program in Texas.**

This committee was fortunate in having as a guest speaker Dr. John H. Gray, Regional Epidemiologist for Veterinary Services, APHIS, USDA. Dr. Gray presented a detailed account on the current status of cattle tick fever (*Boophilus annulatus*) eradication in Texas, with specific emphasis on wildlife surveillance. This report also is scheduled for presentation before the General Session of the 83rd
Annual Meeting of USAHA to be included in the proceedings of this conference.

After considerable discussion following Dr. Gray's excellent report, the membership of this committee unanimously concurred in commending all parties involved in the current eradication program. Specific recognition is given to the cattlemen in the affected area, who are sharing the brunt of burden and responsibility in combating this threat to a significant segment of the domestic livestock and big game animal resources of the United States. Appreciation also is expressed for the excellent cooperation from the Texas Parks and Wildlife Department, Austin, Texas, through which extensive wildlife surveillance is being coordinated and realized.

Recommended Action: That a resolution in support of cattle fever tick eradication and control in Texas be adopted by the United States Animal Health Association.

B. Wildlife disease surveillance to augment Veterinary Services' animal disease control and eradication programs.

Regulatory veterinarians of this and numerous other countries of the world have long recognized that the success or failure of domestic livestock and poultry disease control or eradication programs often is interdependent upon the occurrence of infectious diseases in wild animals either as principal reservoirs or amplifying hosts. Some historic and current examples of this concern are foot-and-mouth disease on the Stanislaus National Forest, California, in 1924; cattle fever ticks in Florida during the late '30s and early 40s; Venezuelan equine encephalomyelitis in Texas during the summer of 1971; exotic Newcastle disease in California, Florida, and Texas in 1972-73; recent introduction of African swine fever into the Dominican Republic and Haiti; the current cattle fever tick situation in Texas; prevalence of pseudorabies and brucellosis in wild and domestic swine of the United States; and bluetongue in wild Cervidae, cattle, and sheep of all regions of this country. Indigenous livestock disease control programs are hampered because of inadequate information on possible wildlife involvement, and eradication of exotic livestock diseases well may be entirely dependent upon acquisition of information in this area. Few animal disease control or eradication programs can be expected to be successful without adequate epizootiologic information on a variety of wild birds and mammals.

Monitoring and surveillance of wildlife populations with parallel transmission studies are necessary prerequisites to prevention and control of indigenous and exotic diseases of major significance to animals and man. These collective efforts may mean the difference between successful eradication or allowing an exotic disease to become established.

It is for these and numerous other reasons that on October 2, 1979,
the USDA, APHIS, Advisory Committee on Foreign Animal and Poultry Diseases introduced and unanimously adopted the following resolution:

WHEREAS, the wildlife fauna of North America share varying degrees of susceptibility to numerous native or foreign diseases that affect domestic livestock and poultry; and

WHEREAS, wildlife populations frequently interact in a multiplicity of ways with domestic livestock and poultry that afford opportunities for disease transmission; and

WHEREAS, the epizootiologies of many indigenous animal diseases are such that wildlife may serve as latent carriers of diseases of domestic livestock and poultry; and

WHEREAS, significant numbers of questions concerning wildlife involvement remain unanswered for current State/Federal cooperative disease control or eradication programs; and

WHEREAS, upon introduction into the United States, many of more than forty major foreign animal diseases could be harbored and spread by native wild birds and mammals; and

WHEREAS, in the event of foreign animal disease introduction, there will be an immediate need for disease surveillance among potentially exposed wildlife populations; and

WHEREAS, past and current wildlife disease surveillance has been vital in planning and implementing domestic livestock and poultry disease control or eradication programs;

NOW BE IT RESOLVED, that this Advisory Committee on Foreign Animal and Poultry Diseases recommends that Veterinary Services, APHIS, USDA, seek immediate means for providing sufficient funding to establish and maintain an efficient program for monitoring and investigating diseases of wildlife that potentially relate to domestic livestock and poultry.

