PROCEEDINGS

EIGHTY-EIGHTH ANNUAL MEETING

of the

UNITED STATES ANIMAL HEALTH ASSOCIATION

P.O. Box 28176
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THE HYATT REGENCY FORT WORTH HOTEL
Fort Worth, Texas
October 21-26, 1984
This 1984 Proceedings of the U.S.A.H.A. is dedicated to the memory of Dr. Leonard A. Rosner, Jefferson City, Missouri and Dr. William R. Hinshaw, Frederick, Maryland.

These distinguished veterinarians served the U.S.A.H.A. in leadership roles for many years as members and/or chairman of committees. Dr. Rosner was President of U.S.A.H.A. in 1964 and represented Missouri on the Executive Committee.

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<th>City, State</th>
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<tr>
<td>W. W. Adams</td>
<td>Gainesville, GA</td>
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<td>R. E. Baer</td>
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<td>C. W. Beard</td>
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<td>S. B. Clubb</td>
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<td>J. O. Pearce, Jr.</td>
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<td>J. R. Ragan</td>
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<td>S. T. Wilson, Jr.</td>
<td>Washington, DC</td>
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<td>George Edwards</td>
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<td>D. D. Gingerich</td>
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<td>L. W. Hinchman</td>
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<td>Donald Hoogestraat</td>
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<td>C. L. Kanitz</td>
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<td>C. W. Monsees</td>
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<td>Thomas Thurber</td>
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<td>Willard Waldo</td>
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<td>F. M. Applehans</td>
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<td>Neal Black</td>
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<td>R. L. West</td>
<td>Schaumburg, IL</td>
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<td>Hans Drayer</td>
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<td>Fort Dodge, IA</td>
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<td>Al Strating</td>
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<td>R. D. Glock</td>
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<td>C. D. Murphy</td>
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<td>C. R. Weston</td>
<td>Walpole, NH</td>
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Gene Erickson, Ames, IA
D. P. Gustafson, Lafayette, IN

R. E. Hall, Madison, WI
D. L. Harris, Rothville, MO
G. W. Hausman, Ames, IA
H. T. Hill, Ames, IA
C. L. Kanitz, W. Lafayette, IN
M. H. Lang, Des Moines, IA
Norman Lichtman, Sewell, NJ
Vincent Marshall, Omaha, NE
J. W. McVicar, Southold, NY
C. W. Monsees, Jefferson City, MO
Committee on Tuberculosis and Johne's Disease—1985

Chairman: S. B. Hurley, Madison, WI
Vice Chairman: V. P. LaBranche, Boston, MA

Committee on Wildlife Diseases—1985

Chairman: E. Tom Thorne, Laramie, WY
Vice Chairman: V. F. Nettles, Athens, GA
Committee on Zoological Animals–1985

Chairman: M. S. Silberman, Atlanta, GA
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Milton Friend, Madison, WI
Allan Furr, Laurel, MD
E. E. Grass, Fair Oaks, CA
D. E. Herrick, Bowie, MD

Werner Heuschele, San Diego, CA
C. J. Mikel, Oklahoma City, OK
G. W. Patterson, Brentwood, TX
J. B. Payeur, Burleson, TX
G. P. Pierson, Glenn Dale, MD
Jeanne Roush, Washington, DC
K. C. Sherman, Topeka, KS
E. Tom Thorne, Laramie, WY
D. J. Williams, Athens, GA

R. J. Yedloutschnig, Southold, NY
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P. E. BRADSHAW  
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J. C. SHOOK  
*Secretary-Treasurer*
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<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tbody>
<tr>
<td>Sept. 27-28, 1897†</td>
<td>Fort Worth, Tex.</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. D. O. Lively, Fort Worth, Tex.</td>
</tr>
<tr>
<td>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>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>Oct. 2-3, 1900</td>
<td>Louisville, Ky.</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>Oct. 8-9, 1901</td>
<td>Buffalo, N.Y.</td>
<td>Dr. E. P. Niles, Va.</td>
<td>Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>Sept. 23-24, 1902</td>
<td>Wichita, Kan.</td>
<td>Mr. W. H. Dunn, Tenn.</td>
<td>Mr. Wm. P. Smith, Monticello, Ill.</td>
</tr>
<tr>
<td>Aug. 23-24, 1904</td>
<td>St. Louis, Mo.</td>
<td>Dr. J. C. Norton, Ariz.</td>
<td>Mr. Wm. P. Smith, Monticello, Ill.</td>
</tr>
<tr>
<td>Aug. 15-16, 1905</td>
<td>Guthrie, Okla.</td>
<td>Mr. Wm. P. Smith, Monticello, Ill.</td>
<td>Dr. S. H. Ward, St. Paul, Minn.</td>
</tr>
<tr>
<td>Sept. 16-17, 1907</td>
<td>Richmond, Va.</td>
<td>Dr. D. F. Luckey, Columbia, Mo.</td>
<td>Dr. C. E. Cotton, St. Paul, Minn.</td>
</tr>
<tr>
<td>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>Dec. 5-7, 1910</td>
<td>Chicago, Ill.</td>
<td>Dr. C. E. Cotton, St. Paul, Ill.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>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>Dec. 3-5, 1912</td>
<td>Chicago, Ill.</td>
<td>Dr. Macyck P. Ravenel, Madison, Wis.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Dec. 2-4, 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>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>Dec. 2-3, 1915</td>
<td>Chicago, Ill.</td>
<td>Dr. J. I. Gibson, Des Moines, Iowa</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Dec. 5-7, 1916</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Springfield, Ill.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Dec. 3-5, 1917</td>
<td>Chicago, Ill.</td>
<td>Dr. J. G. Wills, Albany, N.Y.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Dec. 2-4, 1918</td>
<td>Chicago, Ill.</td>
<td>Dr. M. Jacob, Knoxville, Tenn.</td>
<td>Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Nov. 29-30-Dec. 1, 1920</td>
<td>Chicago, Ill.</td>
<td>Dr. S. F. Musseiman, Frankfort, Ky.</td>
<td>Dr. D. M. Campbell, Chicago, Ill.</td>
</tr>
<tr>
<td>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>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>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>
</tr>
<tr>
<td>Dec. 3-5, 1924</td>
<td>Chicago, Ill.</td>
<td>Dr. J. G. Ferneyhough, Richmond, Va.</td>
<td>Dr. O. E. Dyson, Kansas City, Mo.</td>
</tr>
<tr>
<td>Nov. 30-Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>Dr. L. Van Es, Lincoln, Neb.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
</tr>
<tr>
<td>Dec. 4-6, 1929</td>
<td>Chicago, Ill.</td>
<td>Dr. Chas. G. Lamb, Denver, Colo.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
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<td>44. Dec. 4-6, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wyo.</td>
<td>Dr. Mark Welsh, College Park, Md.</td>
</tr>
<tr>
<td>45. Dec. 3-5, 1941</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. A. Crossman, Boston, Mass.</td>
<td>Dr. Mark Welsh, College Park, Md.</td>
</tr>
<tr>
<td>46. Dec. 2-4, 1942</td>
<td>Chicago, Ill.</td>
<td>*Dr. I. S. McAdory, Auburn, Ala.</td>
<td>Dr. Mark Welsh, College Park, Md.</td>
</tr>
<tr>
<td>47. Dec. 1-3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>50. Dec. 4-6, 1946</td>
<td>Chicago, Ill.</td>
<td>*Dr. William Moore, Raleigh, N.C.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>53. Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. J. P. Brandenburg, Bismarck, N.D.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>57. Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>58. Nov. 10-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W. Va.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</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>
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Dr. J. W. Connaway, Columbia, Md.
*Dr. A. E. Wight, Wash., D.C.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. Walter Wisnicky, Madison, Wis.
*Dr. L. Enos Day, Chicago, Ill.
*Dr. J. L. Axby, Indianapolis, Ind.
*Dr. H. D. Port, Cheyenne, Wyo.
*Dr. E. A. Crossman, Boston, Mass.
*Dr. R. W. Smith, Concord, N.H.
*Dr. E. Westmoreland, Frankfort, Ky.
*Dr. J. L. Miller, Topeka, Kan.
*Dr. William Moore, Raleigh, N.C.
*Mr. W. B. Miller, Topeka, Kan.
*Dr. A. R. Snoddy, Salt Lake City, Utah
*Dr. J. P. Brandenburg, Bismarck, N.D.
*Dr. C. P. Bishop, Harrisburg, Pa.
*Mr. F. E. Mollin, Denver, Colo.
*Dr. Ralph L. West, St. Paul, Minn.
*Dr. T. Childs, Ottawa, Canada
*Dr. T. C. Green, Charleston, W. Va.
*Dr. H. F. Wilkins, Helena, Mont.
*Dr. A. L. Brueckner, Baltimore, Md.
*Dr. G. H. Good, Cheyenne, Wyo.
*Dr. John G. Milligan, Montgomery, Ala.
*Mr. F. G. Buzzell, Augusta, Me.
*Dr. J. R. Hay, Chicago, Ill.
*Dr. A. P. Schneider, Boise, Idaho
*Dr. W. L. Bendix, Richmond, Va.
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<tr>
<td>80. Nov. 7-12, 1976</td>
<td>Miami Beach, Fla.</td>
<td>H. E. Goldstein, Columbus, Ohio</td>
<td>80. Nov. 7-12, 1976</td>
</tr>
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+This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION AND MEMORIAL SERVICE

A. J. Roth, DVM
Richmond, Virginia

Heavenly Father, we thank Thee for the honor of being together at the 88th annual meeting of the United States Animal Health Association and the 27th annual conference of the American Association of Veterinary Laboratory Diagnosticians. We feel very fortunate to be able to feature this week 100 years of Animal Health. Hopefully you will guide and assist us in the making of decisions and developing policies that will be beneficial and rewarding to the livestock and poultry industry which we serve.

We are so appreciative of the guidance you have provided us in the past. May it continue for this meeting and other animal health meetings in the future.

Amen

Memorial Service

Mr. President, Members of the Association, Ladies and Gentlemen:

Each year the United States Animal Health Association takes time to pay tribute to those members who have passed away since our last meeting. They are:

Dr. Alan E. George — Mt. Airy, Maryland — February 6, 1983
Dr. Sam Guss — State College, Pennsylvania — January 7, 1984
Dr. Leonard Rosner — Jefferson City, Missouri — February 21, 1984
Dr. William R. Hinshaw — Frederick, Maryland — April 18, 1984
Dr. Howard Johnson — Onancock, Virginia — April, 1984
Dr. B. D. Spahr — Lincoln, Nebraska — August 4, 1984
Dr. Robert Morgan — Bedford, Texas — September 12, 1984

Would everyone please rise and bow your head for a moment of silent prayer.

Amen
WELCOME TO TEXAS
Robert F. Bartlett, Chairman
Texas Animal Health Commission
Austin, TX

I would like to express the regret and the good wishes of Governor Mark White who is unable to be present with us this evening and for whom I am substituting. I also want to take this opportunity to thank you for selecting Texas as your meeting place. I understand you have not met in Texas for more than 87 years, your last meeting having been held here in 1897. I hope we treat you better this time and your visits won’t be so far apart in the future. As Chairman of the Texas Animal Health Commission and as a Texan myself, I cannot pass up the opportunity to give ya’ll a few basic and interesting facts about our great State.

With over 267,000 square miles in area and 254 counties, Texas is number one in a number of categories relating to agriculture:

1. First in the number of cattle and calves.
2. First in every category relating to sheep and goats.
3. First in the number of farms and ranches as well as farm and ranch land.
4. First in cattle and calves on feed.
5. First in cash receipts for sale of cattle and calves.

Though agriculture remains a vital part of our Texas economy we are a changing state. In the last two generations we have moved from 80% rural population to 80% urban. Texas is the fastest growing state in the nation at twice the national average to the point that today we are the third most populous state. This increase is a result of the strength of our economy and the rapid growth of “high tech” industry and centers of knowledge.

The Governor and I hope your stay in Texas will be enjoyable and you can find time to visit some of the historical points of interest as well as enjoy the other advantages of Fort Worth. You are not very far from a historical site, the Fort Worth Stockyards, founded in 1889. In October 1897, at these yards, a meeting took place that led to the formation of the United States Animal Health Association (USAHA). Present were representatives from six states, the Oklahoma Territory and the Bureau of Animal Industry. Therefore, it is fitting and proper that special recognition be called to that historical meeting. It is my pleasure at this time to recognize the states and the United States Department of Agriculture (USDA) who were involved in the formation of what we know as the USAHA. As I call out the names I would appreciate it if a representative from these states would please join me: Illinois, Kansas, Nebraska, Missouri, Colorado, Texas, Oklahoma and the USDA. I have an official memorandum from Governor White recognizing those states and the USDA. Let me now read the document.
On September 27 and 28, 1897, representatives of Livestock Sanitary Boards from Illinois, Kansas, Missouri, Nebraska, Oklahoma Territory, Colorado, Texas, and the Bureau of Animal Industry met at the Stockyards in Fort Worth.

An organization, which later become known as the United States Animal Health Association, was founded based on a common charge to the officers and boards of the various states in controlling and eradicating contagious and communicable disease in domestic livestock. Thorough organization was deemed necessary for disease programs as well as the cooperation of the Livestock Sanitary Board of all states with the Bureau of Animal Industry in the United States Department of Agriculture.

It is fitting and proper that during this Eighty-eighth Annual Meeting of the United States Animal Health Association, we commend the Association for its efforts to control disease and increase production of healthy domestic livestock. It is also fitting and proper that we call attention to those states who in 1897 founded the Association.

Therefore, I, as Governor of Texas, do hereby proclaim the week of October 22, 1984, as ANIMAL HEALTH WEEK in Texas.”

It gives me great pleasure to give you these tokens of recognition for all the efforts this association has made toward better animal health in the United States.

In closing, let me remind you in 1986 Texas celebrates the 150th anniversary of its independence. In 1836 the Republic of Texas declared its independence from Mexico. The Sesquicentennial Commission was created by the 66th Legislature to coordinate the celebrations which will highlight the historic, patriotic, economic, cultural, environmental and social aspects of Texas. We hope you will return in 1986 to help us celebrate our 150th anniversary.

Thank you for being here with us in Texas!
RESPONSE TO WELCOME
Joan M. Arnoldi, DVM

Mr. Bartlett, on behalf of the membership and guests of the USAHA and AAVLD, I wish to thank you for your warm welcome to Texas.

TO THOSE OF US FROM THE NORTH COUNTRY — TEXANS HAVE LONG BEEN NOTED FOR THEIR HOSPITALITY. IF THESE PAST TWO DAYS ARE ANY INDICATION — THEN THAT LONG-STANDING REPUTATION IS CERTAINLY HOLDING TRUE AND WE ARE LOOKING FORWARD TO THE REMAINDER OF THIS WEEK.

As a relative newcomer to these two great organizations, I believe I have a different perspective from that of my predecessor — I am humbled by both the diversity and magnitude of your accomplishments. You have indeed made it difficult for those of us recently recruited; you all have provided us with a hard act to follow, and a true challenge to provide the same caliber of direction and leadership in the control of animal diseases in the eighties, nineties and beyond. I, for one, don't take that responsibility lightly. The challenges will be different, but the responsibilities are as crucial as they have been in the past.

How well we meet these challenges may well have a significant impact on the economy of this country as we work toward providing healthy animals and animal products not only for this country, as in the past, but also to satisfactorily compete in the world market.

Only by continued cooperative efforts in meetings such as these involving industry, federal and state governments can we achieve these goals.

Now, on behalf of America's Dairyland, the Great State of Wisconsin, I wish to extend an invitation to join us for this meeting in 1985 in Milwaukee, Wisconsin. We look forward to providing you with true Wisconsin GemutlichKeit and another productive meeting in 1985.
100 YEARS OF ANIMAL HEALTH
(THE ROLE OF USAHA)

By Dr. Frank J. Mulhern
Former Director Animal Health
Inter-American Institute for
Cooperation on Agriculture
Former Administrator USDA–APHIS

You have seen the documentary that describes the advances of Animal Health by the Bureau of Animal Industry. I wish to spend some time dwelling on the interrelationships and accomplishments of the Bureau of Animal Industry and what is now called the United States Animal Health Association. It's important to do this because this interdependence of the two organizations was largely responsible for the significant accomplishments that have been achieved.

There are two purposes in mind for focusing on these relationships: One, many of us grew up, so to speak, either a part of these two organizations or associated with them. We have been affected one way or another by their existence so they are part of our “roots” in our development over the years. I want to reflect on that and recommend how we should react to these roots. Second, as Santayana said years ago, “Unless we recall the past we will be forced to relive it.” When we examine the past and study the events surely we can profit by the many experiences that were successful and not have to repeat those that were not.

Having made a recent review of the history of these organizations, it was obvious that during the past hundred years, one organization needed the other as well as the organizations and individuals that were involved and that premise is still true today. Back in the late 1850's some state leaders pleaded to have a federal veterinary force established to combat contagious pleural pneumonia. States like Massachusetts and others recognized that because of interstate movements, they were not able to effectively prevent or control the spread of animal diseases individually.

As Great Britain was taking action to prevent imports of cattle from the United States because of contagious pleural pneumonia, Congress responded to the demands being made and established the Bureau of Animal Industry. Prior to that time, the states had become quite frustrated trying to get on top of the situation.

As the record shows by 1892, the disease was eradicated. Few countries in the world at that time would have accepted the original challenge of eradication as a realistic goal but being relatively a nation in its embryo-state, they didn’t know any better, so they did it. I repeat they didn’t know it couldn’t be done and they did it. I repeated this for emphasis because many times we don’t challenge a problem because we think we know so much about it that such an objective would be impossible to reach, so we don’t even try.
However, reaching that objective must have convinced others to consider that such an approach would apply to other diseases and pests. It must have had a positive effect on the attitude of Congress because they heaped a great deal of praise upon those who conducted the program.

Surely the effective results of that program must have been in the minds of the leaders who called for a meeting of the Interstate Association of Livestock Sanitary Boards to be held at Fort Worth, Texas November 27–28, 1897.

It is noted that the first meeting was called to order by Robert J. Kleberg, outstanding cattleman who was the owner of the King Ranch, but also represented The Texas Livestock Board. This was a meeting of state livestock boards led by a mixture of cattlemen, state veterinarians and Bureau of Animal Industry veterinarians and researchers from the experimental stations of the Universities of Texas and Missouri.

In 1887, the Bureau of Animal Industry working with the Missouri Experiment Station and the Texas Experiment Station developed a method to prove that dipping cattle to rid them of ticks was effective. This was a major breakthrough. The records shows that ticks as the cause of tick fever was claimed to be true in a meeting of cattlemen at Springfield, Illinois as early as 1868. Naturally even after eleven years and the research was completed, the concept was not readily acceptable. “Preposterous” said one official. “Makes no sense” said another.

Mr. Kleberg had requested that the Secretary of Agriculture seek funds to combat Texas Fever. This was an influential cattleman doing all he could to get some constructive action going to combat a pest that was causing great losses domestically. When I recall this action and the participation of people like John Armstrong, J. O. Pearce, Bert Hawkins, Jack Dahl, etc., in the Brucellosis program it makes me wonder, “what is new?”

Referring to “ROOTS,” there was a national problem confronting the livestock industry. In order to combat it, leaders of the livestock industry, State and Federal governments and universities became involved and developed a workable program. This was eighty-seven years ago and it’s not too different as to how programs are initiated today.

These types of meetings and others that followed allowed for open communication despite differences of opinion and motivation. The reason for the successful record was that there was an overriding commitment by most of the leadership to reach the objective. Despite obstacles known to those with vested interest, the leaders always found ways to progress through modifications of programs that would still accomplish the final objective.

Motivation when a disease is on a rampage and the losses are obvious is easy except to those who are overwhelmed by them. No one needs to be impressed with the data to become alarmed when large numbers of animals are sick and dying. Most people are eager to respond to leadership that will show the way to solve the crisis. The real challenge in any
eradication program is the motivation to complete the job. It is the ability to overcome the final or apathy state of such programs.

Read the history of tick eradication that began in 1897 and it is obvious what the frustration of producers must have been when they were trying to raise the market cattle in the tick area or ship cattle to the north of the tick area. People like Kleberg fully realized that if the cattle industry in the southern states was going to develop, tick fever had to be eradicated. To achieve that many thought it would be impossible. It would require good epidemiology to identify specific problems as they were encountered and identify research needs. It would require a program that would be drastic and enforceable but it must be practical enough that livestock owners could comply. More importantly, in order to acquire cooperation of those involved, it was absolutely necessary that they participate in the planning, the modifications of the program and above all be kept currently informed.

When one reads the history, the pattern of the programs of the past repeat themselves. For example, there were those who grew up with ticks and tick fever who considered them part of the environment. These were pests sent there by God and this balance of nature was not to be tampered with. There were those like Mr. Kleberg who said “If I can free several thousand acres, I can free the rest.” Others who said, “let’s define the problem first through research” (hopefully we won’t have to do anything). There were others who said, “Lets do something now and have research continue along with it.”

Read the history of tick eradication and recognize the gratification that took place by owners, regulatory officials and researchers as States started to free their lands of tick and tick fever. What a tremendous effort this must have been. How satisfying it must have been as they recognized that what appeared to be impossible when they began gradually was being accomplished before their very eyes.

People were realizing that a miracle was taking place. You could ship cattle from the tick infested states into those that were free and not produce disease and death losses. You could not raise cattle and develop breeding programs for pure bred cattle in the tick infested states without having them wiped out with tick fever. Read the annual reports about the early optimism of some and the pessimism of others and eventually the exhilaration when the good results come forth.

Dr. Peter F. Bahnsen, State Veterinary of Georgia said in 1915, “Had this association accomplished nothing except to aid in advancing the cause of tick eradication it would not have been created in vain. But it has done more — much more. As an organization we may point with pride to the better understanding and a determination to help one another which is a natural outgrowth of our annual meetings and discussions. Not only do we understand each other better but the public understands us better and respects us more.”
Later in this history as time marched on some change in attitude was taking place. During the USLSA meeting of 1925 Dr. Cary of Alabama complains that the organization looks at tick eradication as a chronic situation of the south. He threatened to leave the association and form another association because they were being ignored. He complained that the Federal tick law was outdated. He said, “Last year and the year before there were trainloads of cattle shipped through Alabama to Texas and other states. They came from down there where they have never done anything about tick eradication. If state “X” sits there and has a territory where it is not doing anything then let them keep its cattle. It is not going to ship anyone through my state.” He complained about changing state veterinarians every two years. He said they couldn’t learn what they needed to learn about tick eradication in two years.

He said, “You ask what we want? We want your backing and your stimulation and your help to push this thing over and get rid of it. Why? We are going to have thousands and thousands of you northern people looking to the south to raise cattle. Why? It is cattle country. You need not side track it right here by trying to side track our little talks here in the meeting. You are going to have to fight it. I want to warn you again, if you don’t give us a better hearing next year on this program we are going to quit you and go where we can get it.”

Also in 1925, Dr. J. H. Bux, State Veterinarian of Arkansas said, “The indifference in mind in tick eradication is that manifested by the general lack of interest of members of this association especially the livestock sanitary officials of the various tick free states is the problem of cattle tick eradication in the remaining tick infested areas of the south.”

As you can imagine since the tick program was initiated other disease and health issues surfaced and demanded the major attention of the state officials as well as the public and the industries that were involved. For example, the so-called “WHITE PLAGUE” or tuberculosis both in people, cattle and swine was everywhere not to mention glanders in horses at a time when horses were being used extensively for work and transportation.

Also the meat scandal was uppermost in everyone’s mind. In 1907, Dr. D. F. Luckey of Columbus, Missouri said, “During the present year we have witnessed the installation by the federal government of the most perfect system of meat inspection now in existence in the world.” At that time he urged the states to set up their meat inspection systems. Also he urged that in the control and eradication of contagious diseases of animals and the encouragement of livestock protection there ought to be perfect cooperation between states and the federal government. He added, “The social feature of a meeting of this kind is of great importance. We get inspiration from one another and learn from one another how to deal with various vexatious problems which arise in our work.”

In 1925, Dr. Veranus A. Moore, State Veterinarian of New York commented. There is no other protection service in connection with food
production that has been more difficult to establish that has had greater obstacles to overcome that has made more rapid progress and that has protected more people than our present meat inspection program.

During this same period horses in the big cities were coming down with glanders in great numbers. In addition human cases had occurred with many deaths being recorded so these are some of the reasons the focus of this association was being diverted from the tick eradication program.

Dr. John R. Mohler, Chief of the BAI at the 1926 meeting made these remarks, “The real test of ability among individuals in an organization is not so much in conceiving and understanding work as it is preserving the initial enthusiasm until the job is finished.” (That should ring all the bells.) The biggest challenge that has faced the Brucellosis program has not occurred in the past it is right “now.” How do you get the enthusiastic support and commitment to finish the job as soon as it is practical to do so?

Aren’t the conditions repeating themselves in the Brucellosis Program? Haven’t we been through the stage where losses were abundant and programs were initiated. Haven’t we been through the stage whereby many of the States are free. Hasn’t indifference crept into our reaction to the need to finish the job as soon as possible. Hopefully at this meeting we will set the goal to free two thirds of the States as soon as possible. Setting goals is relatively easy but commitment of our effort to see that goals are met — is what’s needed. Our predecessors met this challenge. Are we willing to do the same? Can’t we ask ourselves the same question about Tuberculosis eradication?

Dr. Peter F. Bahnsen, state veterinarian of Georgia, gave some important words of advice in 1915. Associations grow more numerous day by day, the very atmosphere in which we live breeds organizations but one after one, old and many newly hatched associations die of inertia or their memberships are assimilated by other, more active organizations.

The United States Livestock Sanitary Association, what a wonderful scope for useful activity, yet how specific its very purpose. Its preamble fixed by the constitutions reads as follows.

“The purpose of this association shall be the study of sanitary science and the dissemination of information and methods pertaining to the control and eradication of infectious diseases amongst livestock.”

“It is true many bodies have assembled and organized on principles so broad and interests so diversified as to force the various special interest in question to segregate. This last named condition often happens where special interest in one form or another are to be served. But is far better that all factions be represented. Nothing helps in broad public questions like the “Get together and pull together spirit.”

“Pull together” was the needed ingredient but all recognized that in order for this to happen effective communication needed high priority. The editors of the agricultural press were very involved in the organization and contributed significantly to its progress by pointing out its deficiencies in
communication. The editor of the Twentieth Century Farmer Magazine, said something like this, the tuberculin test is not 100 percent so quit telling producers that it is. They can still appreciate it if it is only 95 percent accurate. Tuberculin is being used to prevent reactions to the test and these cattle are then sold as negative and introduce tuberculosis in herds. There are unscrupulous people in the industry both cattlemen and veterinarians — let's face up to it. State veterinarians and other sanitary officials could do wonders in the way of satisfying the stockmen and getting their cooperation if they would be more diplomatic. An official order can be so worded that it will at once make the man affected want to fight or it can be just as official and produce the desired result by clothing it in language that will make the stock owner feel that the official is his true friend and is really trying to assist him instead of bearing down on him.

This occurred in 1917 and some of the same attitudes were uncovered when the Brucellosis Advisory Committee made their review in 1978.

In 1921, Dr. Kierran, Chief of TB eradication said, "There is no element in this work that is forcing it to be done. There is no law compelling this work to be done. There is but one element and that is public sentiment and the support of the livestock industry.” In all of the successful programs of the past, this ingredient stands out as one of the most important factors.

Another unrelated point but one that makes me wonder if any change is new. In 1919 at one of the first meetings of a consortium of farm organizations that later became the Farm Bureau Federation the following statement was made “As a result of this condition we have grown lopsided in our national viewpoint towards agriculture. We are just beginning to find out that a cheap food supply in this country is a thing of the past and we must look forward to some new arrangement whereby we can so safeguard the interest of agriculture and give a square deal to the farmer so that people will stay on the farm.”

In 1920 the editor of Hoard’s Dairyman stated, “We need to broaden the United States Livestock Sanitary Association to include feeds and feeding, etc. We need to bring resource speakers to broaden our outlook in areas like nutrition and genetics. We need three types of programs:

1. Those that facilitate the work of regulatory officials.
2. Those that are designed to discover and establish scientific data.
3. Those that will disseminate information through the Agriculture Press and veterinary practitioners.

Leaders recognized the need for the content of these meetings to change with the times.

Then in 1920, the secretary's report among his recommendation included one that we will recognize. “During the year I have been impressed more than ever with the need of a central agency for the collection of animal morbidity and mortality statistics.” Seems in the year of 1984, we are still struggling with this problem.
The sequence of problems encountered in tick eradication applied equally to tuberculosis eradication. Even though it was discussed at the first meeting of the Livestock Boards in 1897 nothing significant was done to combat it until 1917 when the criteria for an accredited herd were established. Also during 1918 the term accredited veterinarian was recognized.

As far as TB was concerned the need was obvious. In 1917 out of a population of 40,000,000 swine close to 4,000,000 had TB lesions at the time of slaughter and 203,193 cattle.

Mr. Oscar Meyer, President of the American Institute in 1925 stated, "Gentlemen it is difficult to imagine a more beneficial work than which is represented by the people in this room. The eradication of this great scourge that has harassed humanity since the beginning of time is a work of the greatest importance. Since the inauguration of the Federal Inspection Act of 1906 although more than twenty years ago, the inspection for disease in slaughtered animals has been of great rigidity and justly so but this has thrown all the more burden on the packing industry that burden has ran all the way from $20–40 million a year. Four or five years ago, the TB situation with reference to hogs was so bad and we were put to such a disadvantage on account of the extreme losses that we were suffering though condemnations that we were virtually being put out of business." During a five year period prior to June 30, 1925, 34,000,000 swine carcasses were retained."

Can you imagine in those days, TB sanitoriums were growing by leaps and bounds to deal with the incidence in humans. The milk supply was heavily contaminated which not only accounted for some of the disease in humans, but also for calves and swine. The disease was being widely disseminated among cattle and most of the pure bred breeds were infected.

In 1919, Senator Hackney of Illinois had to show preserved lesion specimens in their Senate to get them to believe that they could actually see lesions in cows that reacted positive to the TB test.

In 1919, the Commissioner of Agriculture Rasmussen of Pennsylvania made an astute evaluation, "I do not believe that we will ever eradicate tuberculosis until you have every man who has his herd tested for TB look upon the tuberculosis question with the same ardent belief and spirit in its eradication as was expressed by Dr. Kiernan, Chief of Tuberculosis Eradication Program." In other words you have got to have tuberculosis free men as well as tuberculosis free herds from the standpoint of their mental attitude towards their work. If that is the case one of the fundamental challenges is the education of the individual farmer (you need men who can properly and effectively inform the farmer)."

Can you imagine what the reaction of some of those pioneers would be if they were around today and we told them that we don’t have the will or the resources to eradicate the disease when the level is less than 13 infected animals per million.

The outbreaks of emergency type disease were handled very effectively because they were recognized promptly, the response action was swift and
they were eradicated relatively quick. Even though it took seven years to eradicate Foot-and-Mouth Disease in Mexico considering the size of the problem and the conditions encountered it can be said that it was eradicated promptly. The same is true to those diseases such as vesicular exanthema, Venezuelan Equine Encephalometis, Virulent New Castle Disease and Avian Influenza. However they all went through three stages namely: panic, action and apathy. In the programs mentioned, the leadership met the challenge and got us through the apathy stage and completed the task.

As one reads the evolution of the Brucellosis, hog cholera, scabies and screwworm the cycles keep repeating. Fortunately most of the programs endured the challenges and were either successfully completed or are currently underway. Don't we need to seriously look at the Brucellosis and Tuberculosis Programs and give them new life and thrust and get them eradicated and out of the way? Then we can concentrate on the problems of this decade not those we inherited that began years and years ago.

**WHAT CAN WE LEARN FROM OUR PAST?**

The present system is capable of producing effective results. This is very important but the efficiency of the system can and should be improved.

There are some fundamental basic factors that cannot be overlooked especially if we are going to learn from the past.

**PRO**

Programs evaluated annually at meetings.
Programs define problem areas.
Specific research directed to resolve problems.
Excellent competence technically and administratively.
Program results important from health and economic standpoint.
Communication aires differences and programs modified to be acceptable.
Meetings cause better interrelationship & communications.
Proceedings provide valuable technical information.

**CON**

Modify programs to extent where objectives can’t be reached or too costly.
Process takes too long.
Decisions to create change are not implemented promptly.
Erratic funding has traumatic effect on program progress.
Resources unavailable to do what is necessary.
Political influence adversely effects programs progress.

My review of the eighty seven preceedings left me with these thoughts for your consideration.
Justification of Animal Health Programs

The justification for Animal Health Programs should be to protect human health and increase animal production. The need to maintain efficient production is related to trade whether domestically or internationally.

Therefore it’s necessary to keep aware of factors that affect trade so that production can meet those needs. It seems to me that the opening of these annual meetings should contain resource speakers who could define the needs of trade both from a short range and long range viewpoint.

It would create an environment whereby all of us coming from many different factions that are involved in livestock production would realize the real purpose of our deliberations. Since trade plays a big part in our economy one can easily understand why these types of programs deserve a high priority and why these meetings are important.

Leadership of Livestock Industry

The leadership of the livestock industry historically has provided the balance necessary to make programs more applicable. It has provided a bridge between a purely technical approach to the problems and what is acceptable to those being regulated. It has been able to resolve differences between the technical leadership of USAHA and USDA and those within the livestock industry with extreme viewpoints.

However if there is basic agreement that the objective should be attained, then that leadership must guard against modifying the program that would make that objective impossible to obtain.

Reading the history and from personal experience over the last thirty five years, I have often marveled at the personal sacrifice, involvement, dedication and commitment of some of the leaders of the livestock industry.

When I speak of livestock industry I refer to producers, pure bred breeders, packers, livestock markets, transportation companies, agricultural organizations, biological and pharmaceutical companies, and the Animal Welfare Interests and the Agricultural medias.

Future programs need to be planned and proposed by the leadership of the livestock industry so their commitment is not in doubt. Once there is agreement on the objective, the program should be implemented and completed in the shortest time that is acceptable to the industry thereby keeping the overall cost to the taxpayers to the minimum needed. When the National Animal Disease Survey (NADS) is functioning it will provide the basis for future programs. It will highlight what conditions are causing the greatest losses in livestock production and if valid, commitment of the industry should be assured.

Leadership of the State & Federal Regulatory Officials

Historically the records show that there has been outstanding leadership in this area. There were times I am sure when there were socalled
“turf” problems but the more astute were well aware that both groups are vitally needed to get the job done. In fact these programs work the best when there is outward evidence that each group recognizes and respects each other. If one group is dominating eventually the effectiveness will be destroyed.

If you want to be inspired by the effective relationship that has taken place over the years I recommend that you read the history of this organization. There have been some outstanding leaders on both sides who have been largely responsible for the success story of the past 100 years of animal health accomplishments which includes Animal Welfare and Meat & Poultry Inspection.

Research Universities

When people ask about the reason animal health programs have been largely effective in the states, one readily recognizes that the program have been supported by the research conducted at the universities, the biological industries and the federal government. The researchers involved have concentrated on the technical problems responsible for the prevention, perpetuation and spread of disease. This organization’s meetings provided an annual forum where the specific research needs were often identified because they were blocking the progress of the programs. In addition researchers had the capability of determining the future problems most likely to occur in the programs and planned their research accordingly. In order to support this point I refer you to the annual proceedings that have become so important to all who have interest in animal health in the United States.

Diagnostic Laboratories

Many of you recall the fifties. Do you recall the state of the science at the most of the various state diagnostic laboratories located throughout the United States at that time? Perhaps you should travel and observe some of the laboratories that exist in some of the less developed countries of the Americas in order to realize and appreciate how far they have come. The federal laboratories for most of these years carried the biggest load and did it quite well. The state and federal diagnostic laboratories today provide a network that is second to none in the world.

The phenomenal growth and development of the AAVLD in the last thirty years is proof of the great need and the recognition and appreciation for the services they are providing. Here again the continual good leadership contributed significantly to the effectiveness of the programs of the state and federal government.

Communications

Thank the Lord, the leadership of the Agricultural press, radio and other forms of the media have participated in these programs. The history records the involvement of many editors of agricultural publications that cautioned time and time again on the need to improve communication
primarily between the regulatory officials and the livestock industry. The zeal of some of these leaders is obvious and their commitment indicated by their continuous involvement and participation provided direction that enabled the past program to overcome some of their major obstacles that were blocking progress. Needless to say the role of the medias will only increase in importance as the programs of the future are implemented.

Importance of United States Animal Health Association

Dr. D. E. Salmon, the first chief of BAI addressed this body at its third meeting and I quote “I do wish to say a few words in the first place to impress upon the members of this association so far as I can, the importance and value of the organization to which they belong. There was a great need for cooperation between the sanitary authorities of the different states in order to secure uniform action and to enable the owners and shippers of livestock to get their animals to market without unnecessary or burdensome restrictions and this is one of the most important steps towards securing such results.

The federal government has done something to make the regulations uniform but in order to satisfy those in the various parts of the country, it was desirable to have something more; it was desirable that those who are interested should have some voice in the conclusions that were reached. The Department of Agriculture has endeavored to follow so far as possible, the recommendations which have been made from time to time by your organization at its past sessions. It has done so partly because it recognized the fact that such recommendations should be given great weight, and partly because it desired to do as much as it possibly could to raise the prestige and standing of your organization.”

That statement was made in 1899. I could have made the same statement from 1961 to 1980 when I was either directly or indirectly responsible for the USDA participation in those programs because I have always had the same sentiments. That is why I established the APHIS administrator’s award to USAHA. I’m sure Bert Hawkins feels the same way. I would like to close with the following:

CONCLUSIONS

1. History repeats itself.
2. Work together — each segment is important.
3. Effective communication — up, down and across.
4. Recognize and define problems.
5. Develop practical programs — maximum disease reduction minimum trade interference.
6. Evaluate programs and define specific research needs.
7. Modify programs when necessary to reach objectives.
8. Positive attitude and commitment to reach objectives.
9. Flexibility to accept change when necessary.
10. Forecast and plan for the future.

Are we going to learn from the past or be forced to relive it? That is what we should have uppermost in our minds this week and at each of these meetings during the next 100 Years of Animal Health in the United States.

CONCLUSIONS

- HISTORY REPEATS ITSELF
- WORK TOGETHER – EACH SEGMENT IS IMPORTANT
- EFFECTIVE COMMUNICATION – UP, DOWN AND ACROSS
- RECOGNIZE AND DEFINE PROBLEMS
- DEVELOP PRACTICAL PROGRAMS – MAXIMUM DISEASE REDUCTION MINIMUM TRADE INTERFERENCE
- EVALUATE PROGRAMS AND DEFINE SPECIFIC RESEARCH NEEDS
- MODIFY PROGRAMS WHEN NECESSARY TO REACH OBJECTIVES
- POSITIVE ATTITUDE AND COMMITMENT TO REACH OBJECTIVES
- FLEXIBILITY TO ACCEPT CHANGE WHEN NECESSARY
- FORECAST AND PLAN FOR THE FUTURE
### PRO

- Programs evaluated annually at meetings
- Programs define problem areas
- Specific research directed to resolve problems
- Excellent competence technically and administratively
- Program results important from health and economic standpoint
- Communication aires differences and programs modified to be acceptable
- Meetings cause better inter-relationship & communications
- Proceedings provide valuable technical information

### CON

- Modify programs to extent where objectives can't be reached or too costly
- Process takes too long
- Decisions to create change are not implemented promptly
- Erratic funding has traumatic effect on program progress
- Resources unavailable to do what is necessary
- Political influence adversely effects programs progress
Mr. President, distinguished guests, members of USAHA and AAVLD, ladies and gentlemen.

With a program the caliber of this one so far, I know the last thing you want to hear is a report from the Secretary-Treasurer. I would be remiss, however, if I did not make some public acknowledgements and announcements.

This has been a great year. J. O. Pearce has truly been a working president. He is always up in the morning ready to get the job done and he never quits until it is completed. He has been extremely active in attending meetings and making sure his committees are facing their responsibilities. I personally have appreciated his dedication and enthusiasm.

The office has had the busiest year ever. With our fine gals Ella and Linda the additional demands were taken care of and this meeting is their proof of capping a banner year. They are great people to have on our staff.

The registration is going great in large part to the tremendous effort and contributions of Norm and Jay Powers. They both deserve a big thank you from our two organizations and the memberships. There is no way to measure the real worth of their presence each year.

Dave Walker has been a tireless president elect and we are looking forward to another successful year under his leadership. The board of directors has been untiring in providing the necessary guidance in the association business activities.

You recall that under the Ragan administration — that’s John Ragan — we changed our fiscal year from October 1 to September 30 to a calendar year fiscal year. This means we have no formal treasurer’s report at this meeting. The formal report as prepared by the auditor will be printed in the proceedings, copies will be sent to members of the Executive Committee and any member who requests a copy from the office. We can report that to day our cash flow has been in excess of $100,000 and our income has been sufficient to more than meet our obligations.

The Board of Directors have met several times during the year with representatives from AAVLD and our program format appears to be successful. The local committee has arranged a fine meeting and we hope all of you are looking forward to the rest of the week. The hotel accommodations appear quite good and we believe we have a fine program planned.

There are still tickets available for the Round-Up Inn tomorrow night. We need full participation to make it a success. We believe you will have a great time.

The Foreign Animal Disease Committee has done a super job of revising our “gray” book on foreign animal diseases. We have a limited supply with us available at the registration desk for $12.00. This saves postage and handling.
We remind you that Wednesday's general session starts promptly at 8:00 AM.
Thank you.
STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR PERIOD
JANUARY 1, 1984 through DECEMBER 31, 1984

CASH BALANCE — DECEMBER 31, 1984:

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Ella R. Blanton 727.00
Linda B. Ragland 319.00
Dr. J. C. Shook 269.00
Rent — Office Space 4,100.00
Other Meetings 855.95
American Association of Veterinary Livestock Diagnosticians 10,625.00
Virginia Unemployment Insurance 261.26
Surety Bond 32.00
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Shipping Materials 100.00
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Auditing Books 600.00
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CASH BALANCE — DECEMBER, 31, 1984

Cash on Hand — December 31, 1984 $ 4,300.28
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Richmond, Virginia
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Henry H. Budd
Accountant
Distinguished guests, ladies and gentlemen of the United States Animal Health Association and American Association of Veterinary Livestock Diagnosticians, let me seize upon this moment to most sincerely thank you Mr. Bartlett, Texas state officials and the people of Texas for inviting us to the Lone Star state. Your hospitality and gracious offers of help have made all the difference here at Fort Worth.

It is with considerable humility and boundless pride that I address this distinguished assembly as President-Elect of the United States Animal Health Association on this the occasion of its eighty-eighth anniversary and the anniversary year of one hundred years of animal health for APHIS. This is the first meeting at Fort Worth since the birth of this great organization in 1897 and it is fitting that a too long overdue return should come on this momentous occasion. Both the United States Livestock Sanitary Association and the United States Bureau of Animal Industries were founded in stressful times, responding to disastrous disease situations and each has been integrally influential upon the other. Yes, the names have changed but the ideal, the primary goal remains intact; to assure a better, more abundant, more healthy livestock industry producing a healthful, safe food supply for the American people which is second to none other on this earth. Much has been said concerning this important subject far more eloquently by others seated on this stage and I shall defer to them. Suffice it to say that we owe a sizable debt to those who came before us.

USAHA functions as an effective and important interface between state government, federal government and industry offering the common ground for thoughtful deliberation contributing the practical judgement leading to informed, effective disease control programs. It is critical that each of these three be constantly informed, — and that all are pulling equally together. Therefore, one of the primary goals of USAHA and one of my goals is to assure adequate and proper dissemination of information. We are in a unique position to perform this function. In this regard, I see this process utilized to great advantage in strengthening support for APHIS in its quest to better control entry of animals into this country, both wild and domesticated, and the vectors associated therewith. Through this wonderful medium of education, we are learning to act before the fact; not to react after another costly disease has entered the United States. The North American FMD Bank is an excellent example. The State–Federal Relations Committee in 1982 expressed concern that strict enforcement must continue to be employed regulating animal imports into this country. Animal health programs require continual monitoring in order that we:

1. Preserve a credible international livestock market.
2. Prevent incursion of foreign animal diseases.
3. Control production losses with domestic livestock.
As President-Elect, I would like to commend APHIS, state officials, the Avian Influenza Technical Collaborating Committee and the poultry industry in Pennsylvania, Virginia, Maryland, New Jersey and Delaware for outstanding performance and cooperation in first confirming and then eradicating highly pathogenic Avian Influenza. This highly commendable accomplishment was achieved at times under difficult circumstances.

Cooperative program planning, involving industry, is a very necessary element in establishment of reasonable, affordable disease control standards. Necessity dictates that scientific knowledge be applied in a safe, practical manner which is acceptable to our vast livestock industry. There is no doubt in this regard that our committee system, involving some 30 standing committees, is the strength of USAHA. We are blessed with an abundance of scientific expertise, recognized worldwide and programs developed through this system are held in international esteem.

Significant advances just now emerging on the horizon promise even more spectacular development in coming years. Progress will come, but not without problems, setbacks and risk. USAHA with its cohesive melding of government, industry and scientific communities is uniquely suited to influence future livestock disease control development in the best interest of this nation. Worldwide exchange of genetic material through embryo transfer offers great promise for many species with lessened potential for disease spread. Fractionated cell vaccines promising greater reliability are in the works, and an array of other scientific advances are about to become reality. Future progress will demand ever greater cooperation to foster an enlightened industry participation. Through all this there must remain that vehicle of one extra step to compromise where structured policy, technical knowledge and practical implementation clash. USAHA is best suited to be that vehicle.

Changing the fiscal year to January 1 through December 31 has proven beneficial in allowing our treasurer to present a more up-to-date financial report at the annual meeting. Dues for the ensuing year are payable on or before January 1. As you know, this organization has attempted, over many years, to operate on a pay-as-we-go budget. Therefore, your prompt remittance of dues is encouraged. My goal is to see that each committee member is a USAHA member in good standing. March 31 is the delinquent date.

Your board of directors met with AAVLD on several occasions during 1984. There was general agreement that restructuring of the program format offered considerable benefit at last year's meeting. It was also generally agreed that a joint meeting of AAVLD and USAHA add strength to both organizations.

USAHA membership has expanded over the past year thanks to the effort of our Public Relations Committee and individual members, reflecting a health organizational condition. Indeed we are fit as a fiddle. There are areas for improvement, but radical surgery is not indicated.
It does appear to me that an assessment must be made by this organization concerning whether there needs to be a change in the livestock interstate health certification process and, if so, to determine and recommend implementation of those changes. That process is beginning.

I wish each of you a stimulating, productive meeting.

I challenge this great organization over the next year to be aggressive in deliberations, thoughtful in assessments and conclusive in recommendations as expressed in the words of this poem.

I hate to be a kicker, I always long for peace;
But the wheel that does the squeaking is the one
that gets the grease.
It's nice to be a peaceful soul, and not too hard to please;
But the dog that's always scratching is the one
that has the fleas;
The art of soft soap spreading is the thing that palls and stales;
But the guy that wields the hammer is the one who drives the nails.

Anonymous.
Dr. David U. Walker, President-Elect, presents plaque to outgoing President, Mr. J. O. Pearce, Jr., for his outstanding leadership for 1983-1984.
PRESIDENT'S REMARKS

J. O. Pearce, Jr.
Okeechobee, FL

Ladies and Gentlemen, Members of USAHA, AAVLD, and Guests!!

This year we are meeting in Fort Worth and will be 87 years old. It was on September 29, 1897 that concerned animal health officials met in Fort Worth and organized the Interstate Association of Livestock Sanitary Boards. Out of that came the birth of the National Association of State Livestock Boards. From this organization came the U. S. Livestock Sanitary Association which drew together State Regulatory Animal Health People, Federal Regulatory People, Researchers in the field of animal health from all walks of life and the Industry that was affected by animal health problems. The Association was the forum providing for the study of National and International animal disease problems, with input from all interested segments providing for use all of the information available regarding specific problems. In 1968 this group was renamed the U. S. Animal Health Association as we know it today. The Association has endorsed and supported the beginnings of the concept that has been successful in eradicating animal plagues from this Continent when sufficient information and know-how was available. Our goal has always been eradication, but until such information is made available, our goal of necessity has been limited to containment and control. Each within the limits of our ability as related to manpower and finances has been pursued for more than three quarters of a century. These efforts have contributed materially toward making this the best fed nation in the world and now in the last quarter of the 20th Century, our efforts are increasingly important towards making the United States the prime supplier of high protein food of animal origin around the world. Over the years this organization has become involved with all phases of animal health and welfare. We have to stop and think “what we can do to protect our country from the importation of various foreign diseases that would plague our industries with unwanted problems even to the smallest species.”

This year has been very successful in many ways. We are in the best financial condition that we have been in for several years. This is due to several things:

(1) The response you gave me as President when I requested you pay your dues promptly. I hope you will continue to do this as it will help keep us financially stable. I sincerely appreciate your cooperation.

(2) We printed the Foreign Animal Disease Book and had good response on its sale. At this time I would like to thank each and every one of you who had a part in putting this book together. A special thanks goes to Dr. Gil Trevino who put a lot of effort into developing
material at the beginning and taking his time to come to the USAHA office to help get it started. Also, Dr. Jack Hyde who came down to the office and did the last editing and approved it for the printers. We had a real busy year in other areas. We have been working on health certificates and hope to have made some significant progress in this area. We had a severe outbreak of T. B. in some buffalo herds in South Dakota. As they were not covered by T. B. regulations, we had to make several trips to Washington to get a program approved for them to protect the beef cattle population in this area and over the United States as a whole. I have worked very closely with all phases of our Association trying with all of my ability to use the powers of President with dignity and authority. I have deeply enjoyed serving as your President this past year. It was an honor and I promise my continued support to the Officers who are coming into office. Thanks to each Committee Chairman for the fine cooperation they have given me in this past year. It is through working together that the Association can grow and prosper. I especially want to thank Ella and staff for the splendid job that they have done for us. No one knows better than I, the things that have been accomplished in USAHA, due to their willingness, support and devotion to our Association. The many things that we undertook could not have been successful without their support. I am very grateful to them for their cooperation. Before I close I would like to tell you about a letter that I received from Dr. Erskine Morse, who as many of you know was active in the USAHA for many years and served as Chairman of the Salmonella Committee. He sends his best wishes for a successful meeting and hopes that he will be able to join us again in the near future.

Thank you.
Mr. B. W. Hawkins, Administrator, USDA, APHIS, VS, presents Animal and Plant Health Inspection Service’s Animal Health Award to Dr. J. F. Hudelson, State Veterinarian of Colorado for Dr. William C. Tobin, former State Veterinarian of Colorado.
REPORT OF THE COMMITTEE ON NOMINATIONS
AND RESOLUTIONS

The Committee on Nominations and Resolutions presents the following slate of officers for your consideration.

This report will be posted on the bulletin board at the registration desk for 24 hours and presented again at the close of tomorrow's general session prior to final action by the Executive Committee as provided for in the By-Laws of this organization:

President ...................................... David U. Walker
Montpelier, Vermont

President-Elect ............................... N. W. Kruse
Lincoln, Nebraska

First Vice-President ......................... John Hudelson
Denver, Colorado

Second Vice-President ...................... John Cobb
Atlanta, Georgia

Third Vice-President ....................... Phil Bradshaw
Griggsville, Illinois

Treasurer ................................. John Shook
Annapolis, Maryland

Regional Delegates
Northeast ..................................... Everett Bryant
Storrs, Connecticut
Victor LaBranche
Boston, Massachusetts

North Central .............................. Don Gingerich
Parnell, Iowa
Bill Gallagher
Highmore, South Dakota

South ......................................... Joe Finley, Jr.
Encinal, Texas
William Baisley
Dalton, Georgia

West .......................................... Olin H. Timm
Dixon, California
R. H. McCapes
Davis, California
RESOLUTIONS
United States Animal Health Association
Passed, October 26, 1984
Fort Worth, TX

RESOLUTION No. 1
Source: Committees on Salmonella and Transmissible Disease of Poultry
Subject Matter: Establishment of National Animal Salmonella Research Reference Center

Resolution

BE IT RESOLVED that the U.S. Animal Health Association recommends that a National Animal Salmonella Research Center be established by USDA and an epidemiologist be added to the staff to pursue and coordinate epidemiologic investigations of Salmonella outbreaks in cooperation with State Animal Health authorities and Veterinary Diagnostic Laboratories. The ultimate goal is to control and reduce Salmonella infections in livestock and poultry as well as reduce Salmonella infections in humans that are related to animal products.

RESOLUTION No. 2
Source: Import/Export Committee, Epizootic Attack Committee
Subject Matter: Embryo Regulations

Resolution

BE IT RESOLVED that:

1. The Secretary of Agriculture urges his counterparts to consider delaying the issuance of rigid regulations, resolutions and recommendations until additional research data becomes available; and

2. USDA sponsor directly or indirectly an international conference of research leaders, regulatory officials and industry to exchange information on current research data and its implications.

RESOLUTION No. 3
Source: Import/Export Committee, Epizootic Attack Committee
Subject Matter: Embryo Transfer

Resolution

BE IT RESOLVED that:

The Secretary of Agriculture be urged to ensure, support, and foster increased basic, applied, and developmental research on disease transmission by embryo transfer in domestic and zoological animals and that a work group of research, industry and USDA personnel be formed to aid the Secretary of Agriculture in determining these research priorities.
RESOLUTION No. 4
Source: Committee on Zoological Animals
Subject Matter: TB Testing of Non-Domestic Ruminants

Resolution
BE IT RESOLVED that U.S. Department of Agriculture (USDA) develop a program to collect and analyze data related to the problem of tuberculosis in non-domestic ruminants and eventually evaluate the testing methodology so that appropriate and adequate tests can be prescribed.

RESOLUTION No. 5
Source: Committee on Zoological Animals
Subject Matter: TB Testing of American Bison

Resolution
BE IT RESOLVED that tuberculosis testing of American Bison, as prescribed under the Uniform Methods for domestic cattle, be instituted for all bison moving interstate. States should require negative tuberculosis tests before allowing entry of this species into their area.

RESOLUTION No. 6
Source: Committee on Infectious Diseases of Cattle
Subject Matter: Importation of Bovine Semen

Resolution
BE IT RESOLVED that USDA, APHIS be requested to establish test procedure regulations for the importation of bovine semen into the USA which are consistent with (a) existing state regulations, and (b) the Minimum Requirements for Health of Bulls Producing Semen for AI of Certified Semen Services, a subsidiary of the National Association of Animal Breeders.
RESOLUTION No. 7
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species
Subject Matter: USAHA Sponsor Second International Symposium on Avian Influenza

Resolution

BE IT RESOLVED that USAHA in cooperation with USDA, other governmental agencies and poultry industry sponsor the Second International Symposium on Avian Influenza in 1986.

FURTHER BE IT RESOLVED that the USAHA Committee on Transmissible Diseases of Poultry and other avian species serve as the generating force for the Second International Symposium on Avian Influenza similar to the role played for the First Symposium.