Recommended Action: That further credence be reflected on the above resolution through adoption of an identical resolution by the United States Animal Health Association.

C. General considerations relating to legal annual harvest of surplus wildlife populations.

American sportsmen are this nation's foremost conservationists. Fees paid for hunting, fishing, and trapping licenses constitute the major financial support of wildlife conservation in this country. Yet, a growing number of self-styled animal preservationists intend to abolish all that has been accomplished.

The anti-game management crusade in this country is master-minded by richly funded national and international organizations. Financial support, running into millions of dollars annually, is
acquired through crusading advertising campaigns based on emotional appeals that: (1) wildlife should have the same rights as humans, is cute, possesses human characteristics a la Disney, and the American sportsman is cruel and inhumane; or (2) wildlife is endangered and in dire need of protection from greedy agribusinessmen, furriers, commercial fishermen, and bloodlusting hunters. Contributors typically have no vote on how their donations are used. Many assume from the advertisements that the money is used for conservation purposes. In reality, it is used primarily for administration, fund-raising, more public relations crusades, and for political activities.

The anti's systematically fill the media with horror stories of alleged wildlife cruelty and exploitation. They consistently pressure state legislatures and the Congress to adopt anti-hunting, anti-trapping, and anti-scientific wildlife management measures. They methodically bring court suits to harass and burden federal and state wildlife agencies throughout America. Through such highly organized enterprises supported by an ever-increasing number of well meaning but misinformed people, millions of dollars are spent for accusation, for advertising, and for lobbying ... but very little is spent for constructive conservation measures that will benefit our wildlife resources.

The anti-trapping crusade continues to be the self-styled anti's favorite strategy. Abolition of leghold traps will not accomplish the noble purposes suggested. Instead, trapping will be replaced by over population, with the final outcome being famine and disease involving numerous wildlife species. In addition, unrestrained wildlife populations will create an untenable situation which wildlife interests, livestock producers, row crop farmers, and consumers of agricultural products will be unable to tolerate.

Trapping ban efforts are underway throughout the United States ... by legislation and even by constitutional amendment. Sportsmen recognize these efforts merely as the tip of an iceberg that comprises a concerted first step toward stopping the consumptive use of all renewable wildlife resources and abolishing the fundamental principles of wildlife management. Hound dog hunting will be next on the agenda, followed by legislation against bow hunting, then the shotgun, and finally the rifle, to once and for all end sport hunting in this country.

This committee does not condone suffering and inhumanities any more than the most avid protectionists. It is for this and many other reasons that trapping and sport hunting are chosen as the most effective and humane means of controlling wild animal populations ... in lieu of disease and pestilence accompanied by a complete breakdown in wildlife conservation throughout the United States.
To reaffirm this committee's position in the above regard, it is recommended that the United States Animal Health Association use its influence in urging all contiguous states of the United States to enact laws on the time interval between establishing a land trapset and that time when the trap is checked for humane dispatchment or release of any animal that has been caught. Unless obviously impractical as a consequence of adverse weather conditions, tides of the oceans, or remoteness of areas designated by states in the West and in the northern sections of the North Central and Northeastern States, this period of time should not exceed 24 hours. In select areas or sectors delineated in the above designated exceptions, the time interval in question should not exceed 48 hours.

Recommended Action: That a resolution in support of state and federal authority for scientific management of this nation's wildlife resources be adopted by the United States Animal Health Association.
84th ANNUAL MEETING
November 2-7, 1980
GALT HOUSE HOTEL
Louisville, Kentucky

85th ANNUAL MEETING
October 11-16, 1981
SHERATON HOTEL
St. Louis, Missouri

86th ANNUAL MEETING
November 7-12, 1982
RADISSON PLAZA HOTEL
and
HYATT REGENCY HOTEL
Nashville, Tennessee