RESOLUTION No. 8
Source: Tuberculosis and Johne's Disease
Subject Matter: American Bison

Resolution

BE IT RESOLVED that the USAHA goes on record as strongly recommending that all bison raised under agricultural conditions shall be handled under the same tuberculosis testing, indemnity, and depopulation conditions as cattle, except that prior to January 1, 1987, state classification will not be affected by *M. bovis* infection in bison herds.

RESOLUTION No. 9
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species
Subject Matter: An Apparently New Paramyxo-Virus in Pigeons

Resolution

BE IT RESOLVED that the USAHA requests that the USDA determine the extent of the infection in U.S. pigeons, advise the pigeon and poultry interests of their findings and initiate whatever measures the USDA, the involved states, and two industries deem appropriate.
RESOLUTION No. 10
Source: Biologics Committee
Subject Matter: Animal Biologics Regulation

Resolution
BE IT RESOLVED that USAHA strongly supports the general concept of uniform regulation of veterinary biologicals with specific comments to come upon the introduction of legislation.

RESOLUTION No. 11
Source: Committee on Sheep and Goats
Subject Matter: Bluetongue Sentinel Herds

Resolution
BE IT RESOLVED that the USAHA encourages the USDA to support the continuation of the Florida sentinel cattle herd program and the extension of the sentinel cattle herd concept into selected areas of the U.S.

RESOLUTION No. 12
Source: Bluetongue and Bovine Leukosis Committee
Subject Matter: Relocation of Bluetongue Research Lab

Resolution
BE IT RESOLVED that the USAHA strongly recommend that industry participate in the decision-making process before any plans are finalized by ARS to relocate and/or reduce the program.

RESOLUTION No. 13
Source: Committee on Sheep and Goats
Subject Matter: Ovine Footrot

Resolution
BE IT RESOLVED that the USAHA assign to footrot a very high priority among diseases of sheep and support funding of research in footrot vaccine at the highest possible level for at least 3 to 5 years.
RESOLUTION No. 14
Source: Committee on Pharmaceuticals, Pesticides and Related Toxicology
Subject Matter: Salmonella Article, New England Journal of Medicine

Resolution
BE IT RESOLVED that the USAHA urges increased cooperation and communication among scientists, the livestock industry, drug manufacturers, the veterinary profession and the Food and Drug Administration in addressing the issues relative to any alleged effect of the use of drugs in livestock upon human health.

RESOLUTION No. 15
Source: Import/Export Committee
Subject Matter: User Fees

Resolution
BE IT RESOLVED that the USAHA urges Congress to make the necessary changes to enable the costs of such testing to be paid for by the exporter in the form of a reimbursement to APHIS instead of a cost to the American taxpayer.

Further, that USAHA urges USDA to work with industry to establish a mechanism whereby this testing can be done by private industry in a federally-approved laboratory.

RESOLUTION No. 16
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species
Subject Matter: Avian Influenza as a Reportable Disease

Resolution
BE IT RESOLVED that the USAHA urges each state to make Avian Influenza a reportable disease and this resolution be forwarded to the appropriate animal health regulatory agency in each state.
RESOLUTION No. 17
Source: Foreign Animal Disease, Infectious Diseases of Cattle and Animal Disease Surveillance
Subject Matter: Foreign Animal Disease and Ectoparasites Research and Diagnosis

Resolution
BE IT RESOLVED that the USAHA endorses the NRC report, "Long Term Planning for Research and Diagnosis to Protect U.S. Agriculture from Foreign Animal Diseases and Ectoparasites", on research and diagnosis of FAD&E and encourage the USDA to give full consideration to the recommendations of the NRC and seek widespread discussion and input from the livestock industry.

RESOLUTION No. 18
Source: Animal Welfare Committee
Subject Matter: Animal Welfare Act — Enforcement

Resolution
BE IT RESOLVED that the USAHA reaffirms its full support to the USDA in carrying out at all times quality inspections at all animal facilities under their jurisdiction in order to assure the health and well-being of animals so dependent on humans for good care and compassionate treatment.

RESOLUTION No. 19
Source: Animal Welfare
Subject Matter: Animal Behavior Research

Resolution
BE IT RESOLVED that the U.S. Animal Health Association encourages the continued research of farm animal behavior.

RESOLUTION No. 20
Source: Committee on Salmonella and Transmissible Diseases of Poultry
Subject Matter: Five Statements on Symptoms of Salmonella that were agreed on at the International Symposium on Salmonella.

Resolution
BE IT RESOLVED that USAHA accepts the five statements in respect to Salmonella agreed to at the International Symposium on Salmonella, July 19–20, 1984, at New Orleans, Louisiana.
RESOLUTION No. 21  
Source: Foreign Animal Diseases, Epizootic Attack Committees  
Subject Matter: Importation of Zoo Animals  

Resolution  

BE IT RESOLVED, that this committee strongly supports the principles expressed in the interim report of the Veterinary Services Committee on the Importation of Zoological Animals for procedures to be implemented to safeguard against the introduction of such diseases and parasites; and,  

Recommends that the United States Department of Agriculture in concert with the U.S. Department of the Interior, proceed with drafting of new and modification of existing laws and regulations needed to regulate the importation of all animals, birds, poultry, reptiles, and amphibians with respect to matters of diseases and pests and the agents and vectors thereof and to establish such conditions of importation as are deemed appropriate according to the species and health status with respect to disease and pests of the country of origin and that they also be empowered to refuse the importation of such species where adequate safeguards cannot be established.

RESOLUTION No. 22  
Source: Foreign Animal Diseases Committee  
Subject Matter: Importation of Zoo Animals  

Resolution  

BE IT RESOLVED that USAHA strongly supports the recommendations of the Veterinary Services Committee on the Importation of Zoological Animals for procedures to be implemented to safeguard against the introduction of such diseases and parasites; and,  

Recommends that the United States Department of Agriculture be empowered to regulate the importation of all animals, birds, poultry, reptiles, and amphibians with respect to matters of diseases and pests and the agents and vectors thereof and to establish such conditions of importation as are deemed appropriate according to the species and health status with respect to disease and pests of the country of origin and that they also be empowered to refuse the importation of such species where adequate safeguards cannot be established.
RESOLUTION No. 23
Source: Foreign Animal Diseases Committee
Subject Matter: Calici Virus Research

Resolution
BE IT RESOLVED that the USAHA strongly endorses continued research support at the past ($225,000/year) or increased levels for the next 3–5 years with special emphasis on developing research through cooperative agreement with laboratories which have already demonstrated a special expertise in calici virus research.

RESOLUTION No. 24
Source: Environmental Residues Committee
Subject Matter: Chemical Emergency Preparedness

Resolution
BE IT RESOLVED that the USDA strongly supports the development of a National Center for Emergency Preparedness on Chemical Disasters in food animals. The USAHA supports the development of a regional system of veterinary toxicology centers linked with the National Center for Emergency Preparedness to provide:

1) National and regional animal toxicology telephone hotlines linking veterinary practitioners, other professionals, and regulatory agencies at all levels,

2) Emergency investigation capabilities,

3) Comprehensive laboratory services to support the regional and national investigation centers,

4) Epidemiologically oriented computerized records and an animal toxicology information database networked between the regional and national center,

5) Feed analysis and residue monitoring programs, including producer-oriented feed specimen banking, for prospective and retrospective evaluations to alert producers and feed manufacturers of contaminants in livestock and poultry feeds,

6) A chemical disaster management training program designed to develop broad competence in management of chemically induced livestock and poultry problems.

It is not the intent of this resolution to restrict or inhibit the further development of private or public capabilities, either laboratory or field investigative in nature. It is also urged that the legal and liability aspects of the development of such a National Center for Emergency Preparedness be investigated.
RESOLUTION No. 25
Source: Committee on Infectious Diseases of Horses
Subject Matter: Equine Viral Arteritis (EVA)

Resolution
BE IT RESOLVED that research should be done in the following areas:
1. Characterization of the EVA virus.
2. Improvement of aids to EVA diagnosis (serological tests).
3. Introduction of a practical EVA vaccine.

Epidemiological studies should be pursued with special reference to the presence of the EVA virus during the quiet periods between the sporadic outbreaks.

RESOLUTION No. 26
Source: Committee on Transmissible Diseases of Poultry and Other Avian Species
Subject Matter: USDA Financial Support of Laboratory Personnel and Facilities for Increased Research Efforts on Avian Influenza Virus

Resolution
BE IT RESOLVED, USAHA recommends that USDA provide financial support to laboratory personnel and facilities which would contribute to the epidemiologic understanding of the nature of avian influenza in poultry populations. Research should be emphasized which would provide necessary information to predict the potential virulence, spreading qualities, pathogenicity and economic impact of the avian influenza isolates.
RESOLUTION No. 27
Source: Environmental Residues Committee
Subject Matter: Mycotoxins as Environmental and Food Animal Contaminants

Resolution

BE IT RESOLVED that the United States Animal Health Association supports continued activities to reduce real or potential damage from mycotoxins as follows:

1) The several services of USDA concerned with different aspects of mycotoxins continue and increase those efforts in their respective areas, specifically;

   a. That Agricultural Research Service (ARS) increase allocations and activities for research on biologic effects and decontamination methods for common mycotoxins,

   b. That appropriate federal agencies develop and expand activities to monitor occurrence and evaluate the epizootiology of common mycotoxin problems, as well as begin to determine the incidence of less common mycotoxicoses affecting food animals, and

   c. That CSRS continue to develop priority areas for mycotoxin research through the special research grants program.

2). That USAHA provide a representative to the appropriate committees of ARS, APHIS, and CSRS involved with developing priorities and allocating resources pertinent to toxicological and residue problems.

3) That FDA through the Center for Veterinary Medicine proceed toward approval of a model aflatoxin control plan for state management of aflatoxin contamination in corn, so that a framework is available to states needing guidance when large scale aflatoxin contamination in corn may occur. This model could include acceptable aflatoxin levels over 20 ppb when used in feed for mature beef cattle, swine, and poultry.

RESOLUTION No. 28
Source: Tuberculosis and Johne’s Disease Committee
Subject Matter: Tuberculosis in Buffalo

Resolution

BE IT RESOLVED that the United States Department of Agriculture and the buffalo associations should provide support for research on tuberculosis (Mycobacterium bovis infection) in buffalo with particular emphasis on improving diagnostic tests for field use in identifying infected animals.
RESOLUTION No. 29
Source: Epizootic Attack Committee
Subject Matter: Carcass Disposal Problems

Resolution
BE IT RESOLVED that the USAHA urges livestock and poultry interests and state and federal animal health officials to seek solutions to problems of carcass and manure disposal during emergency disease eradication programs. Authority for USDA to use appropriate public land for carcass burial should be pursued as one alternative.

RESOLUTION No. 30
Source: Epizootic Attack Committee
Subject Matter: Relocating Wildlife

Resolution
BE IT RESOLVED that USDA, in time of national animal disease emergency, be authorized to expend funds for the purpose of relocating sufficient animals to provide a reproductive nucleus (seedstock) to reestablish a publically owned wildlife species that has been depopulated in order to prevent establishment of a foreign animal disease in the United States.

RESOLUTION No. 31
Source: Epizootic Attack Committee
Subject Matter: FAD Training of State Employed Veterinarians

Resolution
BE IT RESOLVED that the United States Animal Health Association recommend to the USDA that they take appropriate action to enable adequate funding for increasing the number of Foreign Animal Disease Diagnostic classes and that the purpose of such an increase be for training substantially greater numbers of veterinarians employed by the states.

RESOLUTION No. 32
Source: Anaplasmosis Committee
Subject Matter: National Survey for Anaplasmosis

Resolution
BE IT RESOLVED that a national survey for anaplasmosis be conducted by USDA, APHIS utilizing anonymous serum samples from the brucellosis program, thus requiring no additional field personnel.
RESOLUTION No. 33
Source: Anaplasmosis Committee
Subject Matter: Additional Research Support

Resolution

BE IT RESOLVED that additional research support is needed to accelerate these efforts which are on the verge of providing new, very effective means of control.

RESOLUTION No. 34
Source: Bluetongue and Bovine Leukosis Committee
Subject Matter: Movement of Ruminants from U.S. Possessions in the Caribbean to the U.S. Mainland

Resolution

BE IT RESOLVED that the USAHA strongly recommends:
1) That research be undertaken to determine what exotic serotypes of bluetongue virus and other exotic infectious agents are present in the ruminant populations of U.S. Possessions in the Caribbean; and, 2) that regulations be changed to require that livestock moved from U.S. Possessions in the Caribbean be tested for the same diseases as livestock moving to the U.S. mainland from non-U.S. Possessions in the Caribbean.
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

Chairman: W. B. Fairchild, Baton Rouge, LA
Vice Chairman: K. L. Kuttler, Moscow, ID

J. Lee Alley, AL; R. D. Anderson, NV; J. F. Badger, MO; D. M. Bedell, GA; G. M. Brown, IA; G. M. Buening, MO; A. A. Cuthbertson, NV; C. A. Gipson, MD; R. I. Hail, KY; R. F. Hall, GA; R. L. Hartin, OK; T. J. Holt, MD; J. A. Howarth, CA; J. D. Huber, NV; M. M. Jochim, CO; E. W. Jones, MS; K. Kocan, OK; Stuart Lincoln, ID; D. G. Luther, LA; M. L. Main, SD; Duane Miksch, KY; W. G. Nelson, ID; J. O. Pearce, Jr., FL; M. Ristic, IL; R. C. Searl, IA; N. R. Swanson, WY.

Dr. Travis McGuire of Washington State University gave a report concerning the development of a subunit vaccine for anaplasmosis at Washington State University (WSU). His report indicated that the vaccine is currently being used in limited trials to determine its effectiveness in preventing the spread of initial bodies.

Dr. Ivan Reed gave an historical review of anaplasmosis in Canada and reported on the anaplasmosis eradication program which is being conducted in southern Saskatchewan. He stated that progress has been made. He anticipates total eradication by 1985 or 1986. He stated that their eradication efforts were based on test and slaughter with indemnification based on the owner receiving full market value.

Dr. Zaugg's paper was presented describing a survey conducted to determine the incidence of anaplasmosis in Idaho. The survey revealed prevalence of 12 percent infection in the beef cattle population. He also stated that the disease appeared to have spread to areas of the state not previously infected. The overall prevalence seemed to be less in some areas.

Dr. Gene Luther reported on the findings of a similar survey conducted in Louisiana where the incidence was found to be 9–10 percent. The committee discussed the possibility of a national anaplasmosis survey.

Dr. Kuttler gave a review of anaplasmosis in wildlife. He reported that anaplasmosis is found in most wild ruminants. Epidemiologically, it is found that black-tail deer and possibly mule deer could serve as reservoirs of infection.

Dr. Kocan reported on her studies of ticks as vectors of anaplasmosis. The anaplasmosis organism has been identified in tick tissues and the developmental cycle described.

The committee adopted two resolutions: one, made by Dr. Bob Hartin and seconded by Mr. J. O. Pearce; and one, made by Dr. Nelson and seconded by Dr. A. A. Cuthbertson.
REPORT OF THE COMMITTEE ON ANIMAL WELFARE

Chairman: E. M. Stewart, Lincoln, Nebraska

Vice Chairman: Neal Black, St. Paul, Minnesota

Committee members present were: Mr. E. Mickey Stewart, Lincoln, NE; Mr. Neal Black, South St. Paul, MN; Dr. L. G. Billingsley, Sacramento, CA; Mr. Dewey Bond, Washington, D.C.; Dr. G. C. Cilley, Concord, NH; Mr. Robert Gadd, Ft. Pierre, SD; Ms. Ann Gonnerman, Kansas City, MO; Dr. Carl Graham, Kansas City, MO; Mr. Donald Jones, Netawaka, KS; Dr. David J. Meisinger, Des Moines, IA; Ms. Ronnie Polen, Sewell, NJ; Dr. D. C. Randall, Evergreen, CO; Dr. Robert A. Rice, Raleigh, NC; Mr. Grover W. Roberts, Sacramento, CA; Dr. J. D. Roswurm, Sacramento, CA; Dr. M. S. Silberman, Atlanta, GA; Dr. Nancy E. Wiswall, New Carrollton, MD.

The chairman called the meeting to order at 1:30 p.m., October 23, 1984, Ft. Worth, Texas. He explained how the meeting would be conducted and asked that any resolutions to be presented be brought to the podium. There were 70 members and guests present.

Dr. Mort Silberman visited with the committee about the gray areas and the problem areas in the enforcement of the Animal Welfare Act. He stated that it will take time to establish a track record on the interpretation of the act. The limited funding by Congress has put severe pressure on USDA personnel and their enforcement capabilities.

Mr. Frank Touhy, Sea World, San Diego, California, gave a fine presentation on the research and the establishment of breeding and environment facility for penguins. The "Penguin Encounter" that has been established in San Diego is a very exciting example of man's effort to know, understand and display this unique creature. This is an outstanding facility available to the public.

Dr. Wood-Gush, ethologist from the Edinburgh School of Agriculture, Edinburgh, Scotland, made a very interesting presentation on animal behavior.

Much of his talk describes his "pig park" in which he observes the behavior pattern of swine in an unrestrained, outdoor setting. He described its nesting, rooting, farrowing, feeding and leisure habits. He then translated this environment into a housing facility in order to house the animals in a more "natural confinement."

Dr. Ted Friend, Animal Science Department, Texas A&M University, presented his information on stress as it relates to animal behavior. He presented the relationship of different housing and handling of calves to the health and behavior pattern.

He also described some of the behavioral research being directed by USDA.

Dr. Ole Stalheim, Ames, Iowa, presented information about a new group which will address itself on ways of coping with natural and man-made "animal" disasters. This effort is a result of an AVMA resolution.
Our committee expressed interest in the idea and asked to be kept informed.

Ann Gonnerman presented a concern about the enforcement level of the Animal Welfare Act. A specific example referred to a dog breeding facility. There was considerable discussion about constructive action that our committee should take.

It was agreed that there would be greater communication between USDA and this committee. It was further agreed that animal welfare is a most important issue. It will be necessary for everyone to cooperate so that we will have the very best utilization of the resources available to maximize the effectiveness of the enforcement of the Animal Welfare Act. We should not tolerate conditions of animal abuse or mistreatment.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

Chairman: Vaughn A. Seaton, Ames, IA
Vice Chairman: Kenneth D. Weide, Columbia, MO

W. F. Alexander, OK; A. A. Andersen, IA; R. P. Azelton, MO; H. T. Barron, TN; D. E. Bartlett, WI; Joe Bearden, MS; L. N. Brown, WA; E. A. Carbrey, IA; C. S. Card, PA; R. W. Cellon, Jr., FL; R. A. Crandell, TX; R. P. Crawford, TX; G. L. Crenshaw, CA; J. F. Evermann, WA; R. W. Fulton, OK; G. D. Gurss, KS; R. F. Hall, GA; W. T. Harrer, MT; N. W. Kruse, NE; G. Lambert, IA; A. J. Luedke, CO; M. L. Main, SD; C. S. McCain, OK; A. W. McClurkin, IA; C. A. Mebus, NY; Joyce Mittness, CO; M. A. Mixson, AL; B. F. Newcomb, MT; P. A. O'Berry, IA; B. I. Osburn, CA; S. L. Reynolds, TX; J. A. Schmitz, OR; R. D. Schultz, WI; L. M. Siegfried, WI; W. L. Sippel, FL; Richard Smith, KS; P. L. Spencer, IL; Dan Suther, CA; N. R. Swanson, WY; M. Van der Maaten, IA.

The meeting convened at 1:30 p.m. on October 25, 1984. Twenty of forty members were present. The following reports were presented and are attached as exhibits A, B, C, D, and E:

Exhibit A  Bovine Chlamydiosis
Exhibit B  Persistant Bovine Viral Diarrhea Virus Infection in Cattle and its Relationship to Mucosal Disease
Exhibit C  Public Health Aspects of Listeriosis in Milk
Exhibit D  An Update on Fescue Toxicity
Exhibit E  Resolution: Importation of Bovine Semen

The meeting adjourned at 3:30 p.m.

BOVINE CHLAMYDIOSIS

Lynne M. Siegfried

Chlamydia psittaci is a unique obligate intracellular organism having some of the characteristics of both bacteria and viruses. It has been placed within the order Rickettsiales because of its small size and the fact that it reproduces by binary fission. Chlamydia psittaci produces a variety of disease syndromes covering most avian and mammalian species. Each of the organisms involved has the same group-specific antigen, which is also the one detected by the complement fixation test (the standard laboratory diagnostic assay system) and unique serotype-specific antigens which generally elicit weak immune responses.

C psittaci infections may result in pneumonia, encephalitis, abortion, arthritis, diarrhea, or conjunctivitis. All of these manifestations of the disease have been described in cattle. The question seems to be what is the real impact of chlamydiosis within the cattle industry. The answers will require new information about the organism and the disease. The fact that there is not a reliable analytical means to assess the disease in cattle is
related in part to the unique size and growth characteristics of the organism, its antigenicity, the absence of good serotyping capabilities, and certain immunologic features of infection. Research is in progress in several laboratories aimed at developing laboratory tools for increased understanding of the scope of the disease, its pathogenesis, and factors of its transmission in the field.

Anticipated new research tools include the use the ELISA technique to increase the sensitivity and perhaps the specificity of chlamydial serology. Separation and comparison of protein antigens and nucleic acid digestates will allow for more definitive serotyping of chlamydial isolates than was previously possible. Monoclonal antibodies produced against unique antigens to each serotype will be valuable reagents for epizootiologic studies of the disease and to determine possible vectors and reservoirs of transmission. Studies of cellular and humoral immunity may ultimately result in more effective vaccine production, working around the problems of disease latency and hypersensitization related to previous exposures to chlamydial antigens. Much work has already been done with *C. trachomatis*, primarily a human pathogen, and certain research applications have been successfully used for *C. psittaci*. It is believed that important answers will be obtained in the near future clarifying the role of *C. psittaci* in cattle.

**PERSISTENT BOVINE VIRAL DIARRHEA VIRUS INFECTION IN CATTLE AND ITS RELATIONSHIP TO MUCOSAL DISEASE**

STEVEN R. BOLIN, D.V.M., Ph.D.
National Animal Disease Center, USDA/ARS

Bovine viral diarrhea (BVD) occurs in 2 clinically recognizable forms: an acute form with high morbidity and low mortality, and a chronic form with low morbidity and high mortality. Bovine viral diarrhea virus (BVDV) may also produce an inapparent, persistent infection following exposure of the bovine fetus to BVDV before day 125 of gestation. This third form of BVD is seldom detected by the producer or veterinarian. Recently, several investigators have reported that mucosal disease occurs when cattle persistently infected with noncytopathic BVDV become superinfected with cytopathic BVDV. A series of studies conducted at the National Animal Disease Center has provided experimental evidence supporting the above etiology for mucosal disease.

**Experimental Reproduction of Musosal Disease**

Eight cattle persistently infected with 1 of 3 isolates of noncytopathic BVDV were superinfected with cytopathic BVDV. All of the cattle developed clinical signs of mucosal disease, 2 of the cattle produced neutralizing antibodies to BVDV, and 1 cow survived. Previously, 3 of the 8 cattle had been superinfected at monthly intervals with 4 different isolates of noncytopathic BVDV. No signs of disease occurred following superinfection with the noncytopathic BVDV. Similarly, normal cattle without
neutralizing antibodies to BVDV showed very mild or no clinical signs following inoculation with the cytopathic BVDV.

Isolation of Noncytopathic and Cytopathic BVDV from Field Cases of Mucosal Disease

Virus isolation procedures were performed using splenic tissues from 39 field cases of mucosal disease. The spleens came from 13 herds, 8 of which had been recently vaccinated for BVD. Noncytopathic and cytopathic BVDV were isolated from individual spleen samples from all herds and from 29 of the 39 spleens that were examined. In most cases, noncytopathic BVDV was separated from cytopathic BVDV by titration of splenic suspension in roller tube cell cultures. The noncytopathic BVDV was usually 10 to 100 times more concentrated in splenic tissues than cytopathic BVDV.

Prevalence of Persistent BVDV Infection

Blood buffy coat and serum samples from 3,157 cattle, representing 66 herds, were tested for antibodies to BVDV and for the presence of BVDV. Antibodies to BVDV were detected in 89% of the serum samples. Noncytopathic or cytopathic BVDV were detected in 60 blood buffy coat samples from 6 herds. This was approximately 2% of the cattle and 9% of the herds tested. Large groups of 23 and 31 persistently infected cattle were found in 2 herds. Subsequently, all of the group of 31 cattle died of mucosal disease.

Vaccination of Persistently Infected Cattle with Commercial Vaccines for BVDV

Eight cattle persistently infected with noncytopathic BVDV were vaccinated with 1 of 2 modified live virus (MLV) vaccines for BVD or with a killed virus vaccine for BVD. Clinical signs of BVD were not seen during a 6-week period following vaccination. At 6 weeks after vaccination, the cattle were inoculated with cytopathic BVDV. All the cattle died of mucosal disease 2 weeks to 7 months after inoculation with cytopathic BVDV. All of the cattle had from 100 to 100,000 cell culture infectious doses of noncytopathic BVDV per ml of serum at necropsy. The cattle vaccinated with the MLV vaccines produced detectable levels of antibodies that neutralized cytopathic BVDV. None of the cattle produced detectable levels of antibodies that neutralized the persistently infecting noncytopathic BVDV.

PUBLIC HEALTH ASPECTS OF LISTERIOSIS IN MILK

George Lambert
National Animal Disease Center, ARS, USDA
Ames, Iowa 50010

In August of 1983, the Massachusetts Department of Public Health noted an increase in the number of isolates of Listeria that they were receiving for bacteriologic confirmation. They requested assistance from
the Center for Disease Control in Atlanta. Cases were defined as patients with either a blood or cerebrospinal fluid culture positive for *Listeria monocytogenes*, or a positive placental culture related to illness in the newborn. All microbiological laboratories in the State were contacted and requested to send all isolates to CDC for serotyping.

Between June 30 and August 30, 1983, 49 patients were hospitalized in Massachusetts with meningitis or septicemia caused by *Listeria monocytogenes*. The outbreak began in late June, peaked in mid-July and tapered off during August. Seven of the 49 patients were infants, 42 were adults ranging in age from 24 to 101 years old. All of these adults had previous underlying illnesses resulting in immune system compromise. Of the 49 patients, 14 died, an overall mortality rate of 29 percent. Isolates from 40 of the 49 cases were available for serotyping. Of these, 32 were serotype 4b which has been defined as the epidemic strain.

The epidemiology of the outbreak was difficult to determine. There was no evidence to support person to person, airborne or waterborne transmission of the disease. There was no association of disease with any of 32 specific food items studied. Interestingly, cases appeared to be more likely than controls to shop at a particular food chain. Subsequently, 5 of the patients were taken to the food chain stores and lists of items purchased, were compiled. The chain's brand of pasteurized whole or 2% milk was found to have been purchased by all cases and was the only item common to all lists. Analysis of cases and controls by CDC revealed a very strong association between consumption of the chain store's whole or 2 percent milk and illness.

It was subsequently learned that 50 percent of the controls drank less than one-third glass per day whereas 40 percent of the cases drank one or more glasses per day. Furthermore, consumption of the chain store's skim milk or 1% milk appeared to be protective.

During the outbreak in Massachusetts, thirteen patients with listeriosis were hospitalized in Connecticut. Three of these 13 patients had purchased the chain store's milk. No association with any food or food chain could be shown for the other 10 patients.

The milk implicated in this outbreak was pasteurized by a single processing plant in Massachusetts, which in turn received its milk from a Co-Op of 450 farms in Vermont. Milk shipment flow into the Co-Op resulted in a different set of farms supplying whole and 2% milk than those supplying 1% and skim milk.

Veterinarians reported that in early summer four cases of listeriotic encephalitis had been diagnosed in dairy cows supplying milk to the Co-Op. *L. monocytogenes* type 4b was isolated from the bulk tank of one of
these farms. This farm supplied milk to the Co-Op. The question as to how did this contaminated milk make it by the pasteurizer was the subject of much study. Multiple plant inspections failed to identify a defect which could have resulted in either improper pasteurization or post-pasteurization contamination. Phosphatase testing, a measure of proper pasteurization was performed by both the state and by the company during the outbreak and was consistently negative. A continuing active surveillance for listeriosis in Massachusetts and Vermont has been initiated.

Research has been initiated cooperatively between CDC, FDA, and USDA to determine the ability of Listeria to survive pasteurization as currently recommended.

Prior to this outbreak, ARS and the Office of International Cooperation (OICD) of USDA were cooperating with researchers at the University of Novi Sad’s Veterinary Research Institute in Yugoslavia on Listeriosis research. The objectives were to improve diagnostic procedures and study pathogenesis of listeriosis in dairy cattle. Subsequently, we have initiated two new research projects on listeriosis, one in Yugoslavia and another at the University of Vermont.

The Vermont research is directed toward the development of a rapid test for detecting *Listeria monocytogenes* in raw milk and dairy products. This will include the development of repair-detection procedures for the isolation of *L. monocytogenes* from heat treated milk. The potential for sublethally injured *L. monocytogenes* to survive pasteurization, repair and grow in milk or dairy products will be evaluated.

The Yugoslavia listeriosis research will be expanded to include experimentally and naturally infected cattle. Bacteriological and serological studies of such cows will be examined for evidence of shedding *L. monocytogenes* in milk and seroconversion. Additionally, milk from cows shedding *L. monocytogenes* will be used for the preparation of certain types of cheeses. The survival and resistance of *L. monocytogenes* in the cheeses will be monitored. Different regimes of pasteurization on the survival of *L. monocytogenes* will be studied also.

Although limited spiking of milk samples with *L. monocytogenes* has been done and various heat treatments have indicated effective killing of the organisms, there is little available information on the survival of the organism when shed in the milk of naturally or artificially infected cows.

Acknowledgements: The cooperation of Dr. David Fleming of the Special Pathogens Epidemiology Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Public Health Service, Centers for Disease Control, Department of Health and Human Services is gratefully acknowledged. Dr. Fleming kindly supplied most of the epidemiologic data.
AN UPDATE ON FESCUE TOXICITY

H. J. Bearden
Dairy Science Department
Mississippi State University

A Summary

Tall fescue was first described by a German botanist, Schreber, in 1771, but was not tested in the United States until the early part of this century. Chemical analyses of the plant material indicated that it should be an excellent forage plant for both beef and dairy cattle. However, performance records have traditionally contradicted those predictions. Even so fescue is the most widely grown species of grass in the U.S. with 35 million acres being grown. It has wide adaptation, persistence, good yield, long growing season, has tolerated mismanagement and can be grown from seed.

A wide range of pathological symptoms develop in cattle grazing this crop. These include lowered animal performance, rough hair coats, elevated body temperatures, rapid breathing, desire to stay in the shade close to water, nervousness, lameness and sloughing of tail and feet. For many decades scientists looked for some toxic material contained in the plant itself that might cause these symptoms. Essentially no progress was made until the decade of the seventies when scientists finally discovered that the cause was actually a toxin produced by the fungus \textit{Acremonium coenophialum}. Fungi of the species \textit{Fusarium} and \textit{Aspergillus} have also been found in fescue plants. \textit{Aspergillus terreus} has been shown to cause the foot symptoms associated with fescue toxicity but apparently does not cause other symptoms nor reduced performance. Some scientists are currently thinking that the foot symptoms caused by \textit{Asperigillus terreus} may be a synergistic effect with \textit{Acremonium coenophialum}.

\textit{Acremonium coenophialum} is an unusual fungus in that it has never been found outside of the fescue plant and seeds. It apparently never goes into a reproductive state to produce spores but remains in the vegetative state at all times. It is only transmitted to new plants through the seed. Fairly simple diagnostic techniques have been developed to determine whether either seed or vegetative materials are infected with \textit{Acremonium coenophialum}. The endophytes of this species are easily differentiated from endophytes or mycelium of other species.

Several experiments with fescue using both beef and dairy animals indicate that for each 10% of infection daily weight gains are reduced by one-tenth of a pound. This occurs even though clinical symptoms of fescue toxicity are avoided. We are recommending in Mississippi that infected stands of fescue be renovated and replaced with fungus free stands. This has presented no problems since fescue seeds dropped on the ground are viable for only a few months, thus essentially no reseeding occurs in nature. Mississippi cattlemen have experienced no problem in buying all
the fungus free fescue seed needed. The State of Mississippi has a regulation which requires that all fescue seed sold be labeled as to the percentage of infection with *Acremonium coenophilum*. Alabama will probably adopt a similar regulation before the end of this year and other states are considering regulations.

It seems logical that with moderate effort fungus could be eradicated from fescue. This probably will never be done since 85% of all fescue seed sold are used for lawn purposes. Fungus infected fescue is more disease and insect resistant in lawns, hence more desirable.
PRACTICAL METHOD FOR PERMANENT IDENTIFICATION OF IMPORTED FEEDER STEERS

Prepared by:
Dennis G. Olson
Gene H. Rouse
Iowa State University
for:
USAHA Identification Committees

A practical method needs to be developed to identify beef carcasses that come from cattle which entered the United States as Feeders. With method carcasses, for example, that have evidence of tuberculosis can be easily identified as coming from domestic or nondomestic cattle.

The first phase of this project included the development of methodology of injecting colorant and identifying carcasses of animals injected.

The instrument used for injection was a portable, needleless injector (Pedo-Jet, Model POJ-6515-00-910-0097) with an orifice of 0.011 inches and intensifier attached which delivers 2.25 cc of solution. This instrument allows for easy and accurate delivery of colorant and should be easily adapted to field use. No other delivery system was utilized in the project.

The choice of colorants to be used in marking the carcass was the most difficult aspect of this phase of the project. Initially, aluminum lake dyes FD&C Blue #1 and FD&C Red #3 (carcass stamp ink) were used. Cattle were injected in the muzzle and masseter muscle region with these dyes one day and one week before slaughter. Following examination after slaughter, the dyes gave a purple color along the cartilage of the muzzle but it appeared very diffuse and followed the fasia along the cartilage. Since the diffusion was so extensive it was decided to perform histological examinations to determine if the colorants were retained inside any tissue cells.

Black branding ink (iron oxide) was considered a possible colorant dye to it permanent connective tissue stain. However, no adequate solution could be made using water, alcohol or propylene glycol such that the Pedo-Jet orifice would not become plugged. Hence this colorant was no longer considered an alternative.

With the collaboration of Mr. Robert Strub, Colorcon, Inc., Mayer Blvd., West Point, PA 19486, several dye dispersions were tried. The dye dispersions (DD) used were DD-3261/green (FD&C Yellow #5 and FD&C Blue #1 in glycerin) and DD-4701/purple (FD&C Red #40 and FD&C Blue #1 in sugar solution). These colorants were injected in five cattle in different locations one day prior to slaughter. After slaughter the muzzle was examined. The colorants injected in the muzzle appeared quite diffuse. Subsequent histological examinations showed that no colorant was retained within any cells. Hence, these colorants would most likely be cleared by the body over long term trials.

With further assistance from Colorcon, Inc., a special formula number X-02825/purple containing FD&C Red #40 and FD&C Blue #2 with sugar
was provided. A 70% aqueous solution of X-02825 was prepared and injected into the muzzle two weeks prior to slaughter. Samples were removed from the muzzle after slaughter and examined histologically using frozen sections. The frozen sections showed that these colorants (primarily FD&C #40) were retained in the connective tissue. This was the first success in finding a colorant that has the potential to be maintained in the muzzle during growth. Five additional cattle were injected with Formula No. X-02825 (Colorcon, Inc.) one month prior to slaughter. Muzzles were examined after slaughter and the purple stain was easily found in the connective tissue and 1.5 inches from the muzzle exterior. The purple color that was found is in some muzzle locations not easily distinguished from the dark red muscle background. Colorcon, Inc. has indicated that a similar approved stain having a green color may be more easily distinguished. Currently, purple and green dyes are being compared on slaughter steers.

Various injection sites were used during the trials. These sites include the muzzle (one inch above the nostril), masseter muscle (in the center of the cheek), splenius muscle (center of the neck) and left or right of the tail head. In all sites except for the muzzle, the colorant penetrated the hide but did not extend beyond the hide. Hence when the hide was removed, the colorant was also removed and no carcass identification could be made after hide removal. These results were obtained with cattle at maturity and market weight. In these cattle the hide is too tough to allow penetration of the colorant beyond the hide using the Pedo-Jet. In younger cattle the hide may be more easily penetrated. However, the colorant located in the muzzle would probably result in the least economic devaluation of the carcass.

With the results from the first phase, it appears that the injection of Colorcon X-02825 using a Pedo-Jet has the potential to permanently identifying carcasses of calves injected at an early age. The second phase, being conducted currently, involves the injection of calves 18, 12, and 6 months before they will be slaughtered to determine the effectiveness of the colorant retained in the muzzle during the growth cycle of cattle. A third phase will involve a cooperative field testing program with APHIS of this new procedure.
REPORT OF THE COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: Harold Mindermann, West Des Moines, IA
Vice Chairman: R. E. Nelson, Brattleboro, VT

J. B. Ashcraft, CO; Dewey Bond, DC; P. E. Bradshaw, IL; D. R. Bridgewater, CO; H. F. Embry, IL; G. B. Estes, VA; Robert Gadd, SD; Bill Gallagher, SD; H. E. Goldstein, OH; Tom Haas, KY; J. N. Huff, CO; G. M. Jones, NM; Ralph Jones, SD; Dee Likes, KS; Marlin Main, SD; N. F. Powers, Jr., NY; E. C. Roukema, VA; Raymond Schnell, ND; G. L. Seawright, NM; R. S. Sechrist, OH; G. R. Snyder, VA; W. E. Stemler, IL; F. E. Sterner, CO; J. R. Taylor, TX; J. E. Thomas, AR.

The Committee held its meeting Wednesday, October 19 with the following members present:


Dr. Cecil Watson, USDA-APHIS, reported on the review of Part 78 of CFR in keeping with previous action of the committee to determine if it was adequate or could be strengthened to deter tampering with eartags or identification of animals moving interstate. He stated that the current provisions in Section 71.18 and 78.30 require that eligible animals be individually identified and that these individual identification devices remain on the animals for the interstate movement.

There is authority in the animal quarantine laws to require that individual identification devices on animals moving interstate remain on the animals while they are being moved in interstate commerce. Utilization of this authority would mean that the devices would be required to remain on the animals while such animals are being moved from the point of origin, interstate to the animal’s final destination, such as a farm or slaughtering establishment, and including any temporary stops prior to movement to final destination; such as stops at a stockyard for feed or sale.

In order for tracing to be effective, it will be necessary to amend Sections 71.18 and 78.30 to require that such identification remain on the animals while they are being moved in interstate commerce.

Present regulations indicate, those persons who are responsible for identification of cattle and swine for interstate movement and that removal or tampering with either identification backtags or eartags from such cattle or tattoo and eartag from such swine is forbidden.

Proposed changes would apply the same responsibilities and restrictions to all persons and extends their application to all identification devices and to all movements in interstate commerce rather than just interstate movement.
Proposed changes would also clarify the authority for removal of identification devices at slaughter by authorized persons.

Therefore, the present regulations will need to be amended to allow the exercise of the full authority of the animal quarantine laws pertaining to the individual identification devices remaining on animals moving in interstate commerce until they reach their final destination.

These proposed changes are in the process of being prepared for release as proposed rulemaking in the near future.

Dr. Watson went on to report on the follow-up on other previous action of the Committee requesting a feasibility study on the use of bar codes on backtags and its possible use in the MCI program. He stated that contacts are made with various individuals and firms in an effort to determine what would be required to use bar coding on the backtag.

The Universal Product Code (UPC) used on grocery items was investigated, but it will not work because it is strictly a numeric system. The backtag has two alpha characters and six numeric digits so an alphanumeric code would have to be used.

Further contacts were made and we have let a contract for research and development of a bar coding system and a feasibility study will be conducted to determine whether or not the system will work.

This study will determine the following:
1. The size of a bar code that will be needed.
2. The equipment needed to read the bar code in the laboratory.
3. Whether the bar code can be read electronically through the plastic bag and through the debris that accumulates on the tag surface.
4. Equipment needed to interface with the BIS system so that the backtag information can be put directly into the system.
5. Equipment needed to print or apply the bar code on the backtag.

If this study is successful and this idea of bar coding can be universally adopted there are untold advantages that will accrue to the MCI program.

Errors in transcribing numbers can be virtually eliminated.
Time spent writing up 4-54's will be eliminated.
Time spent inputting data by hand will be eliminated.
The excuses used for not inputting negative data will be eliminated.

We are hopeful, that the research and development of a bar coding system will be successful and that we can report such success at the next meeting of the Committee.

Chairman Minderman reported on the joint meeting of the USAHA and LCI Identification Committees held April 10 in connection with the LCI annual meeting when it made the following observations.

There is general agreement on the need for identification and successful applications of identification to include:
LIVESTOCK IDENTIFICATION

1. Feeder pig ID as a result of the hog cholera program.
2. Boar and sow ID regulations relating to swine brucellosis.
3. Some state branding laws.

Some of the obstacles to a national ID systems are:
1. Organizational policies;
2. Government interference and penalties;
3. Unreasonable tolerance levels for residues;
4. Cost of equipment, labor and recordkeeping;
5. Lack of a permanent ID system that is economical and functional;
6. Need for producer involvement and education;
7. The term mandatory has negative connotations.

Uses of identification would include:
1. Animal health;
2. Herd management;
3. Theft prevention;
4. Residue detection and education;
5. Puts a name on your product;
6. Genetic improvement;
7. Disease eradication.

An identification system should provide for:
1. National in scope;
2. Permanent;
3. National numbering system;
4. Minimum of herd ID, individual ID optimum;
5. Application at the farm level;
6. Methods may vary as long as they meet these qualifications;
7. Ultimate goal a computerized, electronic system.

As to the resolutions passed by the joint session, the committee acted to clarify No. 1 and the three resolutions as follows were ratified as action of this Committee on motions by Ashcraft and seconded by Embry.

1. To continue to pursue a national, uniform means to identify swine back to the farm or ranch (acknowledging that eventually the word "mandatory" will be included), that would be in compliance with a national swine disease regulatory program.

2. That the National Pork Producers Council task force on identification be made aware of Resolution #1 and that it is supported by both LCI and USAHA.

3. To encourage all segments of the industry involved in LCI to inform members of their organizations of their support for total identification and to work toward a uniform policy of all organizations.
Dr. Gene Rouse reported on a method of identifying imported beef cattle in order that their carcasses can be identified as such by injecting a dye in the muzzle with portable needleless injection. The complete paper will be published separately.

Dr. David Meisinger of the National Pork Producers Council updated the committee on the progress made by the Pork Producers Council Task Force on Identification. He reviewed the analysis and observations developed during the course of its just three meetings indicating that conclusions on procedures were yet to come.

Dr. Claude Lavigne of Agriculture Canada reviewed the identification situation in Canada. He pointed out that the Canadian Government identified cattle and sheep, primarily cattle, with eartags through disease programs, the production testing program and through artificial insemination cooperatives.

He referred to diverse groups with unique interests but indicated that methods have changed little though electronic developments were being monitored. He explained that Agriculture Canada invested $100,000 in a project to develop electronic identification in 1979 but nothing materialized. However, they have scheduled a conference to evaluate electronic identification on October 29 and 30.

Mr. Cliff Prough of Identification Devices Inc. stated that his company was pursuing the market from:

1. Implantable transponder and eartags;
2. Data collection devices in research facilities; and
3. Electronic monitoring of carcasses in processing.

He would explain the latter at this meeting. He stated that early in 1984 he toured three separate slaughter operations to establish the practicality of electronic identification in slaughter plants. Satisfied that a significant contribution could be made, he proceeded to locate plants receptive to this idea. At the present time they are designing two systems.

One system is to be installed at a skinning plant processing about 893 animals per hour. This plant has 8500 gambrels and will utilize five reading stations. The system will perform the following functions:

1. Individual carcass lot ID.
2. Individual carcass grade.
3. Individual carcass weight.
5. Identify condemned carcass.

In addition to this information, the company will provide additional data so as to achieve a comprehensive readout of performance by animal and lot.

The second system is to be installed in a bacon processing plant to collect data on bacon slabs as they proceed through the processing operation. Our device will operate as a subsystem to a larger computerized operations system being installed by another company.
The purpose of these installations is to demonstrate practical, useful, and cost-efficient application of electronic identification of individual animals in packing plant operations.

At the present time there are about eight plants, each having in excess of 8000 hooks, that are interested in applying this technology to their operations.

Through continued product development, we now feel that the price for such a system can be justified; continuing interest from the industry worldwide encourages us to pursue this application to a successful conclusion.

Electronics in animal handling has progressed far beyond the idea of the rancher or farmer identifying his livestock as they run across the pasture. Animal handling operations are becoming high tech users to a greater degree all of the time and the benefits of reliable, fast, and efficient data is now viewed as essential to good farm and industrial management. Electronic identification as applied to these and other situations are designed to enhance the quality of this industry. As long as this is kept in focus, the animal handling industry will benefit greatly.

Richard Nelson commented on renewed activity of the National Livestock Electronic Identification Board with a meeting April 11 that attracted 8 participants involved in producing or distributing electronic devices, three of which were from Europe and with Dr. Spohr of the University of Illinois summarizing overall development.

He stated that an effort was going forward on matters relating to standardization through contact with the Instrumentation and Measurement Society of the Institute of Electrical and Electronics Engineers.

In closing, the meeting chairman, Minderman, announced that new responsibilities in connection with a position change made it necessary for him to resign as chairman. It was then unanimously voted that the committee express its appreciation and gratitude for his very significant, effective and productive contribution he has made to the work of the Identification Committee.
The Committee on Pharmaceuticals, Pesticides and Related Toxicology met as scheduled on Tuesday, October 23, 1984, in Citizens Room B, Hyatt Regency Hotel, Fort Worth, Texas. There were 29 persons in attendance: 20 members and 9 guests. Dr. R. T. Roe, an Australian Government Veterinarian, attended in place of member Dr. J. C. Humphries, Veterinary Attache, Embassy of Australia, Washington, D.C.

The meeting opened with the review of two books. The book entitled The Apocalyptics by Edith Efron (Simon and Schuster), published in 1982, was very ably reviewed by committee member A. A. Chadwick.

The Apocalyptics presents an important discussion on the basis for present OSHA, FDA, and EPA policies on cancer prevention, and concludes that these policies are based more on the personal opinions of some important cancer scientists than on scientific fact.

The book gives an historical review of the events which carried the thinking of doomsday prophets like Rachael Carson and Barry Commoner into the scientific community and into the U.S. Regulatory Agencies — thinking which condemned industrial chemicals as the major cause of cancer.

Ms. Efron gives the pros and cons of the No Threshold (one molecule) theory of cancer initiation. She discusses the weaknesses of animal tests in predicting human cancer, and mutagenic tests in predicting carcinogenesis. She finds the risk assessment concept statistically unsound.

Her primary point is that industrial carcinogens account for only 5% of total cancers occurring in the United States, yet U.S. Government agencies including N.C.I. have deliberately led the public to believe industrial carcinogens are the major cause of cancer and a major government effort must be directed toward them at the expense of other cancer work. The book is not pro-industry, but rather a rebuttal to the book, Silent Spring by Rachael Carson and other notable apocalyptics.

The second book entitled Modern Meat by Orville Schell, (Random House), published 1984, was the subject of a detailed review by Dr. T. P. Siburt. The author’s purpose is to alert the public to alleged undiscriminate use of massive quantities of antibiotics, pesticides, drugs, chemical
compounds, hormones, feed additives and other agents in the production of food animals. In the reviewer’s opinion this book, *Modern Meat*, is very poorly written and will, therefore, not have the intended impact upon the reading public that would be expected from a book on this subject.

Dr. Donald A. Gable, Center for Veterinary Medicine, Food and Drug Administration (CVM–FDA), Washington, D.C., presented a paper entitled, “Regulation of Biotechnology-Derived Products.” His presentation was limited to products intended for use in animals.

Dr. Gable stated that there has been a dramatic increase at CVM–FDA in the amount of interest and the number of inquiries involving biotechnology-derived products. He indicated that it was possible that one or two FDA approvals could occur next year.

CVM maintains normal review procedures when recombinant DNA products are involved. GMP (Good Manufacturing Procedures) regulations as they apply to the manufacture of biologics are the most appropriate for the manufacture of recombinant DNA products. Environmental concerns focus upon the biocontainment of all product components.

Biotechnology-derived products that are immunizing biologicals will be regulated by Veterinary Services Biologics Program. Those which have therapeutic applications will be regulated by CVM–FDA.

Dr. George T. Edds, Waco, Texas, presented a paper entitled “Selenium — Vitamin E Relationship to Drug Metabolism, Toxicosis and Immunity.” This paper appears elsewhere in these proceedings. Selenium and Vitamin E are antioxidants. Selenium is a essential constituent of the enzyme glutathione peroxidase. It enables the immune system to perform more effectively. It restores complement levels as well as phagocytic capabilities of leucocytes. Selenium has a very narrow therapeutic index. It has a protective function against aflatoxicosis as well as toxicoses involving mercury, arsenic and cadmium. A dietary source of selenium should be provided according to species and geographic location (amount of selenium in soil/plants).

General recommendations:

- All species: .1 ppm
- Turkeys: .2 ppm
- Young, growing swine: .3 ppm

An informal discussion period included the following subjects discussed by Dr. William Bixler, Center for Veterinary Medicine Food and Drug Administration, Washington, D.C.

(A) Second generation medicated feed regulation. Status Report. In process. It is not expected that the regulation will issue before mid-1985. Some administrative changes are anticipated. For instance, new medicated feed application (Form 19 OV) will be used for category 2 drugs.

(B) Causal Review. What criteria does FDA use to “trigger” a causal review of an approved drug?

Dr. Bixler stated that a causal review is a problem solving exercise. It is
not cyclical in nature. It is instituted only when a serious problem exists. The Drug Experience Report (DER) is usually a basic source of information concerning problems with an approved drug.

(C) Extra-label Drug Use Update

If a veterinarian elects extra-label use of an animal drug, an understanding between him (her) and his (her) client is necessary, said Dr. Bixler. It is hoped that the veterinarian, when confronted with this situation, would use a higher dose of the (approved) drug previously administered as the next extra-label step. If this does not produce satisfactory results, then the veterinarian may find it necessary to use an approved drug for the species, but in an “extra-label” manner, i.e. indications, dosage, etc.

Chloramphenicol has been determined to be unsafe in any species of food producing animal. It is not to be used in food animals under any circumstances.

FDA is “going after” flagrant violations of chloramphenicol abuse and misuse, i.e. distributors, veterinarians, etc.

(D) Importation of Illegal Animal Drugs

FDA is attempting to deal effectively with the illegal import of animal drugs across our borders.

Dr. Bixler also addressed the subject of the veterinary prescriptions. He stated that the public comment period has been extended until December 31, 1984. He urged that the veterinary profession respond with comments. Prescription drugs are in jeopardy. Concern was expressed that if the problem of veterinary prescriptions is not resolved, the policy on prescription drugs may not survive.

Dr. Donald A. Gable, CVM–FDA, addressed several agenda topics:

1. IR-4/FDA status of human food safety with special reference to metabolitic data requirements.

   Dr. Gable reported that the status is unchanged. Sheep are classified as a major species with respect to human food safety requirements.

2. Environmental impact requirements for drug clearances. Dr. Gable discussed this briefly. It appeared to be the consensus of those present that, in general, veterinary drugs have very little environmental impact and that this should be recognized and subjected to critical review insofar as present requirements are concerned.

The committee discussed a study by Holmberg, et al, published in the September 6, 1984, New England Journal of Medicine, which reportedly proves a link between subtherapeutic use of antibiotics in animal feed and antibiotic resistant bacterial disease in humans.

The report has received extensive publicity in the news media and is heralded as linking human illness to the use of drugs in livestock. However, committee members expressed concern that rather than establishing
such a link, the investigation was, in fact, incomplete in several important respects. As a result, the committee believes the report provides no conclusive new evidence which would associate the subtherapeutic use of antibiotics in animals with human health problems.

Due to the publicity received by the report, the committee is concerned that consumers may become unduly alarmed about the safety of the nation's meat supply. This, in turn, could result in consumer pressures to unduly restrict the use of drugs in livestock.

The committee recommended increased cooperation and communication among scientists, the livestock industry, drug manufacturers, the veterinary profession, and the food and drug administration in addressing the issues relative to any effect of drugs used in livestock upon human health. The committee hopes that such communication would result in future discussions concerning the use of drugs in livestock, and would be based on sound scientific evidence and a broad understanding of the issues involved.

**SELENIUM/VITAMIN E RELATIONSHIP TO DRUG METABOLISM, AFLATOXICOSIS AND IMMUNITY**

George T. Edds, DVM, PhD, Visiting Research Professor, Texas A&M University

In 1931, it was found by the USDA that selenium was responsible for the toxicity of certain wheat grown in South Dakota. The selenium was taken up from the seleniferous shales. The quantities of selenium present in random selections from the wheat markets varied from 0.1 to 1.9 ppm. Certain samples of wheat were highly toxic to white rats and other animals and would probably be toxic for humans. Researchers have shown that the presence of sulfur in the soil is an inhibiting factor and that as the sulfur in the soil is increased, as by use of fertilizers, the selenium absorbed from the soil is decreased. Wheat from a small flat in Wyoming contained 2.7 ppm. and wheat from plots in South Dakota varied from 8-26 ppm. When the diet contained 3 to 6 ppm. the growth was depressed and reproduction ceased.

Jaffe,7 1967, reported that of the 113 bovine serum samples analyzed 72 contained more than 3 ppm., 39 more than 10 ppm., and 2 more than 5100 ppm. He stated that levels greater than 0.3 to 0.5 ppm. in feeds or foods would be dangerous to animals and mankind. He discovered that in certain states in Venezuela, concentrations of selenium in feeds were present at more than 5 ppm. and 0.5 ppm. was present in water and milk. Malformations and excess elongation of the hooves of cattle were observed in animals receiving feed high in selenium.

Selenium toxicity in horses or cattle is called "bob-tail" disease with a loss of hair from the mane and tail. The animals also developed lesions around the coronary band and in severe cases actually sloughed the hoof.

Mahan and Moxon,8, 1979, reported on experiments to determine the minimum concentrations of dietary and injectable selenium, as sodium selenite to produce selenosis in young swine. Dietary levels up to 2.5 ppm.
resulted in no adverse effects on any criteria evaluated. At 5 ppm. selenium or higher, when fed over a 37 day period, caused depression of feed intake and growth rate, body weakness, loss of hair, and hoof sloughing. A single injection of 0.25 Se/lb bodyweight had no adverse effects but 0.5 mg Se/lb bodyweight resulted in death in 10 hours. Signs of body weakness, vomiting, prostration and dyspnea were evident.

Research\(^1\) from Colombia, S.A., 1947, confirmed that feeds and foods from Boyaca province induced toxic effects in cattle, poultry and even humans including skin diseases with loss of hair and feathering. Barley was found to contain 137 ppm. selenium and peas 136 ppm. The soil samples from the fields where these plant products were grown contained selenium levels of 3.5-7.0 ppm. selenium.

Although selenium at higher levels in feed and foods may induce toxicity in animals and humans, it has been recognized as one of the nutritionally required trace elements at lower levels. (Edds & Bortell\(^3\)). Selenium is an essential constituent of glutathione peroxidase. Each molecule of the enzyme contains 4 atoms of selenium which functions cooperatively with vitamin E to protect the cell from oxidative stress by either the destruction of precursors of active oxygen or by reducing the oxidized cellular components. Two important precursors of active oxygen are superoxide \(O_2^-\) and hydrogen peroxide. These two precursors may promote the oxidation of lipids, proteins and other cellular components. The enzyme glutathione peroxidase is present in various cell types including erythrocytes phagocytic leucocytes, granulocytes neutrophils, basophils, eosinophils, and monocytes as well as alveolar and peritoneal macrophages and in the liver, kidneys, lungs and muscles. To test for delayed hypersensitivity, *Candida albicans* was injected intradermally. There was a delay in hypersensitivity in piglets in the test group given aflatoxin B\(_1\). Antibody production and WBC numbers were not decreased, yet the decreased complement titers indicated that antibody function and phagocytic capacity were impaired. Electrophoretic studies during the trial showed an altered A/G ratio, a decrease in alpha globulins, no change in beta globulins but an increase in gamma globulins. The serum levels for SGPT, SGOT and arginase were increased in the aflatoxin exposed group. There was also a marked increase in bile duct proliferation as well as lymphocytic infiltration in this group. Earlier research reports suggested that added selenium in the diet would reverse or decrease these toxic effects of aflatoxin B\(_1\).

In a recent trial, 50 poulets received a selenium supplemented diet at 0.2 ug/g and 50 poulets received a Se diet at 2.0 ug/g for 4 weeks. These 2 groups were subdivided with 25 poulets continuing at 0.2 ug/g and 25 poulets at 2.0 ug/g while two other groups of 25 received Se at one of these levels plus aflatoxin B\(_1\) at 750 ng/g for these latter groups. The four groups were maintained on these diets for an additional 3 weeks.

Selenium stimulated increased GSH\(P_x\) productions peaks in 3–4 weeks in the turkey poult. There were no obvious differences in physical ap-
pearances nor mortality in either of the 4 groups. But, the livers of most of the poults receiving selenium at 0.2 ug/g and aflatoxin were light brown to tan while those poults receiving Se at 2.0 ug/g and aflatoxin were similar to the aflatoxin negative 0.2 ug/g selenium group. The average body weights of poults of the low level selenium and aflatoxin group were lower than those receiving selenium at the highest level and aflatoxin. Total serum protein, as well as albumin and globulins were depressed significantly in the aflatoxin and low selenium group. Poults in the 2.0 ug/g selenium and aflatoxin group had values comparable to the control group for total serum, albumin, globulins and albumin/globulin ratios. The complemet titers were similar to or higher than those in the control group in the group receiving Se, 2.0 ug/g and aflatoxin B1 750 ng/g in the diet.

Gregory, 4 1984, reviewed the protective effect of selenium against aflatoxin B1, in several species. Newberne and Connor, 1974,9 first reported that selenium supplementation up to a dietary level of 1.0 ppm progressively reduced the toxicity of aflatoxin B1 in rats.

The concentration of aflatoxin B1 used in the diets of treated birds was 750 ng/g diet. Hematological, immunological and histological evidence of aflatoxicosis was produced. Addition of selenium at 0.2, 2.0 and 4 ppm. was effective in reducing the toxic effects. Concentrations of free aflatoxins B1 and M1 were inversely related to the dietary selenium level. In contrast, the concentration of conjugated (aqueous phase) aflatoxins B1, M1 and total (B1 + M1) was higher for the 2.0 and 4.0 ppm. selenium groups than for the 0.2 ppm. group. These results indicate that selenium promotes the in vivo formation of water-soluble conjugates, which may promote the excretion of the toxin. Further research comparing the effect of selenium on metabolic patterns in various animal species would assist in evaluating the overall protective effect of selenium. These and similar data in other species, suggest that selenium at nutritional levels would enhance the resistance of animals to the effects of aflatoxin B1.

Current research indicates that other single as well as mixtures of mycotoxins may also adversely affect animal and poultry productivity. Schlosberg,13 1948, describes an episode involving a laying flock of 2800 hens where new feed containing the trichothecenes, T-2 and HT-2, reduced production in a week to 150 eggs per day. Change to a new non-contaminated feed resulted in recovery to normal production in 15 days. It was the authors opinion that the laying flock was exposed to a relatively small quantity of feed highly contaminated with the trichothecene mycotoxins, while most of the several tons of feed was mycotoxin free.

Gyang,5 et al, 1984 demonstrated that selenium treatment increases the serum antibody titers in cattle. The polymorphonuclear leucocytes of selenium — vitamin E injected cows killed Staphylococcs aureus faster than those from selenium deficient cows because of the lowered GSH.Px activity in the latter group. The accumulating toxic peroxides in the latter damages the neutrophils and proteins. The production of hydrogen peroxide and superoxide O2 is dependent on the availability of NADPH, dihydronicotinamide adenine dinucleotide phosphate in the neutrophils
which, in turn, is influenced by the GSH.P.X activity associated with adequate selenium levels.

Aziz,2 1984, demonstrated that adequate levels of selenium in the diet of goats was essential for normal neutrophil phagocytic activity. In goats fed selenium deficient diets, the neutrophils demonstrated decreased random migration under agarose, decreased leukotaxis toward serum chemotoxins and decreased phagocytosis. Incubation of the neutrophils with selenium resulted in marked functional enhancement. Such varied functions were associated with corresponding alterations in blood, serum and intracytoplasmic GSH.P.X activities.

South,14 1982, reported on research at the University of Idaho where cattle received salt mixes containing 20, 50 and 90 ppm. selenium. (A 90 ppm. Se-salt mix is produced by adding 4 grams of reagent grade sodium selenite to 50 lbs. salt). After 60 days on the selenium-salt supplement GSH.P.X tests on blood samples indicated adequate tissue selenium levels, while cattle receiving the 20 ppm. mixture showed little change. Calves born to cows on the 90ppm. Se in salt were tested at 1-3 days after birth and shown to be Se adequate while calves from a control group were inadequate. Since FDA prohibits the use of oral Se concentration over 20 ppm. in commercial products, a veterinary prescription is necessary to allow the feed manufacturer to supply higher concentrations. It is noted that alfalfa and clover legumes are poor absorbers of selenium.

Harris,6 1980, suggested that the selenium level of the hair of cattle is a useful indicator of the status of the animal. Cattle with hair values below 0.25 ppm. need supplementation while those with values more than 5 ppm. suggest selenium toxicity. Work at Ohio State University showed that retention of the placenta in dairy cattle could be prevented by giving an intramuscular injection of 50 mg. selenium as selenite plus 680 IU of vitamin E 21 days pre-partum, or by feeding 1.0 mg selenium per day as selenite the last 60 days of the dry period.

Novi,11 1981, reported that reduced glutathione (GSH) a harmless, natural antioxidant, produced a regression of aflatoxin B1 induced liver tumors in rats when given at the late stages of tumor progression. The aflatoxin dosing regimen induced hepatocellular carcinomas in 100% of rats after 1 year. After 16 months, the rats were divided into 2 groups, one group receiving 100 mg of GSH per day in 2.5 ml. of distilled water. All rats treated only with aflatoxin B1, died of liver tumors in 20 months after discontinuing the carcinogen, whereas 81% of the rats treated with AFB, and followed by GSH were alive and healthy after 20 months.

Finally, selenium is an essential nutrient, is a constituent of glutathione peroxidase and evidence suggests there is a homeostatic mechanism regulating blood selenium levels where dietary intake is adequate, but not in excess, to provide this critically important element. The FDA NRC guidelines suggested levels of 0.1 ug/g for humans, broilers, layers and cattle, 0.2 ug/g for turkey pouls and 0.3 ug/g for young swine for best growth and performance including immunocompetence.
REFERENCES

15. Wozniak, B.F., Immunocompetence of Swine Exposed to Aflatoxin B1, M.S. Degree, University of Fla., 1980.

TRACE ELEMENTS IN HUMAN HEALTH AND DISEASE

Selenium Values

In Foods:
- Fish products 1 ug/g
- Grains — seleniferous area 1 ug/g
- Animal meats 0.2 ug/g

In Serum (ug/ml):

<table>
<thead>
<tr>
<th></th>
<th>Deficient</th>
<th>Adequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equine</td>
<td>0.05</td>
<td>0.22</td>
</tr>
<tr>
<td>Bovine</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Porcine</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
<td>Ovine</td>
<td>0.02</td>
<td>0.29</td>
</tr>
</tbody>
</table>
**TABLE 2**

**AVERAGE SERUM SELENIUM CONCENTRATIONS OF FOALS NURSING MARES GROUPED BY SELENIUM STATUS**

Average of 31 Foals Nursing Deficient Mares: \(0.112\ ppm \pm 0.088\ ppm\).

Average of 61 Foals Nursing Marginal Mares: \(0.124\ ppm \pm 0.075\ ppm\).

Average of 20 Foals Nursing Adequate Mares: \(0.127\ ppm \pm 0.076\ ppm\).

**TABLE 3**

<table>
<thead>
<tr>
<th>Selenium concentration in U.S. Swine Feeds.(^1)</th>
<th>Ingredient</th>
<th>Origin</th>
<th>No. of samples</th>
<th>Range (ppm)</th>
<th>Mean (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>Ill.</td>
<td>31</td>
<td>0.02-0.15</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Ind.</td>
<td>17</td>
<td>0.01-0.15</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Iowa</td>
<td>25</td>
<td>0.02-0.16</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Kan.</td>
<td>1</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Mich.</td>
<td>17</td>
<td>0.01-0.09</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Minn.</td>
<td>23</td>
<td>0.02-0.19</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Mo.</td>
<td>4</td>
<td>0.02-0.09</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Nebr.</td>
<td>6</td>
<td>0.04-0.81</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>N.Y.</td>
<td>1</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>N. Dak.</td>
<td>5</td>
<td>0.09-0.26</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Ohio &amp; Ind.</td>
<td>5</td>
<td>0.09-0.15</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>S. Dak.</td>
<td>9</td>
<td>0.11-2.03</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Wisc.</td>
<td>5</td>
<td>0.02-0.13</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Meat meal</td>
<td>Iowa</td>
<td>1</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat meal</td>
<td>Ohio</td>
<td>2</td>
<td>0.13-0.24</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>SBM (44)</td>
<td>Ill.</td>
<td>2</td>
<td>0.20-0.21</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>SBM (44)</td>
<td>Iowa</td>
<td>1</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBM (44)</td>
<td>Ohio</td>
<td>4</td>
<td>0.05-0.13</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>SBM (49)</td>
<td>Ind.</td>
<td>1</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>Ohio</td>
<td>1</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

1. From information presented by Ullrey (1974).
### TABLE 4

Selenium-vitamin E interrelationships.\(^1\)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Animal</th>
<th>Vitamin E</th>
<th>Selenium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive failure:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryonic degeneration</td>
<td>Female, Hen,</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ewe</td>
<td>No(^2)</td>
<td>Yes(^3)</td>
</tr>
<tr>
<td>Sterility</td>
<td>Male: Dog</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Cock, Pig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver, blood, brain &amp; capillaries:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver necrosis</td>
<td>Pig</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Erythrocyte destruction</td>
<td>Chick</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Blood protein loss</td>
<td>Chick, Turkey</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Encephalomalacia</td>
<td>Chick</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Exudative diathesis</td>
<td>Chick, Turkey</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Steatitis</td>
<td>Mink, Chick,Pig</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nutritional myopathies:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional muscular dystrophy</td>
<td>Duck, Chick,</td>
<td>Yes</td>
<td>Partially</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiff lamb</td>
<td>Lamb, Kid</td>
<td>No(^2)</td>
<td>Yes(^3)</td>
</tr>
<tr>
<td>White muscle disease</td>
<td>Calf, Sheep</td>
<td>No(^2)</td>
<td>Yes(^3)</td>
</tr>
<tr>
<td>Myopathy of gizzard &amp; Heart</td>
<td>Turkey poult</td>
<td>No(^2)</td>
<td>Yes(^3)</td>
</tr>
</tbody>
</table>

2. Not in selenium — deficient diets.
3. When added to diets containing vitamin E.
REPORT OF THE COMMITTEE ON PROFESSIONAL OVERSIGHT

Chairman: P.L. Smith, California
Vice Chairman: H.E. Goldstein, Ohio
C.L. Campbell, FL; J.D. Jefferies, FL; J.O. Pearce, FL; J.R. Ragan, TN;
S. Wilson, Wash. DC; T.F. Zweigart, NC

The USAHA Committee on Professional Oversight met at 1:30 p.m. on Thursday, October 25, 1984.

The committee at its past five meetings had expressed concern regarding the implied warranty involved in signing health certificates.

Recent litigation concerning interstate movement of livestock and issuance of health certificates seems to indicate that the accredited veterinary profession may be legally vulnerable for good faith issuances of health certificates.

A USAHA Ad Hoc Committee on Interstate Health Certificates was formed by the USAHA president to review the problem and make recommendations. A committee of the Western States Livestock Health Association over the past year has been studying the improvement of present health certification procedures. These two committees coordinated their findings and presented uniform recommendations. These recommendations were reviewed and approved by the AVMA Council on Public Health and Regulatory Medicine and by the AVMA Insurance Trust.

At the most recent meetings of the Western States Livestock Health Association and the Western District of the USAHA, these recommendations were approved and noted to be forwarded to the USAHA Professional Oversight Committee for consideration.

Prior to the meeting of the Professional Oversight Committee, the health certificate recommendations were introduced at the Committee on Brucellosis. The recommendations were accepted by that committee and became part of its report.

For the sake of clarity and to place emphasis on these important recommendations, the Committee on Professional Oversight together with the Brucellosis Committee makes the recommendations listed in this report. Further, it should be emphasized that these recommendations involve the health certification for all species of animals.

It is recommended that the current individual states' official health certificates be revised to incorporate the following three points:

1) Name of Certificate:
   “Certificate of Veterinary Inspection”

2) Veterinary Certification:
   “I certify, as an accredited veterinarian, that the above described animals have been inspected by me and that they are not showing signs of infectious, contagious, and/or communicable disease, (except where noted). The vaccinations and results of tests are as indicated on the certificate.”
To the best of my knowledge, the animals listed on this certificate meet the state of destination and federal interstate requirements. No further warranty is made or implied.

s/__________________________
Accredited Veterinarian

3) Owner/Agent Statement: (when applicable)

"The animals in this shipment are those certified to and listed on this certificate."

s/__________________________
Owner/Agent

It is further recommended that the USAHA president mail these proposed health certification changes to all state veterinarians and request their high priority consideration for adoption. It is recommended that the USAHA president request a response from each state veterinarian indicating if he/she will adopt the changes and what will be the planned timetable for adoption.
REPORT OF THE COMMITTEE ON RABIES

Chairman: Leon Russell, College Station, Texas
Vice Chairman: W. R. Miller, Beltsville, Maryland

W. H. Bechenhauer, NE; John Brown, GA; R. R. Brown, AL; D. W. Dreesen, GA; T. J. Galvin, WV; B. Hancock, IA; D. R. Howard, KS; Bruce Kaplan, KY; F. V. McCasland, TX; Robert B. Miller, MD; J. C. New, TN; J. C. Prucha, MD; E. L. Shroyer, OH; J. M. Shuler, IN; Al Strating, NV; W. G. Winkler, GA; Taylor Woods, AR.

The committee met on October 22, 1984, with 18 members and guests present.

Old Business:

The USDA, FSIS requirements for management and slaughter of rabies exposed livestock was discussed. A motion was made and passed that data be collected and evaluated for preparation of a resolution on the slaughter management of rabies exposed livestock. This resolution will be presented to the Resolution Committee at next year's meeting.

New Business:

1. Dr. D. R. Howard, Manhattan, Kansas, presented the current status of the Kansas State University 3 year study of Intradermal Human Diploid (Merieux) rabies vaccination.

2. Dr. D. R. Howard, reported the availability of RFFIT titers through the Diagnostic Laboratory at Kansas State University.

3. Dr. Robert B. Miller, USDA, APHIS, VS Biologics, Maryland, discussed the study of proposed changes in Animal Rabies Vaccines Licensing requirements.

4. The Rabies Committee commended the Centers for Disease Control, Atlanta, Georgia, for their recent intensive effort in developing uniform proficiency in rabies diagnostic procedures at animal disease diagnostic laboratories and public health laboratories throughout the United States.

5. A positive discussion was stimulated concerning numerous, potential papers for next year's Rabies Committee program.

The committee was adjourned.
REPORT OF THE COMMITTEE ON
STATE FEDERAL RELATIONS

Chairman: D. U. Walker, Montpelier, VT

R. G. Burnett, OR; J. A. Cobb, GA; H. E. Goldstein, OH; R. L. Hartin, OK; J. F. Hudelson, CO; N. W. Kruse, NE; J. O. Pearce, Jr., FL; J. R. Ragan, TN; J. C. Shook, MD; M. A. Van Buskirk, PA.

The State-Federal Relations Committee of the United States Animal Health Association held its annual meeting at the Quality Inn, College Park, Maryland, January 30 through February 3, 1984.

The Committee met with representatives from the Agriculture Research Service, Bureau of Veterinary Medicine, Food and Drug Administration, Food Safety Inspection Service, Animal and Plant Health Inspection Service, both Veterinary Services and Plant Pest Quarantine.

The Committee commends the representatives of these agencies for the effort and preparation in sharing program policy and budgetary agendas. Communications were presented most openly and committee member input was courteously received.

We seriously request that constructive comments and suggestions made by this committee during oral discussion of presentations be considered by those making decisions.

This committee was most pleased with responses to the various resolutions presented by USAHA at the 1983 Annual Meeting.

The USAHA pledges total recognition to the USDA celebration of 100 years of Animal Health.

This report does not attempt to address every item considered. Following are those items which constitute the major concerns of this committee.

1. BRUCELLOSIS BUDGET—The fiscal 1985 brucellosis budget put forth by USDA is a national disgrace. It is clear that the department charged with providing leadership and support for this important program is, in fact, for the third consecutive year making every effort to dismantle and destroy the program.

   Critical elements such as indemnity for infected herd depopulation are already limiting the progress of the national effort as a result of inadequate funding.

   This committee continues to strongly support a comprehensive, adequately funded national program for the eradication of brucellosis. The USDA budget clearly does not provide for that.

   To gamble the existence of a vital disease program on the unlikely success of an ill-defined checkoff scheme is, at best, grossly irresponsible.

2. AVIAN INFLUENZA—The committee commends USDA officials for declaration of an Extraordinary Emergency involving the recent outbreak of Highly Pathogenic Avian Influenza which had its apparent origination in Lancaster County, Pennsylvania. All
efforts should be made to contain and eradicate the H5 Avian Influenza virus in the existing quarantine zone and in areas where infection may have resulted from initial foci of infection. The poultry industry of Pennsylvania, Maryland, New Jersey and Virginia and the respective state regulatory officials of each state are also to be complimented and encouraged to continue cooperation with the Task Force to attain ultimate eradication of this disease in as short a time as possible. Every effort should be taken to assure the poultry industry of this nation that the disease will not be experienced outside existing quarantine zones.

3. REGIONAL OFFICES—VETERINARY SERVICES-APHIS—
The present concept of Regional Offices in Veterinary Services, APHIS, appears to provide an unnecessary and expensive administrative level which is not serving the original purpose intended when the Regional Directors were moved out of Hyattsville to the various regions.

This committee is not supportive of the present concept and suggests that Regional Offices be moved back to Hyattsville as they were in the past. As an alternative, we would suggest that an immediate study be made to determine the advantages and disadvantages of the present concept versus the old concept. This study would be made by a committee which would include state, federal and industry representatives with necessary costs and expenses paid by APHIS. The committee would make recommendations to the Administrator suggesting a plan which would produce the needed and desired use of the Regional Offices which would be most cost effective.

4. BIOLOGICS LEGISLATION—We reiterate concerns expressed in the USAHA Biologics Committee discussions and this committee’s briefings regarding the broad, all-inclusive nature of the proposed legislation. We urge that specific exemption be included in the legislation for diagnostic reagents prepared and used in public agency laboratories.

5. APPROVAL OF DRUGS AND BIOLOGICS—The committee supports continuing efforts to expedite in every possible manner the evaluation and approval of new animal drugs and biologics. Further, we support the concept that all animal drugs and biologics which can be demonstrated safe for over-the-counter sale should be so approved.

6. DRUGS—SIMULTANEOUS APPROVAL—We encourage FDA to continue examining the feasibility of simultaneous approval of animal drugs in all developed countries.

7. IMPORT OF FOREIGN ANIMALS—There is a dire need to enhance regulatory communications between federal import officials and state regulatory officials when imported animals have final destination in the respective states. It is strongly recom-
mended that each state official have advance knowledge when animals are leaving export stations and moving into that state.

8. **PROGENY OF IMPORTED ANIMALS**—This committee is concerned about the freedom of progeny of imported ruminant animals going into wildlife parks and hunting preserves adjacent to domestic livestock and the potential threat of such diseases as Malignant Catarrhal Fever and others.

9. **FOREIGN EXPORTS**—The Committee was most pleased with the efforts of USDA to stimulate export of livestock semen, embryos and animal food products to other nations. This would enhance agriculture productivity and provide additional economic assistance to the livestock industry.

10. **TB HERD DEPOPULATION**—We strongly recommend that the policy of depopulating all *M. bovis* confirmed cattle herds be maintained, and are concerned that program officials appear to have retreated somewhat from that time-tested policy.

11. **TB—MEXICAN CATTLE**—We urge that APHIS, in conjunction with border states continue vigorously to seek necessary procedures and authorities to prevent Mexican feeder, stocker, or rodeo cattle from becoming the vehicle for introducing tuberculosis into the United States herds.

12. **TALMADGE-AIKEN MEAT PLANTS**—This committee requests that FSIS reimburse state meat inspection programs for 100% of the cost of the T. A. program.

13. **COMMUNICATION—FSIS-APHIS**—Communications between federal agencies should be improved. In particular FSIS and APHIS-VS should consider each others objectives and problems when reviewing their respective programs. APHIS needs the support and cooperation of FSIS for livestock disease surveillance programs which depend upon the MPI, MCIP and MSIP programs.

14. **APHIS-ARS COMMUNICATIONS**—The committee is most complimentary in addressing the apparent increased dialogue between APHIS and ARS involving research needs and clearly defining priority needs.

15. **INTERSTATE REGULATIONS BOOK**—The present system of printing a book containing the various states import requirements is very costly and is always out-of-date. We suggest that other methods be considered to make this information available. Consideration might be given to putting this information into the BIS where it could be readily obtained and changed.

16. **N.V.S.L. TRAINING**—The committee is most appreciative of the availability and training opportunities offered to the states by N.V.S.L. and strongly encourages continuation of these services.

17. **FEDERAL PERSONNEL—CROSS UTILIZATION**—Based on recommendations of the WBBS Task Force Study of 1983, it is the
general feeling of this committee that cross-utilization of federal personnel in certain budget line items in several regions of the country appears to be reasonable and a benefit to the states.

18. **VESICULAR STOMATITIS AND AVIAN INFLUENZA**—This committee recommends ARS continue research into Vesicular Stomatitis and research directed toward elucidating the pathogenesis of Avian Influenza.

19. **INTERNATIONAL OPERATIONS**—We support a high priority for the international disease control efforts of ARS and APHIS including the proposed expansion of APHIS monitoring of animal disease situations in overseas locations.

20. **SWINE IDENTIFICATION**—The swine identification program has provided some real improvement and it is suggested that further effort be made to provide a satisfactory means of accomplishing this goal.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

Chairman: J. P. Kluge, Ames, IA  
Vice Chairman: D. G. Thawley, Columbia, MO

L. G. Biehl, IL; Neal Black, MN; C. E. Boyd, SC; P. E. Bradshaw, IL; John Brown, GA; Jesus Castaneda G., Venezuela; R. A. Crandell, TX; A. M. Creswell, TN; P. B. Doby, IL; Gene Erickson, IA; D. P. Gustafson, IN; E. O. Haelterman, IN; R. E. Hall, WI; D. L. Harris, MO; G. W. Hausman, IA; H. T. Hill, IA; C. L. Kanitz, IN; M. H. Lang, IA; Norman Lichtman, NJ; Vincent Marshall, NE; J. W. McVicar, NY; K. E. Myers, IA; C. W. Monsees, MO; P. A. O’Berry, IA; Carson Rogers, NE; Linda Schlater, IA; G. M. Schloer, NY; L. W. Schnurrenberger, MD; W. C. Stewart, IA; D. L. Thompson, CA; R. E. Thompson, AR; Wesley Towers, Jr., DE; C. D. Van Houweling, VA; J. D. Villari, NJ; M. W. Vorhies, SD; B. D. Ward, NY; Fred Wertman, IA

The Transmissible Diseases of Swine Committee of the United States Animal Health Association convened at 1:30 p.m. on Wednesday, October 24, 1984. Eighteen (18) members were present together with seventy-two (72) guests.

The theme of this year’s committee meeting was centered around an identification of the most important current and emerging disease problems within the swine industry. To this end a panel consisting of the following was formed and asked to address the issue:

<table>
<thead>
<tr>
<th>Participant</th>
<th>Viewpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. J. Meisinger</td>
<td>NPPC</td>
</tr>
<tr>
<td>D. Gingerich</td>
<td>Producer</td>
</tr>
<tr>
<td>George Lambert</td>
<td>ARS</td>
</tr>
<tr>
<td>L. W. Schnurrenberger</td>
<td>APHIS</td>
</tr>
<tr>
<td>E. A. Carbrey</td>
<td>Federal Diagnostic Laboratory</td>
</tr>
<tr>
<td>D. C. Hoefling</td>
<td>State Diagnostic Laboratory</td>
</tr>
<tr>
<td>R. D. Glock</td>
<td>Private Diagnostic Laboratory</td>
</tr>
<tr>
<td>R. A. Schultz</td>
<td>Swine Practitioner</td>
</tr>
<tr>
<td>R. E. Hall</td>
<td>Swine Practitioner</td>
</tr>
</tbody>
</table>

Specific results of surveys and priorities of the NPPC (Appendix A), AASP (Appendix B), Dr. G. A. Erickson of NVSL (Appendix C), and Dr. D. C. Hoefling of Galesburg Animal Disease Laboratory (Appendix D) will be presented in total in the proceedings. There was general agreement that preweaning enteric diseases and pneumonias currently represent the greatest threat to swine health. The top ranked conditions of economic importance as listed by the majority of participants were: pneumonias, preweaning scours, atrophic rhinitis, salmonellosis, Strep. suis infections, reproductive/infertility problems and problems caused by housing design and environment. The committee also discussed the importance of the multifactoral etiology of several of the emerging conditions.
There was considerable discussion that if implementation of current knowledge could be fostered through more effective communication of preventive procedures many of the disease problems which have been identified could be significantly reduced.

The committee also discussed the increasing need for better information on the cost effectiveness of disease control procedures and the need to develop new and more effective means of producing long-lasting immunological protection for swine during periods of susceptibility.

Several members of the committee raised the question of how the use of new rapid producer-based diagnostic tests will be supervised. A discussion ensued as to the need and place for these tests in official disease control programs. The consensus was that there is a definite need for these tests and industry should be encouraged to develop such tests.

The meeting was adjourned at 4:15 p.m.
The National Pork Producers Council has developed research and education priorities for use throughout the pork industry. The priorities encompass pork production, health, and marketing. No special treatment was assigned financial or economic considerations, but pork producers unanimously agree that profit is their motivation for raising hogs. Therefore, there is concern for economic payback from any new technology utilized in the industry.

For pork producers to know the problem areas needing attention in their industry, they must know the production, marketing, and financial characteristics of their enterprises. This requires compilation and analysis of comprehensive enterprise records. The NPPC strongly encourages education programs to concentrate on the importance of record keeping as a means toward problem solving and enterprise improvement.

Pork producers generally respond to production problems with a systems approach. Where possible, NPPC recommends that research and education programs be developed utilizing an interdisciplinary, multifunctional approach to problem solving, as well. Although they are not prevalent on the priorities list, interactions between various components such as genetics and health, nutrition and environment, etc., are very important to NPPC and to the pork industry.

The list of priorities is flexible and dynamic. It represents an initial effort to direct limited funds for the maximum impact. NPPC has utilized a great many sources from throughout the industry to develop these research and education priorities, and will continue to modify them as problems are solved and new problems arise.
REPORT OF THE COMMITTEE

<table>
<thead>
<tr>
<th>REPRODUCTION IMPROVEMENT</th>
<th>RESEARCH</th>
<th>EDUCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Female Reproduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Failure to conceive/maintain pregnancy</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>b. Rebreeding the first-litter sow</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>c. Embryo survival</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>d. Anestrus</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>e. Heat detection (AI)</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>f. Production scheduling</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>(2) Boar Fertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Evaluation of boar potential</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>b. Semen quality</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>c. Environmental stress</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>(3) Evaluation of Cost Efficiency of Reproduction</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(4) Breeding Values for Reproduction</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>(5) Manipulation of Gametes</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>(6) Crossbreeding Systems for Optimum Reproduction</td>
<td>x</td>
<td>x</td>
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<table>
<thead>
<tr>
<th>FEEDER PIG MANAGEMENT</th>
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<tbody>
<tr>
<td>(1) Factors Affecting Feeder Pig Prices</td>
</tr>
<tr>
<td>(2) Health Certification Programs</td>
</tr>
<tr>
<td>(3) Transportation and Stress</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HEALTH IMPROVEMENT (E - education need; R - research need)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIAGNOSIS</td>
</tr>
<tr>
<td>(1) Respiratory Diseases</td>
</tr>
<tr>
<td>a. Hemophilus pleuropneumonia</td>
</tr>
<tr>
<td>b. Mycoplasma pneumonia</td>
</tr>
<tr>
<td>c. Atrophic rhinitis</td>
</tr>
<tr>
<td>d. Pasteurella multocida</td>
</tr>
<tr>
<td>e. Influenza</td>
</tr>
<tr>
<td>f. Pseudorabies</td>
</tr>
<tr>
<td>g. Hemophilus parasuis</td>
</tr>
<tr>
<td>(2) Enteric Diseases</td>
</tr>
<tr>
<td>a. E. coli scours</td>
</tr>
<tr>
<td>b. Coccidiosis</td>
</tr>
<tr>
<td>c. Clostridium</td>
</tr>
<tr>
<td>d. TGE</td>
</tr>
<tr>
<td>e. Other viral</td>
</tr>
<tr>
<td>f. Thread worm</td>
</tr>
<tr>
<td>g. Salmonella</td>
</tr>
<tr>
<td>h. Dysentery</td>
</tr>
<tr>
<td>i. Internal parasites</td>
</tr>
<tr>
<td>j. Proliferative enteritis</td>
</tr>
<tr>
<td>(3) Reproductive Diseases</td>
</tr>
<tr>
<td>a. MMA</td>
</tr>
<tr>
<td>b. Porcine parvovirus</td>
</tr>
<tr>
<td>c. Leptospirosis</td>
</tr>
<tr>
<td>d. Eperythrozoonosis</td>
</tr>
<tr>
<td>e. Streptococcus</td>
</tr>
<tr>
<td>f. Pseudorabies</td>
</tr>
<tr>
<td>(4) Other</td>
</tr>
<tr>
<td>b. Lameness</td>
</tr>
<tr>
<td>c. Economics (cost effectiveness)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ENVIRONMENTAL MANAGEMENT AND STRESS (including economics)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESEARCH</td>
</tr>
<tr>
<td>(1) Energy Costs</td>
</tr>
<tr>
<td>a. Ventilation</td>
</tr>
<tr>
<td>(a) Rates for optimum production costs</td>
</tr>
<tr>
<td>(b) Control mechanisms/methods for ventilation systems</td>
</tr>
<tr>
<td>b. Heating and Cooling</td>
</tr>
<tr>
<td>d. Energy sources - economics</td>
</tr>
<tr>
<td>(b) Controls</td>
</tr>
<tr>
<td>c. Insulation</td>
</tr>
</tbody>
</table>
TRANSMISSIBLE DISEASES OF SWINE

(2) Health (confinement workers and pigs)  x  x
(3) Facilities and Equipment
   (a) Design features to optimize animal comfort and production  x
(4) Waste Management  x  x
(5) Stress Quantification  

NUTRITION AND FEED UTILIZATION

<table>
<thead>
<tr>
<th>RESEARCH</th>
<th>EDUCATION</th>
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</thead>
<tbody>
<tr>
<td>(1) Total Herd Feed Efficiency</td>
<td></td>
</tr>
<tr>
<td>a. Cost of maintenance of large vs. small body type swine  x</td>
<td></td>
</tr>
<tr>
<td>b. Prevention of feed wastage  x</td>
<td></td>
</tr>
<tr>
<td>c. Nutrition/environment interaction  x</td>
<td></td>
</tr>
<tr>
<td>(2) Nutrition and Nutrient Requirements</td>
<td></td>
</tr>
<tr>
<td>a. High producing sows and developing gilts  x  x</td>
<td></td>
</tr>
<tr>
<td>b. Nursing pigs  x</td>
<td></td>
</tr>
<tr>
<td>c. Early weaned pigs  x  x</td>
<td></td>
</tr>
<tr>
<td>d. Fast growing hogs  x  x</td>
<td></td>
</tr>
<tr>
<td>e. Mineral requirements, interactions and toxicities (mycotoxins)  x</td>
<td></td>
</tr>
<tr>
<td>(3) Feed Alternatives</td>
<td></td>
</tr>
<tr>
<td>a. New crops and varieties  x  x</td>
<td></td>
</tr>
<tr>
<td>b. Byproducts  x  x</td>
<td></td>
</tr>
<tr>
<td>c. Waste feeding  x</td>
<td></td>
</tr>
<tr>
<td>(4) Feed Additives and Growth Promotants</td>
<td></td>
</tr>
<tr>
<td>a. Framework for evaluation  x</td>
<td></td>
</tr>
<tr>
<td>(5) Feed Processing</td>
<td></td>
</tr>
<tr>
<td>a. Farm systems  x  x</td>
<td></td>
</tr>
<tr>
<td>b. Quality control  x</td>
<td></td>
</tr>
<tr>
<td>c. Purchasing strategies  x  x</td>
<td></td>
</tr>
<tr>
<td>d. Equipment design  x</td>
<td></td>
</tr>
</tbody>
</table>

MARKETING (live hog, carcass and product)

<table>
<thead>
<tr>
<th>RESEARCH</th>
<th>EDUCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Product</td>
<td></td>
</tr>
<tr>
<td>a. Carcass marketing systems  x</td>
<td></td>
</tr>
<tr>
<td>(a) Compare and develop  x</td>
<td></td>
</tr>
<tr>
<td>b. New product development  x</td>
<td></td>
</tr>
<tr>
<td>c. Processing techniques  x</td>
<td></td>
</tr>
<tr>
<td>(a) Hot product processing  x</td>
<td></td>
</tr>
<tr>
<td>(b) Irradiation  x</td>
<td></td>
</tr>
<tr>
<td>(c) Electrical stimulation  x</td>
<td></td>
</tr>
<tr>
<td>(d) Restructuring  x</td>
<td></td>
</tr>
<tr>
<td>c. Consumer acceptance factors and trends  x  x</td>
<td></td>
</tr>
<tr>
<td>d. Carcass composition  x</td>
<td></td>
</tr>
<tr>
<td>e. Instrumentation of carcass evaluation  x</td>
<td></td>
</tr>
<tr>
<td>(2) Swine Identification</td>
<td></td>
</tr>
<tr>
<td>a. Innovative methods  x</td>
<td></td>
</tr>
<tr>
<td>b. System to utilize disease data  x</td>
<td></td>
</tr>
<tr>
<td>c. Economic evaluation  x</td>
<td></td>
</tr>
<tr>
<td>d. Benefits from:</td>
<td></td>
</tr>
<tr>
<td>(a) Slaughter check  x</td>
<td></td>
</tr>
<tr>
<td>(b) Genetic tracing  x</td>
<td></td>
</tr>
<tr>
<td>(c) Residue detection  x</td>
<td></td>
</tr>
<tr>
<td>(d) Reduction of losses  x</td>
<td></td>
</tr>
<tr>
<td>(3) Market Alternatives, Strategies and Risk Management  x  x</td>
<td></td>
</tr>
<tr>
<td>a. Market information  x  x</td>
<td></td>
</tr>
<tr>
<td>b. Forward pricing  x</td>
<td></td>
</tr>
<tr>
<td>c. Market opportunities (domestic/foreign)  x  x</td>
<td></td>
</tr>
<tr>
<td>d. Market weights  x</td>
<td></td>
</tr>
<tr>
<td>e. Electronic marketing  x  x</td>
<td></td>
</tr>
<tr>
<td>(4) Structure of the Industry</td>
<td></td>
</tr>
<tr>
<td>a. Plant closings  x</td>
<td></td>
</tr>
<tr>
<td>b. Tax laws  x</td>
<td></td>
</tr>
<tr>
<td>c. Concentration and location  x</td>
<td></td>
</tr>
<tr>
<td>d. Integration  x</td>
<td></td>
</tr>
</tbody>
</table>
A 1984 Swine Health Survey

Conducted by the

American Association of Swine Practitioners (AASP)

Presented by Dr. R. E. Hall

October 23, 1984

USAHA Meeting, Ft. Worth, Texas
1984 (AASP) Swine Disease Survey

**CREDITS**

**George Danchuk** - UW-Extension (WRAP)

**Dr. R. E. Hall** - USAAA Representative (AASP)

**Rob Hall** - UW-Extension (WRAP)

**Dr. Roy Schultz** - President (AASP)

**Dr. Fred Wertman** - Executive Secretary (AASP)
1984 - American Association of Swine Practitioners (AASP)

Swine Disease Survey

<table>
<thead>
<tr>
<th>Respondents</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Practitioners</td>
<td>158</td>
</tr>
<tr>
<td>Teaching/Research</td>
<td>14</td>
</tr>
<tr>
<td>Industry</td>
<td>13</td>
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<tr>
<td>Governments</td>
<td>4</td>
</tr>
<tr>
<td>Extension</td>
<td>4</td>
</tr>
<tr>
<td>Diagnosticians</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>195</td>
</tr>
</tbody>
</table>
1984 (AASP) SWINE DISEASE SURVEY
1984 (AASP) Swine Disease Survey

Respondents Geographic Distribution

<table>
<thead>
<tr>
<th>Region</th>
<th>Count</th>
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</thead>
<tbody>
<tr>
<td>Northcentral</td>
<td>154</td>
</tr>
<tr>
<td>Southeast</td>
<td>35</td>
</tr>
<tr>
<td>East</td>
<td>4</td>
</tr>
<tr>
<td>West</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
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</table>
1984 (AASP) Swine Disease Survey

<table>
<thead>
<tr>
<th>Rank Determination</th>
<th>Points Assigned</th>
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</thead>
<tbody>
<tr>
<td>If respondent made it 1st choice</td>
<td>1</td>
</tr>
<tr>
<td>&quot; &quot; 2nd &quot;</td>
<td>2</td>
</tr>
<tr>
<td>&quot; &quot; 3rd &quot;</td>
<td>3</td>
</tr>
<tr>
<td>&quot; &quot; 4th &quot;</td>
<td>4</td>
</tr>
<tr>
<td>&quot; &quot; 5th &quot;</td>
<td>5</td>
</tr>
<tr>
<td>ETC, ETC</td>
<td>ETC</td>
</tr>
</tbody>
</table>

Rank equals total number of points assigned to each choice of answers to a question ÷ number of respondents answering = Mean.
1984 (AASP) Swine Disease Survey

Q1. Rank according to causes of death loss.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Preweaning Scours (not T.G.E.)</td>
<td>2.189</td>
</tr>
<tr>
<td>2. Baby Pig Mortality (nonspecific)</td>
<td>2.473</td>
</tr>
<tr>
<td>3. Pneumonias</td>
<td>2.606</td>
</tr>
<tr>
<td>4. Agalactia Syndrome</td>
<td>3.196</td>
</tr>
<tr>
<td>5. Transmissible Gastroenteritis</td>
<td>3.233</td>
</tr>
<tr>
<td>(T.G.E.)</td>
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</tr>
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</table>
### 1984 (AASP) Swine Disease Survey

#### Regional Responses to Q1. Death Loss Causes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean</th>
<th>Disease</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td><strong>Northcentral U.S. (n=133)</strong></td>
<td></td>
<td><strong>Southeastern U.S. (n=35)</strong></td>
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<tr>
<td>1. Preweaning Scours</td>
<td>2.25</td>
<td>Preweaning Scours</td>
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<tr>
<td>2. Baby Pig Mortality (PS)</td>
<td>2.26</td>
<td>Pneumonias</td>
<td>2.26</td>
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<td>3. Pneumonias</td>
<td>2.64</td>
<td>T.G.E.</td>
<td>2.9</td>
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<td>4. Agalactia Syndrome</td>
<td>3.18</td>
<td>Baby Pig Mortality</td>
<td>3.16</td>
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<td>5. T.G.E.</td>
<td>3.28</td>
<td>Agalactia Syndrome</td>
<td>3.5</td>
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</table>

1979 (n=331)                                      1984 (n=195)

1. Preweaning Scours                            Preweaning scours (not T.G.E.)
2. Pneumonias                                    Baby Pig Mortality (nonspecific)
3. Agalactia Syndrome                            Pneumonias
4. Postweaning Scours                            Agalactia Syndrome
5. T.G.E.                                        T.G.E.
1984 (AASP) Swine Disease Survey

Q2. Rank according to total economic losses.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean</th>
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<tbody>
<tr>
<td>1. Pneumonias</td>
<td>3.06</td>
</tr>
<tr>
<td>2. Reproduction/Infertility</td>
<td>3.14</td>
</tr>
<tr>
<td>3. Atrophic Rhinitis</td>
<td>3.38</td>
</tr>
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<td>4. Preweaning Scours (not T.G.E.)</td>
<td>3.47</td>
</tr>
<tr>
<td>5. Housing/Environment</td>
<td>3.62</td>
</tr>
</tbody>
</table>
1984 (AASP) Swine Disease Survey

Regional Responses to Q2. Total Economic Losses

<table>
<thead>
<tr>
<th>Northcentral U.S.</th>
<th>Southeastern U.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>1. Atrophic Rhinitis</td>
<td>2.21</td>
</tr>
<tr>
<td>2. Pneumonias</td>
<td>2.78</td>
</tr>
<tr>
<td>3. Preweaning Scours</td>
<td>2.83</td>
</tr>
<tr>
<td>4. Reproduction/Infertility</td>
<td>2.84</td>
</tr>
<tr>
<td>5. Housing/Environment</td>
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</table>
(AASP) Swine Disease Survey

Q2. **Total Economic Losses** - A six (6) year comparison.

1979 \( (n=331) \) \hspace{1cm} 1984 \( (n=195) \)

1. Pneumonias
2. Preweaning Scours
3. Atrophic Rhinitis
4. Reproduction/Infertility
5. Agalactia Syndrome

Pneumonias
Reproduction/Infertility
Atrophic Rhinitis
Preweaning Scours
Housing/Environment
1984 (AASP) Swine Disease Survey

Q3. Rank according to research needed.

<table>
<thead>
<tr>
<th>Disease</th>
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<tbody>
<tr>
<td>1. Pneumonias</td>
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</tr>
<tr>
<td>2. Nutrition/Disease</td>
<td>2.79</td>
</tr>
<tr>
<td>3. Atrophic Rhinitis</td>
<td>2.87</td>
</tr>
<tr>
<td>4. Housing/Environment</td>
<td>2.88</td>
</tr>
<tr>
<td>5. Agalactia Syndrome</td>
<td>2.98</td>
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</tbody>
</table>
1984 (AASP) Swine Disease Survey
Regional Responses to Q3. Research Needed

<table>
<thead>
<tr>
<th>Northcentral U.S.</th>
<th>Disease</th>
<th>Mean</th>
<th>Southeastern U.S.</th>
<th>Disease</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
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<td>1.</td>
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<tr>
<td>2.</td>
<td>Nutrition/Disease</td>
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<td>2.</td>
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<tr>
<td>3.</td>
<td>Atrophic Rhinitis</td>
<td>2.82</td>
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<td>4.</td>
<td>Housing/Environment</td>
<td>3.00</td>
<td>4.</td>
<td>Postweaning Scours</td>
<td>2.81</td>
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</table>
### (AASP) Swine Disease Survey

#### Q3. Research Needed - A Six (6) Year Comparison.

<table>
<thead>
<tr>
<th>1979</th>
<th>1984</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reproduction/Infertility</td>
<td>Pneumonias</td>
</tr>
<tr>
<td>2. Agalactia Syndrome</td>
<td>Nutrition/Disease</td>
</tr>
<tr>
<td>3. Preweaning Scours</td>
<td>Atrophic Rhinitis</td>
</tr>
<tr>
<td>4. Nutrition/Disease</td>
<td>Housing/Environment</td>
</tr>
<tr>
<td>5. Pneumonias</td>
<td>Agalactia Syndrome</td>
</tr>
</tbody>
</table>
1984 (AASP) Swine Disease Survey

Q4. Rank according to information needed.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Atrophic Rhinitis</td>
<td>3.27</td>
</tr>
<tr>
<td>2. Nutrition</td>
<td>3.30</td>
</tr>
<tr>
<td>3. Pneumonias</td>
<td>3.39</td>
</tr>
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<td>4. Housing/Environment</td>
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<td>5. Economics of Herd Health Program</td>
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1984 (AASP) Swine Disease Survey
Regional Responses to Q4. Information Needed.

<table>
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<tbody>
<tr>
<td><strong>DISEASE</strong></td>
<td><strong>MEAN</strong></td>
</tr>
<tr>
<td>1. Atrophic Rhinitis</td>
<td>2.61</td>
</tr>
<tr>
<td>2. Housing/Environment</td>
<td>2.63</td>
</tr>
<tr>
<td>3. Nutrition/Disease</td>
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</tr>
<tr>
<td>4. Pneumonias</td>
<td>2.68</td>
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<td>5. Economics of Herd Health</td>
<td>2.72</td>
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<tr>
<td></td>
<td></td>
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</table>
Q4. **Information Needed - A six (6) year comparison.**

<table>
<thead>
<tr>
<th>1979</th>
<th>1984</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Preweaning Scours</td>
<td>Atrophic Rhinitis</td>
</tr>
<tr>
<td>2. Reproduction/Infertility</td>
<td>Nutrition/Disease</td>
</tr>
<tr>
<td>3. Agalactia Syndrome</td>
<td>Pneumonias</td>
</tr>
<tr>
<td>4. Housing/Environment</td>
<td>Housing Environment</td>
</tr>
<tr>
<td>5. Nutrition/Disease</td>
<td>Economics of Herd Health Programs</td>
</tr>
</tbody>
</table>
SUMMARY

From this survey of eleven state laboratories, clearly baby pig diarrhea is the number one disease problem of swine, and most of those cases are associated with *E. coli* infection. Other etiologic agents include rotavirus (10 labs), TGE virus (10 labs), coccidiosis (6 labs), and cryptosporidia (1 lab).

Salmonella (1 lab) and swine dysentery (7 labs) were frequently reported as significant causes of swine diarrhea. Of the six laboratories that ranked dysentery by itself as a significant problem, 3 ranked it as the second most important disease of swine, 2 as third and 1 as fourth. Dysentery was therefore ranked as the second most important disease.

The third most significant disease was pneumonia, specifically caused by *Haemophilus pleuropneumonia* (3 labs), pasteurellosis (3 labs), mycoplasmosis (2 labs), and salmonellosis (1 lab).

The fourth most prevalent disease was septicemic salmonellosis that resulted in encephalitis and pneumonia. Five laboratories ranked it fourth and 2 ranked it third.

Fifth, pseudorabies has undoubtedly had its greatest impact on swine breeders and was ranked as the fourth most significant disease by Iowa and Illinois. One lab, Minnesota, had Pr ranked second as one of 3 agents responsible for encephalitic disease in pigs. Similarly, Missouri ranked Pr in encephalitic disease that is the fourth most prevalent disease syndrome in its swine industry. Ohio ranked it fifth based on the state's need to keep the disease out of its swine. North Carolina also ranked Pr fifth based on baby pig and feeder pig death losses. Indiana ranked Pr sixth due to the cost of testing required for swine movement. Nebraska reported 35 diagnoses based on pathologic examinations conducted in their laboratory, which ranked Pr around sixth, if Pr was combined with atrophic rhinitis (36 cases) and proliferative ileitis (38 cases). South Dakota reported Pr as a disease control problem with no specific ranking.

Based on the remaining laboratory responses, sixth place was a 4-way tie. Five laboratories ranked mycoplasmosis as first, second, third, fifth, and sixth. Four laboratories ranked proliferative ileitis as second, fourth, sixth, and seventh. Pasteurellosis was ranked second by 2 laboratories and fifth by 1 laboratory. *Strep suis* was ranked fifth, sixth, and fourth, in a
multiple etiology syndrome listed under encephalitis.

**Iowa — Dr. Howard Hill**

1. **Enteric Complex** — comprised by *E. coli* (baby pigs mortality most significant); TGE, rota, and coccidiosis
2. Swine dysentery is second in importance due to the continued need for medication, i.e., $25–$50/ton feed, as well as death losses
3. **Haemophilus pleuropneumonia**
4. Pseudorabies, a devastating disease to the purebred producer, and producers are unable to protect against production losses through vaccination
5. **Strep suis** type 2 pneumonia and encephalitis — cases of this type are far outnumbered by pure pseudorabies outbreaks
6. **Mycoplasma hyopneumoniae** as a primary disease, is not a major problem in Iowa swine unless poor management practices exist such as excess pit gases in housing under poor ventilation conditions

Influenza is still a problem in Iowa swine, but it is not in the top 6 disease problems.

**Illinois — Dr. Doug Hoeftling**

1. Baby pig diarrhea as 10 day scours, caused by colibacillosis, rota, TGE, and coccidiosis, appears as the lactogenic immunity is dropping at that age and it has been very difficult to protect the pigs.
2. Swine dysentery
3. Strep meningitis occurs in Illinois swine at a greater incidence than observed for pseudorabies
4. Pseudorabies — The disease is a significant one and has been made significant by the regulations that require a significant outlay by the producer (breeder) due to testing requirements
5. Salmonellosis
6. **Haemophilus pleuropneumonia**

**Illinois — Dr. Anne Platt, Centralia**

1. Diarrhea
   a. Salmonella and hemolytic *E. coli*
   b. TGE and rota are often found together in herds. Both diseases remain a problem in spite of the available vaccines and due to the erratic duration of colostral immunity

In farrow-to-finish operations under a single roof, diarrhea outbreaks may sweep the entire facility due to 1 of the 4 agents. Rota appears to be endemic in many operations and sudden stress precipitates outbreaks of diarrhea.
2. Pneumonia
   a. biggest problem is due to Haemophilus
   b. atrophic rhinitis appears as an isolated herd problem that persists within those herds.
3. Parvo — Not a major problem except in endemic herds. Endemic herds continue to have abortion problems despite vaccination.

4. Pseudorabies — Not that many cases observed. Usually diagnosed in abortion problems. Previously infected herds are presently vaccinating.

5. Mycoplasma pneumonia

6. Some *Strep suis* problems have been diagnosed. Incidence is below haemophilus and mycoplasma cases.

**Indiana — Dr. Leon Thacker**

1. Enteric disease due to colibacillosis and TGE
2. Swine dysentery
3. Mycoplasmal pneumonia
4. Salmonellosis
5. Haemophilus pneumonia
6. Pseudorabies due to expense of testing required for swine movement. As a disease problem, would not rank as No. 6.

7. Porcine proliferative ileitis

**Minnesota — Dr. Kurtz**

1. Enteric disease — *E. coli* and rota as a disease complex is the most significant problem in Minnesota swine. Cryptosporidia and coccidiosis are also significant agents in this syndrome, but occur with a slightly lower frequency.

2. A grouping of 3 diseases:
   a. Edema Disease causing CNS disease and as a differential diagnosis problem
   b. Pseudorabies in nursing and feeder pigs
   c. Salmonellosis

3. Pneumonia — Pasteurella pneumonia more significant than *Haemophilus parasuis*. Death loss greatest when both agents are present.

4. Proliferative ileitis — Occurs at a higher incidence than swine dysentery.

5. & 6. none given

**Nebraska — Dr. Rodney Moxley**

This is a tabulation of the diagnoses made in our diagnostic laboratory for the year 1983.

<table>
<thead>
<tr>
<th>No. Positive Cases</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>104</td>
<td>Mycoplasmosis</td>
</tr>
<tr>
<td>80</td>
<td>Enteric colibacillosis</td>
</tr>
<tr>
<td>75</td>
<td>TGE</td>
</tr>
<tr>
<td>64</td>
<td>Coccidiosis</td>
</tr>
<tr>
<td>35</td>
<td>Rotaviral enteritis</td>
</tr>
<tr>
<td>75</td>
<td>Salmonellosis</td>
</tr>
</tbody>
</table>
4. Swine dysentery 63
5. Pasteurellosis 60
6. Proliferative ileitis 38
7. Atrophic rhinitis 36
8. Pseudorabies 35

Missouri — Dr. Larry Morehouse

1. Enteric complex — in order of frequency of diagnosis
   a. Colibacillosis — highest cost disease
   b. TGE — A very severe year in 1982–1983
   c. Swine dysentery
   d. Coccidiosis is increasing since 1980. Frequency of diagnosis may now be as great as that observed for dysentery
   e. Rotavirus enteritis — in combination with colibacillosis or TGE

2. Respiratory Disease
   a. *Mycoplasmosis hyopneumoniae* is most significant respiratory pathogen
   b. Pasteurella
   c. Haemophilus
   d. Atrophic Rhinitis

3. Systemic salmonellosis resulting in pneumonia and encephalitis

4. Encephalitis
   a. Pseudorabies
   b. HEV
   c. Strep meningitis

5. Reproductive Disease — Undiagnosed

Ohio — Dr. Ed Bohl

1. Enteritis
   a. *E. coli* — more frequently seen as postweaning enteritis instead of preweaning
   b. TGE — second most significant agent in baby pigs; initially seen in neonate and later persists in nursery
   c. Rota

2. Respiratory Disease
   Primarily see pasteurellosis as a secondary disease agent. Very little acute haemophilus pneumonia is diagnosed and mycoplasma are not significant in respiratory disease unless they are combined with atrophic rhinitis.

3. Perinatal mortality due to agalactia that is observed in over conditioned breeders or gilts bred at too small or too early and age, and mastitis in sows during weaning when they are penned together in dirty pens away from baby pigs.

4. Reproductive disease, i.e., gilts or sows that fail to settle due to management. Previously, major problem was due to parvo but the
parvo vaccine has helped reduce reproductive losses.

5. Pseudorabies — Very little Pr is present in Ohio, but due to its relative absence and desire to eradicate Pr from Ohio it is very important to keep Pr out of the state through testing and other control measures. Main problem area in the state is along the Indiana border.

6. Environmental factors and their effects on swine production.

**South Dakota — Dr. M. W. Vohries**

1. Enteritis — Colibacillosis and rota infections appear to occur in a synergistic relationship. Rota infection may promote adherence of *E. coli*. TGE is a primary disease agent by itself. Coccidiosis occurs and is a problem that is related to poor housing conditions.

2. Proliferative ileitis of 4- to 8-week-old feeder pigs and replacement gilts of the large white pig breed is an emerging problem in South Dakota.

3. Swine dysentery

4. Septicemic salmonellosis resulting in pneumonia

5. *Haemophilus parasuis* infection resulting in chronic weight loss is a far greater problem than that caused by *Haemophilus pleuropneumonia*.

Pseudorabies is a disease control problem for South Dakota.

**Kansas — Dr. George Kennedy**

1. Enteric disease caused by *E. coli*


3. Swine dysentery

4. Septicemic salmonellosis resulting in pneumonia and enteritis. A slightly greater incidence of enteritis due to salmonella infection is diagnosed.

5. Enteritis due to rota and TGE

**North Carolina — Dr. C. W. Pittman**

1. Diarrhea due to colibacillosis (No. 1), and TGE (No. 2). Rotaviral diarrhea is not very common in North Carolina swine

2. Acute pneumonic pasteurellosis

3. Septicemic salmonellosis resulting in encephalitis and pneumonia

4. *Haemophilus pleuropneumonia*

5. Pseudorabies infection seems to be fairly stable at present. Clinical disease is most commonly observed as baby pig mortality in the farrowing house and as death losses of up to 5% in feeder pigs.

6. Infectious arthritis of baby pigs due to streptococcal and staphylococcal infection.
APPENDIX D

What are the Most Important Disease Problems?

D. C. Hoefling
Galesburg Animal Disease Laboratory
Galesburg, Ill.

Disease Diagnosis: 1984 Survey, Galesburg Animal Disease Laboratory

Colibacillosis 14%
Clostridial enteritis 10%
T.G.E. 8%
Coccidiosis 7%
Agalactia 5%
Bacterial pneumonia 5%
Hemophilus pneumonia 5%
Mycoplasma pneumonia 5%
Abortion — unknown 4%
Rotavirus 4%
Salmonellosis 4%
Streptococcus suis meningitis 3%
Swine dysentery 3%
Autolysis 2%
Endotoxic Escherichia coli 2%
Hemorrhagic bowel syndrome 2%
Ileitis 2%
Pseudorabies 2%
Vitamin E — Selenium deficiency 2%
Arthritis — unknown 1%
Arthritis — bacterial 1%
Arthritis — Mycoplasma 1%
Atrophic rhinitis 1%
Edema disease 1%
Encephalitis 1%
Torsion — intestine 1%
Abortion — Lept less than 1%
Abortion — Parvo less than 1%
Abscess less than 1%
Anemia — Epi less than 1%
Anemia — unknown less than 1%
Atresia ani less than 1%
Bull nose less than 1%
Cellulitis less than 1%
Clostridial myositis less than 1%
Crushing less than 1%
Cystitis — nephritis less than 1%
Endocarditis less than 1%
Erysipelas less than 1%
Foot abscess  
Iron related vitamin E —  
Selinium deficiency  
Glasser’s disease  
Greasy pig  
Mange  
Melanosis  
Middle ear infection  
Navel infection  
Nephrosis  
Orchitis  
Over heat  
Peritonitis  
Pityriasis rosea  
Puffer sow syndrome  
Rectal stricture  
Shaker pig  
Suffocation  
Tetanus  
Toxicity  
Trauma

**Major Disease Problem Areas**

- Baby pig diarrhea 43%
  - Colibacillosis
  - Clostridial enteritis
  - T.G.E.
  - Coccidiosis
  - Rotavirus
- Pneumonia 15%
  - Bacterial
  - Mycoplasma

Growing-finishing pig diarrhea 11%
- Salmonellosis
- Swine dysentery
- Ileitis
- Hemorrhagic bowel syndrome

- Abortion 5%
- Agalactia 5%
- Streptococcus Meningitis 3%
- Endotoxic *Escherichia coli* 2%
- Vitamine E — Selenium deficiency 2%
- Pseudorabies 2%

**Subjective analysis:**

7–10 day old pig diarrhea
— immunology, epidemiology
Clostridial enteritis
— immunology
Hemophilus pneumonia
— immunology
Streptococcus suis
— epidemiology, immunology
Abortion
— diagnosis, etiology
Pseudorabies
— regulatory, eradication
VITALITY, USEFULNESS, AND RECEPTIBILITY OF NADDS
Remarks by Dr. J. K. Atwell, Committee on Animal Disease
Surveillance, U.S. Animal Health Association Meeting, Ft. Worth,
Texas, October 25, 1984.

The livestock industry, those associated with it, and those attempting to
serve it are becoming increasingly aware of the need for valid information
concerning the prevalence and economic importance of diseases. The
availability of scientifically and statistically reliable information is essen-
tial to improving the health of animal populations.

Unquestionably, one of the greatest needs of livestock owners and the
veterinary profession throughout the world today is the perfection of a
comprehensive animal disease surveillance system. Surveillance and dis-
ease detection may very well become the single most important area of
"regulatory" veterinary services in the future. A national animal disease
detection system will provide accurate documentation of the importance
of animal diseases and a rational basis for determining research priorities
and actions against livestock diseases. Such a system will also provide the
foundation for many aspects of future cooperative efforts between the
public and private sectors mutually concerned about the health of our
livestock populations.

In order to meet this challenge, the Animal and Plant Health Inspection
Service (APHIS) has accepted the lead in coordinating and developing the
methodology to establish a National Animal Disease Detection System
(NADDS). However, in truth, such an ambitious and vital activity repre-
sents a challenge to everyone concerned about the health of this Nation's
livestock and poultry.

Increasingly, NADDS will require the help and cooperation of a widen-
ing circle of other groups and specialists. A team approach involves an
ongoing commitment in which all members become aware of the different
components of the problem and develop perceptiveness and capabilities far
beyond those they would normally exercise when operating in isolation.
Eventually the livestock industries will be serviced by, and will become
the responsibility of, such an interdisciplinary group.

NADDS not only plans to involve a wide circle of participants but, indeed, demands it. NADDS represents a national activity in which an
increased collaboration of government, both State and Federal, university,
professional, and producer organizations is a natural part of its evolution.
APHIS has taken a big step in coordinating the development and imple-
mentation of NADDS. We look forward to working with other Federal
agencies, such as the Food Safety and Inspection Service (FSIS), the
Statistical Reporting Service (SRS), the Economic Research Service (ERS),
the Agricultural Research Service (ARS), and the Extension Service (ES),
as well as State departments of agriculture and university systems and
colleges of veterinary medicine. We anticipate, seek, and expect valuable
input from all segments of the livestock and poultry industries to assure
that the project and data have utility and applicability. Because of the scope of NADDS, the project will serve many beneficiaries who, by themselves, cannot marshal the resources to implement a national system, but who vitally need the information. The needs of so many groups and individuals have especially compelled us to be involved in this venture.

NADDS represents a new type of activity for a “regulatory” agency such as APHIS. It cannot be considered in the same light of past regulatory programs. NADDS is an integrated effort based on cooperation between the veterinary and livestock professions, without codes or laws which mandate the activity. It represents a forward-looking approach, with a premium placed on information exchange with the industry and those serving the industry.

Our early experiences in Ohio and Tennessee suggest that Veterinary Medical Officers (VMO’s) enjoy dealing with livestock owners on a “non-program” basis and vice versa. NADDS also places an emphasis on preventive veterinary medicine and better utilizes the extensive and diverse training of veterinarians and uses these strengths toward a service-oriented activity. We are certainly heartened by the receptiveness to NADDS by both the field VMO’s and the participating owners and ranchers.

Currently, 75 State, Federal, and university veterinarians in Ohio, Tennessee, California, Iowa, and Colorado are involved in monthly visits to randomly selected herds, where data is being collected regarding all health-related events for the previous month. This data has formed the foundation and basis for evaluating the pilot project and determining its future expansion.

Information concerning demographic characteristics of the farms and ranches, livestock diseases and conditions, and the economic impact of these findings is being transferred into a computer system. Retrievals, data manipulation, and analysis are being explored and evaluated. Further expansion of NADDS is being considered, to be certain that all significant groups of food-producing livestock and poultry are involved in the health information system. The State of Georgia has agreed to be the sixth pilot State to participate, and we will especially focus on bringing the poultry industry into the project during this time.

NADDS data is being collected on farms and ranches by State and Federal VMO’s. This data is based on the signs of disease or final outcomes of such problems, i.e., weight loss, reproductive problems, etc. Thus, the livestock producer and the VMO record and track their clinical impressions over time concerning the occurrences of all health-related events in their herds and flocks. This requires a keen awareness and recognition of disease problems to extend our knowledge of the natural history of disease and make an assessment of its economic impact. As we recognize that diseases and conditions are complex, multifactorial events that involve all aspects of the host, agent, and environment, the importance of utilizing veterinarians in collecting data and making judgments on disease occurrences and the underlying interactions is deemed unequivocally essential.
Most people are amazed that we currently have neither information on the incidence or prevalence of common generic problems such as respiratory infections, diarrheas, or mastitis, nor knowledge of the economic consequences of these problems on a national basis. We believe it is important to quantify these problems, but, at the same time, we realize the necessity of further identifying specific agents of disease where appropriate. A significant number of herds and flocks will require in depth laboratory diagnosis and analysis to classify diseases and measure their occurrences over time. We will be relying on diagnostic laboratories to help us determine which of these agents are commonly found in “healthy” herds, i.e., base-line data for comparisons and to validate the information being collected from the farms. We realize that many potential disease agents infect livestock without resulting in overt clinical illness. These subclinical and/or latent infections can quickly change character, and fulminating, costly problems may result. The factors which can change the outcome of infection must be identified, so that preventive measures can be implemented in high-risk situations. Postmortem findings will also be useful in judging disease problems and further defining problems, and will be accomplished as we follow some herds/flocks to slaughter. A well-designed surveillance and detection system must use laboratory resources to validate the on-farm data that is being reported.

The centennial celebration of the Bureau of Animal Industry allowed us to take some time to reflect on our heritage. Underlying all the success and hard work, a central theme was apparent. Animal health has been, and currently is, in a state of dynamic transition. We now live in a world where changes occur much more quickly than in the past. Transitions are accelerated, and, as such, often do not permit us much time for making decisions. Thus, management, more and more, must rely upon and develop systems that have current and valid information for effective decision-making. This, of course, emphasizes the usefulness and necessity of a NADDS.

Our transition into new activities such as NADDS in no way impinges upon or detracts from our current program commitments. Rather, it enables us to develop simultaneously new activities to better serve the livestock and poultry industries and the consuming public. I believe a stable structure of APHIS personnel will always be essential for preventing and fighting exotic diseases and pests. However, program activities will change priorities as information is acquired on the amount and costs of livestock diseases and conditions. We believe that the development and implementation of a national animal disease detection system, that will occur hand-in-hand with our ongoing program, is mutually beneficial to both. As tuberculosis and brucellosis eradication efforts are brought to fruition, our Agency will be well positioned to move into new service areas; however, with NADDS, we will have the proper documentation and evidence of the amount and cost of disease problems for the first time. Therefore, legislators, the states, the livestock and poultry industries, and APHIS can properly establish future directions and services.
Time and change will surely show that firmness in our commitment to a national animal disease detection system today will pay dividends many-fold over as we progress in effective support of animal health.

Looking back on the last 100 years, one cannot help being impressed with the cooperative efforts among the Federal-State-industry groups. The cooperative work and the shared successes have been due to the quality of the people involved. Hard work and many sacrifices have characterized these people. The Agency has matured, grown, and become better because of this dedication. At the same time, we believe that APHIS must maintain a conducive environment for individual, professional, and personal growth. Just as APHIS has undergone changes, our personnel must also change. Their personal growth is enhanced by meeting new challenges. The NADDS is such a challenge. This nonregulatory activity necessitates that our veterinarians acquire and maintain new skills in the area of preventive veterinary medicine.

In my position as Deputy Administrator I must look at current jobs, think how they might change, and consider how those changes impact on people. I believe that people being affected by a decision must be part of the process that mediates that change. In a like manner, the development of a NADDS must have its basis in the grass roots of each State, with constant feedback from the people involved and affected. This approach is more participatory in concept and emphasizes a decentralization of the activity.

It is comfortable to cling to the past and takes courage to abandon tradition; however, animal health problems change and new priorities surface. The livestock and poultry industries are more diverse and complex, and this requires us to expand our thinking and services, acquire information, and define their problems. Then management and policy options can be suggested to decision-makers that point out risks and consequences of decisions with regard to the improvement of animal health.

Alfred North Whitehead stated that “The art of progress is to preserve order amid change and to preserve change amid order.” We adhere to that tenet as we move forward and evaluate the vitality, usefulness, and receptibility of a new activity such as NADDS.

Part of the challenge that we face in transition is an increasing awareness of the need to expand our export markets. Our balance of trade is favorably influenced through all types of agricultural commodities. Yet, current events portend more open trading and more competition for markets. Already, 75 percent of all U.S. products are in competition worldwide. This will certainly increase in the future. To meet this challenge, a national disease detection system will give the United States a decided edge in competition for exporting our livestock and livestock products.

Productivity and our competitive position in the marketplace will be greatly influenced by our capacity to use our resources efficiently. We need to be constantly working on increasing our efficiency of production. NADDS will prove to be cost-effective by allowing owners to better visual-
ize their losses due to disease conditions and suboptimal production. We also believe that our livestock and poultry industries represent some of the healthiest animals in the world. Monitoring the health status of our animal populations will demonstrate to all importing countries how healthy and desirable our livestock and poultry really are.

As Deputy Administrator, I have always been concerned with the untenable, "Catch-22" position in which economists and legislators often place us. We are constantly being asked to supply them with valid data on animal disease situations and, except for a few national program diseases, the information is not available. However, when we request funding and resources for gathering such data, adequate funding levels are not approved. I am more confident today that legislators see the vital need for statistically and biologically valid information on diseases and conditions of our livestock and poultry. Decisions on funding future research and program activities must be based on proper documentation. NADDS will provide this basis, across the board, for the first time.

While serving as the Deputy Administrator of VSAPHIS, I have seen a new emphasis on international activities. Daily, the world is becoming more and more interdependent because of a global economy. Although there is a great disparity in the level and sophistication of animal health practices throughout the world, animal disease detection systems represent a common theme and goal. The rapid exchange of accurate information between countries concerning the status of livestock diseases has never been more important. NADDS can serve as a prototype system that will have many transferable aspects for use in other countries. We need information to make contingency plans and acquire better lead time to plan and head off potential exotic disease problems. Information must be credible and accessible and must be shared internationally for mutual benefit. The methodology being developed for our NADDS should be shared with other nations that have similar aspirations. NADDS is producing an infrastructure of trained veterinarians with experience in monitoring disease problems. At the same time, it heightens the awareness of the industry to such problems. Both results strengthen our ability to deal with exotic disease threats.

Oliver Wendell Holmes once stated, "The mind once expanded to the dimensions of larger ideas, never returns to its original size." This is a compelling thought for us all to consider and, I believe, sums up the vitality and potential of the NADDS for all of us involved.
THE NATIONAL ANIMAL DISEASE SURVEILLANCE SYSTEM IN CALIFORNIA: IMPLEMENTATION METHODOLOGY


From the Bureau of Animal Health, California Department of Food and Agriculture, Sacramento, California (Heron), and the Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California (Deluyker, Hird).

INTRODUCTION

When California was asked in October 1983 to be in the second tier of states to participate in a pilot project for the National Animal Disease Surveillance system (NADs), we readily agreed. We agreed because we wanted to have some input into the development of the program, and also because we believed that this program would stimulate us to define and develop good livestock population data bases. At that time we had what we considered to be a good population data base (list frame) for dairy cattle—the Dairy Cattle Data Base, maintained by the Department of Epidemiology and Preventive Medicine, University of California, Davis—but not for beef cattle, sheep, or swine. Finally, we believed the time had come to obtain much needed information on the morbidity and mortality patterns of California livestock.

OBJECTIVES

The objectives of the California NADs pilot project were:

1. Logistics development —

   The pilot program would be a simulation of the full program. Logistical problems and obstacles revealed in the pilot would help us plan for a full NADS program, providing information to help us:
   a. obtain cost estimates of a full NADS program,
   b. integrate the NADS program into other established programs,
   c. develop an efficient system for information flow,
   d. determine what kind of priority can we give the NADS program,
   e. determine how to keep the commitment made to the farmer when pressures build.

2. Providing training for VMOs as interviewers —

3. Development of sampling methods and resources —

   We might
   a. develop species population data bases (list frames), and
   b. consider sampling methods other than list frame sampling.

4. Development of programs for collation, processing, analysis and dissemination of California NADS data —

   This would be a time to define and develop tools and techniques, and to
practice using them. The disease information gathered, however, would not be complete or unbiased. When California subsequently embarks on the full NADS program, we anticipate that our efforts in the pilot program will pay dividends by making it possible to obtain valid disease information.

**CHRONOLOGY OF THE CALIFORNIA NADS PILOT PROJECT**

California agreed to participate in NADS soon after initial contacts with Dr. Lonnie King of USDNAPHIS in October 1983. At the end of February 1984, APHIS personnel from Hyattsville conducted a training session for 23 state and federal veterinary medical officers (VMOs) selected to be field interviewers. Field work began in April and May, and in October 1984 a review and evaluation meeting was held for participating VMOs and their supervisors. At that time a questionnaire designed to evaluate some aspects of program progress was administered to participating VMOs.

**DECISIONS THAT WERE MADE IN INITIATING THE PROGRAM**

Due to limited resources, it was not possible to include all species and types of food animals in the pilot study or to sample all areas of the state.

The decisions reached were:

1. To deal with only 4 "species" of livestock — dairy cattle, beef cattle, swine, and sheep — (we consider beef and dairy cattle as separate "species" for our purposes), and to consider only breeding herds or flocks.

2. To sample only areas which were easily accessible from the district offices in the state, or from certain other VMO headquarters. It was decided to sample counties, and of the 12 counties selected, 10 were selected for the reason mentioned, and 2 because of NADS-related studies planned by the University of California, Davis. Due to the nature of the sample, we realized that results from this sample could not be extrapolated to the entire state, but rather only to the counties from which the sample was drawn.

3. For each species sampled we decided to stratify the selection process according to herd size. The strata limits were chosen so as to be compatible with those of the Statistical Reporting Service (SRS), but instead of using 8 herd size categories as SRS does, we used 3 — large, medium, and small (Table I).

4. Since we decided to use breeding stock for the pilot study, we had to decide what animals were to be counted to determine herd size. We again tried to conform to SRS definitions and used the definitions listed in Table II.

5. For the areas chosen, a stratified (on herd size) sample of herds was chosen from a list frame of livestock enterprises. It was intended that all areas be sampled in a random fashion, but inadequate list frames made this impossible on several occasions. The Dairy Cattle Data Base was used for dairy cattle, and the Kardex file (records of herd
visits made by the Bureau of Animal Health) was used for beef cattle
and swine, supplemented by other lists such as Animal Industry
association membership lists, farm advisor lists and livestock market
records.

6. California has many large herds with corporate owners, and other
large, complex livestock operations. It was difficult to categorize
herds on multiple premises, multiple herds on the same premises
(e.g., commercial and purebred herds on the same ranch), herds of
mixed types and herds with multiple owners.

7. In coding the premises identification data on the NADS forms, we
decided to remove the veterinarian identification code (for the VMO
interviewer) from the producer code and to make it a separate item.
This allows us to maintain continuing and retrievable records on a
given herd even though the interviewer might change. It also facili-
tates evaluation of interviewer influence on the data collection pro-
cess.

WHAT WE HAVE LEARNED FROM FIVE MONTHS IN THE NADS
PROGRAM

Five months after the start of collection of field data, an evaluation
meeting was held in October 1984 and a questionnaire administered to
participating VMOs, as previously mentioned. Some conclusions are as
follows.*

1. The first enrollments of producers in the NADS pilot program oc-
curred nearly seven weeks after the training session held in Feb-
uary. Much of the delay in assigning herds to VMOs was due to
difficulty in selecting herds because of the poor population data bases
(list of frames) available. This delay proved undesirable, since a
considerable amount of enthusiasm had been developed in the train-
ing session, and some was lost between training and herd as-
signment.

2. Farmers participating in the NADS program were promised that the
data collected on their farms would be kept confidential. Con-
fidentiality, however, did not seem to be very important to most
cooperating farmers, in the opinion of the VMOs answering the
questionnaire. On a scale of 0 to 5, with 0 indicating no importance
and 5 extremely important, the mean score was 1.8, with one-third of
respondents giving a 0 score. Nevertheless, we view confidentiality
as an essential part of the NADS data gathering strategy. Even fewer
farmers felt it necessary to have the same interviewer each month,
the mean score for this question being 1.6 on the same scale.

*For complete data and analysis of questionnaire results, refer to Deluyker, H. A. R. G.,
3. The integration of NADS activities with other VMO responsibilities was a gradual process, because enrolling a herd in the NADS program involves making a commitment to the cooperator to return once a month without fail to gather information. Figure 1 shows the rate at which California state and federal VMOs became involved in NADS and the rate at which herds became enrolled.

4. Figure 2, which indicates data collection and report submission during the 5-month period April to September, shows a lag in submission of reports from the field to the central office of approximately two months, considering the herd enrollment dates shown in figure 1. This lag period is unacceptably long and shows the importance of a control system for report submission. Such a control system should ensure timely collection and transmission of data.

5. Figure 3, which compares the number of enrolled herds with those contacted but not enrolled for any reason, gives an indication of the importance of good population data bases (list frames) when attempting to select and enroll herds. The ratio of nonenrollees to enrollees is 3.1 to 1 for hogs, 1.5 to 1 for sheep, 1.3 to 1 for beef cattle and 0.7 to 1 for dairy cattle. The reasons for nonenrollment are given, by species, in figure 4. Refusal of the owner to participate was the reason for 11 of the 12 nonenrollments for dairies. Most of these dairy owners said they were too busy to participate. The main reason for nonenrollment for beef cattle, swine, and sheep enterprises was that the ranches, although chosen from a list frame, were out of business. The second most important reason was that the herd failed to meet selection criteria; 85% of these failures were because the herd was too small. The third most frequent reason for nonenrollment for beef, swine, and sheep herds was refusal of the owner to participate, and the fourth was that the VMO was unable to locate the herd. This last reason, along with those out of business enterprises, is an indication of the amount of "dead wood" in the available list frames.

6. One of the main reasons for embarking on a pilot project was to obtain cost estimates, and the most important element of cost is employee time. Figure 5 shows the breakdown of time spent per enrolled herd on herds that did not enroll. Again, swine herds were the most costly in this category because of the difficulty in enrolling herds. For herds that did enroll, the numbers of hours spent finding the herd, getting the mutual agreement signed, completing NADS Form 1, and conducting the monthly interviews were similar for all species. Insufficient information was available for sheep enterprises.

7. Cooperating farmers are paid $25 per month to participate in the program, but by questionnaire response the VMOs believed that farmers thought this not to be an especially important incentive, assigning a mean score of 2.0 on the 0 – 5 scale, with 0 being of no importance and 5 being extremely important.

8. An indication of VMO influence on disease control practices on the
farm was measured by assessing the amount of disease control information given by the VMO interviewer to the farmer, and disease investigations and necropsies performed by the VMO interviewer. In the first five months of the pilot program, 70% of participants received disease control information from the VMO interviewer; 60% of participants had requested this information. Disease investigations and postmortem examinations had been performed on 5% of the farms. On the 1 – 5 scale, VMOs gave a mean score of 3.0 to the importance of their disease investigation skills in obtaining data.

9. Farmers desired feedback from the NADS system. Forty-three percent had asked VMO interviewers for this information.

CONCLUSIONS

Farmers and livestock management systems vary greatly across the country, so California’s experiences with NADS are not directly applicable to all other states. However, we suggest that those of you anticipating participation in NADS update species population data (list frames) and consider three items that are critical in ensuring the success of a NADS pilot project. First, use a realistic, but meaningful number of herds. Too few herds will not yield enough reliable information to be of use for planning a subsequent complete NADS program. Too many herds will result in more problems than solutions; individuals and organizations need time to build the program and to integrate it with other programs. Second, commitment on the part of the VMO interviewers must be instilled. Both federal and state employees must believe that the program will benefit the state and its livestock industries, and that it is not a program that can be abandoned easily for unexpected emergencies or other commitments. Third, state and federal supervisors must give the program sufficiently high priority to accomplish the goals set. They and the VMO interviewers make binding commitments to enrolled farmers, and for NADS to succeed this commitment cannot be taken lightly.

ACKNOWLEDGEMENTS

We acknowledge the many people who have worked to make the NADS program a success in California, especially Dr. W. W. Utterback, USDA/APHIS, Sacramento.
TABLE I—Herd size strata used for classification of of herds selected for the California National Animal Disease Surveillance pilot project, 1984

<table>
<thead>
<tr>
<th>Species</th>
<th>small</th>
<th>medium</th>
<th>large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>25 - 199</td>
<td>200 - 499</td>
<td>500 and over</td>
</tr>
<tr>
<td>Dairy</td>
<td>1 - 199</td>
<td>200 - 499</td>
<td>500 and over</td>
</tr>
<tr>
<td>Swine</td>
<td>10 - 49</td>
<td>50 - 199</td>
<td>200 and over</td>
</tr>
<tr>
<td>Sheep</td>
<td>10 - 99</td>
<td>100 - 499</td>
<td>500 and over</td>
</tr>
</tbody>
</table>

TABLE II—Animals eligible to be counted to determine herd size for the California National Animal Disease Surveillance pilot project, 1984

<table>
<thead>
<tr>
<th>Species</th>
<th>Animals Counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Cows and heifers over 2 years of age</td>
</tr>
<tr>
<td>Dairy</td>
<td>All milking and dry cows that have calved</td>
</tr>
<tr>
<td>Swine</td>
<td>Two options:</td>
</tr>
<tr>
<td></td>
<td>1. Sows, boars and replacement guilts, or</td>
</tr>
<tr>
<td></td>
<td>2. All animals over 8 months of age</td>
</tr>
<tr>
<td>Sheep</td>
<td>Ewes, rams, and replacement breeding stock over 8 months of age</td>
</tr>
</tbody>
</table>
Cumulative California MADs Herds and Veterinarians by Month 1984

Figure 1
Number of months for which data was reported collected compared to reports submitted.

California NADS Pilot Program
April thru September 1984

Figure 2
Comparison between herds enrolled in the California NADS pilot program and those that failed to enroll from April thru September 1984.
REASONS HERDS FAILED TO ENROLL IN THE CALIFORNIA NADS PILOT PROGRAM BY SPECIES APRIL THRU SEPTEMBER 1984

Figure 4
### Average Hours Spent in NADS Program by Activity and Species

**California NADS Pilot Program**  
**April thru September 1984**

<table>
<thead>
<tr>
<th>Activity &amp; Species</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finding Herd - Non Productive</td>
<td>1.2</td>
</tr>
<tr>
<td>F.H. - Productive</td>
<td>3.4</td>
</tr>
<tr>
<td>M.A. &amp; F-I</td>
<td>1.2</td>
</tr>
<tr>
<td>Monthly</td>
<td>3.3</td>
</tr>
<tr>
<td>F.H. - Non-Prod.</td>
<td>5.8</td>
</tr>
<tr>
<td>F.H. - Prod.</td>
<td>3.1</td>
</tr>
<tr>
<td>M.A. &amp; F-I</td>
<td>1.1</td>
</tr>
<tr>
<td>Monthly</td>
<td>1.5</td>
</tr>
<tr>
<td>F.H. - Non Prod.</td>
<td>1.1</td>
</tr>
<tr>
<td>F.H. - Prod.</td>
<td>4.4</td>
</tr>
<tr>
<td>M.A. &amp; F-I</td>
<td>3.5</td>
</tr>
<tr>
<td>Monthly</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**F.H. - Non Prod.** = Average hours (per enrolled herd) on herds that did not enroll in NADS Program.

**F.H. - Prod.** = Average hours spent in finding the herd and setting up first interview.

**M.A. & F-I** = Average time spent on Mutual Agreement (M.A.) and Form 1.

**Monthly** = Average time spent on monthly interview.

*Figure 5*
MODERN TRENDS IN IMMUNODIAGNOSIS WHICH FACILITATE AND EXPEDITE SPECIFIC DIAGNOSES

H. S. Gosser, DVM, PhD and A. R. Pursell, BS

For the past 30 to 40 years, a dramatic increase in technology has occurred which has given us tools used to study and consequently understand more about normal biological processes within man and animals and deviations from these normal processes. Before World War II, diagnostic tests consisted primarily of gross and histopathologic examinations, bacterial cultures, and serology. Immunology and virology came to the forefront in the early 1950's. The electron microscope, which was primarily a research tool at that stage, enabled scientists to visualize and study viruses. Biochemical pathways were better defined, and, as chemistry methodology advanced, faster and simpler diagnostic procedures such as urine test strips were developed which could be used by physicians and veterinarians alike. These strips provided tests that could be applied in their office, and within a few minutes, results were known which enabled a better evaluation of the patient.

One of the earliest techniques which increased the rapidity and accuracy in disease diagnosis was the application of immunofluorescence. This is simply the examination of smears or sections containing antigen which have been treated with appropriate fluorescein labeled antibody and examined under a fluorescent microscope. The first application of this technique in microbiology was Coons in 1942 when he demonstrated that soluble pneumococcal polysaccharide could be stained in sections of tissue from infected mice. In 1958, Goldwasser reported the detection of rabies antigen in the brain by fluorescent antibody techniques. This method provided a rapid, reliable diagnosis for a dreaded disease. Earlier diagnostic methods consisted of either the search for Negri bodies in smears made from brain and was not as reliable as needed, or a reliable but delayed diagnosis made by the inoculation of suckling mice with possibly infected tissue. The fluorescent antibody technique for the diagnosis of hog cholera was developed in the early 1960’s and provided a simple, accurate, rapid, and inexpensive test that was invaluable in the eradication of hog cholera from the United States.

Most laboratory test procedures used today to identify microbiological etiologies of disease consist of an antigen-antibody reaction and an indicator system. These tests and their indicators are as follows:

1) Complement fixations test — Hemolysis of red blood cells.
2) Fluorescein labeled antibody — Activated fluorescein.
3) Hemagglutination — Agglutination.
4) Serum neutralization — Cytopathology of cells.

*Veterinary Diagnostic and Investigational Laboratory, College of Veterinary Medicine, University of Georgia, Tifton, GA 31793.
5) Agglutination tests — Agglutination.
6) Agar gel immunodiffusion — Precipitation lines.
7) Metabolic inhibition test — Color change indicated by pH shift.
8) Hemabsorption test — Hemabsorption of red blood cells.
9) Enzyme-linked immunosorbent assay (ELISA) — Color change.

The above test procedures, while invaluable to a veterinary diagnostic laboratory, require either a great deal of technician time, or special technical skills, or sophisticated instrumentation or hours to days to determine the result. Thus, these tests are of little use to veterinarians in the field for rapid on-the-farm type diagnoses. Often the information gained is in retrospect to the acute problem on the farm, but does provide guidance for future herd management.

Two new technologies have advanced throughout the past decade and now offer the possibility of faster, more accurate diagnostic tests. These are the use of monoclonal antibodies (MAB's) and DNA probes.

1. Monoclonal antibodies in diagnostic medicine

The immune response in higher animals occurs when antibodies are produced in response to an antigen. Specialized cells called B lymphocytes, present in the spleen, lymph nodes, and blood, recognize substances foreign to the body (antigens), and respond by producing antibodies that specifically recognize and bind to those antigens. Each B lymphocyte, when stimulated, produces a plasma-cell clone. The antibody elaborated by this clone is a particular immunoglobulin class and subclass, with defined properties. The conventional method of producing antibodies for diagnostic, therapeutic, and investigational purposes is to inject an antigen into a laboratory animal and, after evoking an immune response, to collect antiserum from the animal. This is termed polyclonal antiserum and contains a mixture of specificities, affinities, classes, and subclasses. This method is widely used today but contains several inherent problems such as the following:

- the slightest contamination of the injected antigen results in a mixture of antibodies in the antiserum;
- a widely varied population of antibodies with concomitant differences in activity may be present, especially when a number of different animals are used to prepare the antiserum; and
- the supply of quality antisera is limited for any given purpose.

The standardization of immunoassays and accumulation of large amounts of reference antisera have been difficult because of the problems associated with standard antibody preparations. Although the difficulties and expense have not prevented the effective use of antibodies as diagnostic tools, the search for new methods and more refined antibody production has brought about the development and use of MAB's.

Monoclonal antibodies are produced when a mouse is immunized with a specific antigen, and a specific clone of B lymphocytes is selected from the
mouse's spleen. The clone-cells are then chemically fused with mouse myeloma cells to produce an antibody of a particular immunoglobulin with defined properties. All antibodies produced by this single cell line have identical properties.3

This method allows the preparation of large quantities of highly specific antibodies against a wide variety of antigens. These antibodies are homogeneous and highly specific. Their production is predictable and repeatable and can be chemically defined, as compared to polyclonal antibodies produced with conventional immunological methods.

The use of MAB's can improve the rapid identification of clinical isolates and permit early diagnosis of many infections by direct testing of clinical specimens. For identification of specific pathogens in tissue culture, tissue culture cells may be incubated briefly with fluorescein-conjugated monoclonal antibodies and examined by fluorescence microscopy. For pathogens that are typically scarce among clinical specimens, rapid diagnostic tests such as ELISA that are more sensitive than immunofluorescence may be required. In human medicine, MAB's are used for identification of viruses, bacteria, fungi, chlamydia, and parasites.5,11,12 Scientists at Genetic Systems Corporation, Seattle, Washington, have developed direct tests for the diagnosis of gonorrhea, chlamydia, and herpes virus infections in humans utilizing MAB's with fluorescein as the indicator. This technique requires only 15 to 20 minutes to perform, whereas previously 3 to 6 days of culture were needed.11 Monoclonal antibodies are being studied and perfected for several animal diseases, and tests are being developed for diseases such as bluetongue, equine infectious anemia, and bovine leukosis. Also, canine parvovirus, canine rotavirus, feline leukemia virus, and dirofilaria are being studied using MAB techniques.3

2. DNA Probes in diagnostic medicine

DNA probe technology, while relatively new, is making rapid advances in the area of disease diagnosis. The basic technique for preparing DNA probes is to remove an identifiable DNA segment from a microbial pathogen and insert this fragment into a DNA "vector," which is usually a plasmid. This plasmid, once inside a host cell, replicates many times, thus providing numerous copies of each donor DNA fragment or probe. The probe is labeled so it can be identified later. When organisms from a diagnostic specimen are placed on a matrix, they are broken open and the DNA isolated. The DNA is then treated with chemicals to separate the strands and bind them to the matrix. At this point, the labeled DNA probes are added to the matrix and hybridize to complementary DNA in the sample. The extra probes are then washed away from the matrix and signal molecules are added to indicate the positive reaction.8,9

DNA probes were originally developed to use as a research tool. The DNA probes were labeled with radioactive nucleotides usually 32P, and identified by autoradiography. This technique did not lend itself to diagnostic use because of special handling requirements and the short lives of radioactive labels. However, Dr. David C. Ward and his colleagues at Yale
University School of Medicine devised a method to attach signal molecules to the probes without impairing their ability to hybridize with complementary sequences. Dr. Ward chemically attached biotin to the DNA probes. Avidin which has a pronounced affinity for biotin has been attached to either fluorescent antibodies or enzymes that will produce a color change in a dye. The labeled avidin binds to the biotinylated DNA probe, coupling the signal molecule to the probe. Horseradish peroxidase and alkaline phosphatase are two of the most commonly used enzymes to attach to the avidin. These produce quantifiable color changes and can serve as an ELISA system.9

Although DNA probe technology has limitations, diagnostic tests are being developed with this technique. Recent developments have created probe systems that are at least 1000 fold more sensitive than serological tests and eliminate the need to culture test samples. Enzo Biochem, Inc., New York, NY is a leader in the DNA probe field in both research and the development of diagnostic kits. Enzo currently offers kits for the detection and identification of herpes simplex virus, Epstein-Barr virus, hepatitis B virus, cytomegalovirus, and adenovirus type II. Technology in this area is advancing also in the food industry. DNA probes have the potential to accelerate testing of food for microbes such as *Salmonella* sp. and *E. coli*. Work is being conducted with DNA probe technology for the diagnosis of human genetic disorders such as cystic fibrosis, prenatal diagnosis of diseases such as Down’s syndrome, tissue typing, detection of damage caused by mutagenic agents such as polyvinyl chloride, and the diagnosis of cancer.9

Use of MAB’s and DNA Probes in Veterinary Diagnostic Medicine

Several diagnostic kits which utilize either ELISA or immunofluorescence technology are marketed for veterinary use. Kits are available for several diseases, including feline leukemia, feline infectious peritonitis, dirofilariasis, and rotavirus infection. Daryl Laboratories Inc., Santa Clara, CA has introduced into veterinary diagnostic medicine a rapid immunodiagnostic system which provides fast diagnostic assays for feline leukemia virus, feline infectious peritonitis, dirofilariasis and others. This system utilizes immunofluorescence technology and a 3 dimensional binding gel. These kits and diagnostic systems have increased diagnostic capabilities for laboratories and allow for faster diagnoses; however, acceptance of the systems by private veterinary practitioners and many diagnostic laboratories has been slow because of the cost per test and technician time required to run the tests.

There is considerable interest in the MAB and DNA probe technology as applied to veterinary medicine, but scientists are not optimistic about the applications as to on-the-farm diagnoses. Several hurdles must be overcome before these types of diagnostic kits are readily acceptable by veterinarians or marketable by commercial firms. While, generally speaking, no expense is spared for human health care products, there is a limit to how much our farmers will pay for health care of their animals. If diagnostic
kits are marketed, they will probably be restricted to high profit animals such as those for export and to companion animals such as dogs, cats, and horses. There may be markets for tests that screen bull semen for contaminants and eventually will screen for desired traits. Also, these diagnostic techniques could substantially assist in large scale disease control programs in both developed and less developed countries. Diseases would be detected, and this information used to develop appropriate disease control programs.8,9

The question has been asked about the feasibility of using “portable” tests by field veterinarians to assess disease frequency rates. Before such diagnostic products are to be effective several obstacles must first be overcome.

1) Tests must be able to recognize the large variety of disease strains to be encountered.
2) Time to complete tests must be reduced to 15 minutes or less. Thirty minutes or an hour is a long period of time to keep a farmer occupied when he has other chores to do.
3) Tests must be economically feasible.
4) Tests must not require instrumentation. Even the need to use a compound microscope can present problems on many farms.

Also, there are problems not related to the tests per se.
1) A particular farm can have serious disease outbreaks but if the field veterinarian is not on the premises until later, the etiologic agent probably cannot be identified.
2) Most of these tests are best for identifying antigens. If sera are examined for antibodies, few diseases can be diagnosed with only one sample. A second sample would be required 10-14 days after the first.
3) If the field veterinarian or data collector does diagnose a disease as to etiology, how does he answer the farmer’s questions? What role does the veterinary practitioner play?

Recently, kits have been developed and are being marketed which are intended for use in monitoring the level of specific pesticides, herbicides, detergents and other organic chemicals used in agriculture and industry, as well as the level of toxic chemicals in the environment, particularly in food and drinking water samples. Environmental Diagnostic, Inc., Irvine, CA is developing these kits utilizing MAB technology and employs the ELISA technique to give color changes to indicate levels of the suspected chemical. Their “Quick-Screen” field test requires approximately five minutes for a qualitative test and twenty minutes for a semiquantitative measurement. To date, “Quick-Screen” kits have been developed for Paraquat, a herbicide, and Triton-X, a surfactant used in detergents.6 This type of application illustrates the potential for rapidity and ease of disease diagnosis in veterinary medicine.

Diagnostic medicine is an extremely dynamic discipline, developing rapidly with constant modifications of methodology as research presents
new and innovative approaches to the diagnosis of diseases. The technology is available today for many on-the-farm tests. However, the market for these products to be used in the diagnosis of diseases of food-producing animals has not developed substantially to encourage manufacturers.

REFERENCES


THE NATURE OF THE ANIMAL DISEASE SURVEY SAMPLING AND ESTIMATION PROBLEM

Victor C. Beal, Jr., PhD.
Chief Staff Biometrician, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Hyattsville, MD.

There are two animal disease surveillance problems which involve national or State-wide populations of animals. Both of these problems can be thought of as involving animal disease or animal health surveillance. One of these problems relates to estimating the level of disease that exists in the population. The other animal disease surveillance problem relates to the detection of foci of infected herds and flocks of animals.

The activities of the Committee on Animal Disease Surveillance (CADS), formerly the Committee on Morbidity–Mortality, of the United States Animal Health Association (USAHA) and of its predecessor committee, the Committee on morbidity and mortality statistics of the U.S. Livestock Sanitary Association (USLSA), have been directed toward the problem of disease estimation. In contrast, the activities of animal disease control and eradication programs have been directed toward the detection and elimination of foci of infected herds and flocks of animals.

DIFFERENCE IN TWO ANIMAL DISEASE SURVEILLANCE PROBLEMS

Both of these animal disease surveillance problems involve some form of the sampling of populations of animals. However, the contrasting nature of these two problems means that two distinctly different approaches of sampling of the population are required.

The problem of disease estimation requires a careful random sampling (statistical probability sampling) of a very small fraction of the herds and flocks in the population. In contrast, the problem of animal disease detection requires the intensive surveillance of large portions of the target population for the detection of the target disease. Consequently, a system developed for estimating the level of animal disease or animal health is of little value in the detection of foci of infection for purposes of disease control or eradication.

The application of the science of sampling statistics helps to distinguish the nature of the difference in these two problems. Also, the science of sampling statistics helps to explain why a system developed for estimating the level of disease is of no use in the detection of foci of infected herds or flocks for purposes of disease control or eradication and for purposes of detecting outbreaks of exotic diseases. In addition, this science of sampling statistics helps to explain why a system of surveillance developed for detecting such foci of infection for purposes of disease control and eradication is often of limited use for estimating the level of the target disease.
The application of sampling statistics to these two problems may be likened to being statistical estimation. The statistical estimation problem relating to the level of disease involves estimating frequency rates of prevalence and incidence of disease. This in turn permits estimating physical losses due to disease. The statistical estimation problem relating to the detection of foci of infection involves determining adequate surveillance levels for the detection of foci of infection for purposes of disease control or eradication.

Veterinary Services (VS) is currently involved in pilot projects in five states designed to develop procedures for the purpose of estimating frequency rates of prevalence and incidence. It is this problem which will be emphasized here. This problem must always involve statistically based random samples (probability samples).

The other estimation problem relates to the detection of foci of infection or to the likelihood of the presence of foci of infection. This problem has been examined by a number of workers in the past (Harvey, 1958; Damon, 1961; Beal, 1977, 1983a, and 1983b). The author has demonstrated conclusively in his Regulatory Statistics, Parts III, IV, and VII (Beal, 1983a) the extensive surveillance coverage needed in order to obtain adequate levels of disease detection for purposes of control and eradication. Starting in 1963, the author expanded upon earlier work by Harvey (1958) and Damon (1961).

The nature of the inadequacy of traditional animal disease surveillance data for purposes of estimating the level of disease has been discussed by various authors (Beal, 1975, 1980a, 1980b, 1983a, 1983b; McCallon, 1981; McCallon and Beal, 1982; and Leech and Sellers, 1979). It has been demonstrated most conclusively by these authors that traditional animal disease data has been totally inadequate for the estimation of disease levels.

As discussed by the author (1983b), some information can be obtained from random samples of market animals. However, the most important information must come from samples consisting of herds and flocks of animals. One of the major problems in estimating the level of disease incidence and prevalence is to determine the adequate size of the sample.

BASIC REQUIREMENTS FOR ESTIMATING DISEASE INCIDENCE AND PREVALENCE

There are two basic requirements in estimating disease incidence and prevalence. They are statistical validity and diagnostic validity. Discussions of the nature of an adequate system for estimating disease incidence and prevalence have tended not to properly recognize the nature of these two requirements.

Diagnostic validity: Diagnostic validity involves two aspects. These two aspects depend upon whether diagnostic samples are being obtained or not. In the absence of diagnostic samples, diagnostic validity consists of
having clinically valid descriptions of the disease being observed. In the presence of diagnostic samples, diagnostic validity consists of accurately relating clinical observations to the laboratory results.

The adequate accomplishment of either of these aspects of diagnostic validity requires the involvement of veterinarians. It has been proposed by some interests that non-veterinary lay enumerators could obtain the required data more cost-effectively than can veterinarians. However, certain past experiences cast doubt on this proposition.

**Statistical validity**: Statistical validity involves in part having an adequate survey design and an adequate sample size. Also, statistical validity requires the accurate recording of the basic data. This means that diagnostic validity is also a part of statistical validity in addition to being a separate factor.

The problem of statistically adequate or inadequate sample size for disease estimation can be compared to the sample sizes which are used for the Statistical Reporting Service’s (SRS) livestock inventory estimation. In fact, the problem of adequate sample size for disease estimation has been related to the sample sizes used for inventory estimation. Samples of 1,000 to 1,500 herds have been mentioned as constituting adequate sample sizes for state estimates of livestock inventory.

Consequently, the thought has occurred to the author that additional aspects of the nature of the animal disease estimation problem need to be discussed. In doing this, past experiences in the field of animal disease can be used.

The author first faced this type of estimation problem shortly after joining the Biometrical Services Staff (BSS) of the Agricultural Research Service (ARS) 21 years ago. The results of two surveys done in the Animal Health Division, a predecessor of Veterinary Services (VS), and reported on in 1966 and 1967 are pertinent in examining this estimation problem.

One survey was for trichinae in garbage fed swine while the other was for salmonella in basic feed mills. In each survey, the basic sample unit was not the herd or feed mill being drawn at random from the population at large but was the individual animal within the herd for the trichinae survey and the individual feed sample from the feed mill for the salmonella survey.

The nature of the estimation problem for the current pilot projects in VS and for the ultimate national system is identical in that the individual herd being drawn at random is not the sampling unit of interest. Rather, the individual animal within the individual herd is the sampling unit of interest. This is in contrast to SRS inventory estimation where there is an individual observation value for the herd which is the number of animals in the herd.

As mentioned above, in examining this problem, one can consider certain of the results from the trichinae and salmonella surveys. Variance and percent positive estimates were computed with the use of cluster

The contrast in computing the estimates for the trichinae and salmonella surveys demonstrates the importance of having a well defined population to sample from. Consequently, distributions supplied by SRS for the five individual state pilot projects have been of great value to VS. In the trichinae survey, a list of garbage feeders was available for each state which contained the number of animals for each herd. This permitted the inclusion of number of swine for each herd and for each state for weighting in the estimation process.

In contrast, for the salmonella survey, while there was available a list of feed mills for each state and an estimate of the finished feed production for each state, the production of total feed or of each feed category for the individual mills was unknown. We also did not know the production of each feed category by state. This meant that feed category production by state or by feed mill could not be used for weighting in the estimation process and that only total finished feed production by state could be used for weighting.

Table 1 shows estimates of percent positive and the standard deviation. Both the theoretical binomial and the actual cluster variance were computed in order to obtain the increase in the variance due to cluster sampling. There were 297 premises sampled in the trichinosis survey out of several thousand premises in the United States. There were 724 feed mills from 26 states out of several thousand total basic feed mills. The average number of samples per mill varied from 2.10 for animal meal to 4.46 for cattle feed. Not all mills had all categories of feed.

It will be noted that the estimate of percent positive for trichinae was 0.50 percent with a standard deviation of 0.13 percent. This was with 297 herds and 5,955 individual samples. This represents a much lower percent positive than many of those that will be estimated in the NADS for animal diseases. More in line with what might be expected in estimating many of the diseases or disease conditions of interest in the NADS is the result for fish meal where the percent positive is 4.72 with a standard deviation of 0.92 percent.

<table>
<thead>
<tr>
<th>Type of Survey</th>
<th>Number of Clusters</th>
<th>Number of Samples</th>
<th>Percent Positive</th>
<th>Standard Deviation of Binomial</th>
<th>Standard Deviation of Cluster</th>
<th>Ratio of Standard Deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichinosis</td>
<td>297</td>
<td>5,955</td>
<td>0.50</td>
<td>0.091</td>
<td>0.13</td>
<td>1.42</td>
</tr>
<tr>
<td>Grain</td>
<td>635</td>
<td>2,698</td>
<td>0.66</td>
<td>0.156</td>
<td>0.19</td>
<td>1.22</td>
</tr>
<tr>
<td>Oil Seed</td>
<td>621</td>
<td>2,629</td>
<td>2.28</td>
<td>0.291</td>
<td>0.32</td>
<td>1.10</td>
</tr>
<tr>
<td>Cattle Feed</td>
<td>582</td>
<td>2,597</td>
<td>0.85</td>
<td>0.180</td>
<td>0.22</td>
<td>1.22</td>
</tr>
<tr>
<td>Swine Feed</td>
<td>502</td>
<td>1,567</td>
<td>3.13</td>
<td>0.440</td>
<td>0.58</td>
<td>1.32</td>
</tr>
<tr>
<td>Poultry Feed</td>
<td>560</td>
<td>1,605</td>
<td>5.23</td>
<td>0.556</td>
<td>0.73</td>
<td>1.31</td>
</tr>
<tr>
<td>Fish Meal</td>
<td>366</td>
<td>805</td>
<td>4.72</td>
<td>0.747</td>
<td>0.92</td>
<td>1.23</td>
</tr>
<tr>
<td>Animal Meal</td>
<td>414</td>
<td>869</td>
<td>31.07</td>
<td>1.570</td>
<td>2.18</td>
<td>1.39</td>
</tr>
</tbody>
</table>
A consideration of these confidence limits brings up an important aspect of the animal disease estimation problem. This is the precision in terms of the width of confidence limits which is required. For example, the January and July 1983 Cattle reports point out that the standard error of the national estimate for total cattle and calves is less than one percent for the January report and about one and one-half percent for the July report.

Experience has shown that much wider confidence limits as a percent of the estimate of the total affected for any given disease or disease condition are acceptable for the national estimate. In addition, the cost of obtaining an individual herd is such that it is not cost-beneficial to obtain confidence limits anywhere as narrow as those necessary for inventory estimation.

In further examining what might be expected in the case of NADS sampling, we can consider disease incidence results from the Doane Agricultural Service Animal Health Market study. It must be emphasized that the Doane data lacks estimates on monetary loss due to morbidity, has an unknown degree of diagnostic validity, and has little information on specific disease agents. This is why their data is not adequate for any of the potential users that desire this information including the U.S. Department of Agriculture.

Certain results from the Doane Agriculture Service for 1979 are shown in Tables 2 and 3. Results are shown for certain classes of livestock and certain disease classifications. The denominator in Table 2 represents the January 1, 1980 inventory plus animals sold and purchased during 1979. This is the denominator that Doane uses in computing death loss rates.

Since I do not agree with this construction of the denominator, the denominator in Table 3 represents only the January 1, 1980 inventory and this denominator is used for the purpose of illustration. In contrast, the Minnesota studies constructed the denominator from animal months at risk. This permitted the construction of an annual rate based upon the average number of animals at risk per month over a 12 month period.

Table 2. Results from Doane Agricultural Service Disease Incidence Package.

<table>
<thead>
<tr>
<th>Livestock Class</th>
<th>Inventory Plus Sales &amp; Purchases</th>
<th>Disease Classification</th>
<th>Number Affected</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Cattle</td>
<td>22,879,671 Total Death Loss</td>
<td>1,514,647</td>
<td>6.62</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; Disease Death Loss</td>
<td>1,232,624</td>
<td>5.39</td>
<td></td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>12,955,127 Total Death Loss</td>
<td>278,478</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; Disease Death Loss</td>
<td>131,543</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>Beef Cattle</td>
<td>139,618,690 Total Death Loss</td>
<td>3,806,957</td>
<td>2.73</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; Disease Death Loss</td>
<td>2,555,230</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>233,046,866 Total Death Loss</td>
<td>15,208,938</td>
<td>6.53</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; Disease Death Loss</td>
<td>10,558,379</td>
<td>4.53</td>
<td></td>
</tr>
</tbody>
</table>
It can be seen from Table 3 that 47,036 (number of clinical cases) beef cattle were estimated to have anaplasmosis. It is this estimate in beef cattle that casts doubt on the Doane incidence estimates as far as diagnosis is concerned. In a probability sample survey for anaplasmosis using brucellosis blood samples in 1973, we estimated that there were 4,890,500 (number of serological positives) beef cows affected for the United States.

Table 3. Results from Doane Agricultural Service Disease Incidence Package.

<table>
<thead>
<tr>
<th>Livestock Class</th>
<th>Inventory</th>
<th>Disease Classification</th>
<th>Number Affected</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Cattle</td>
<td>18,138,504</td>
<td>Total Death Loss</td>
<td>1,514,647</td>
<td>8.35</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Disease Death Loss</td>
<td>1,232,624</td>
<td>6.80</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Intestinal Problems</td>
<td>1,595,955</td>
<td>8.80</td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>10,392,514</td>
<td>Total Death Loss</td>
<td>278,478</td>
<td>2.68</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Disease Death Loss</td>
<td>131,543</td>
<td>1.27</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Mastitis</td>
<td>1,865,872</td>
<td>17.95</td>
</tr>
<tr>
<td>Beef Cattle</td>
<td>75,538,626</td>
<td>Total Death Loss</td>
<td>3,806,957</td>
<td>5.04</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Disease Death Loss</td>
<td>2,555,230</td>
<td>3.38</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Anaplasmosis</td>
<td>47,036</td>
<td>0.06</td>
</tr>
<tr>
<td>Swine</td>
<td>65,149,954</td>
<td>Total Death Loss</td>
<td>15,208,938</td>
<td>23.34</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Disease Death Loss</td>
<td>10,558,379</td>
<td>16.21</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Virus Pig Pneumonia</td>
<td>4,378,246</td>
<td>6.72</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Swine Influenza</td>
<td>8,132,723</td>
<td>12.48</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>TGE</td>
<td>4,834,059</td>
<td>7.42</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td>Intestinal Problems</td>
<td>23,257,424</td>
<td>35.70</td>
</tr>
</tbody>
</table>

Of course, it is obvious that the amount and nature of variation from cluster to cluster is important in considering the sample size in terms of number of herds which will be needed. Conditions such as mastitis and calf scours will occur in most herds and will have different variance characteristics and will require a smaller size in terms of number of herds than will diseases that are spread by the movement of animals.

In considering this problem, we can consider results from the Minnesota validation study. Table 4 shows selected data from the validation report of Diesch, et. al. in 1981.

It can be seen from an examination of Table 4 that some disease conditions and diseases have narrower confidence limits in terms of a percent of the point estimate than do other disease conditions and diseases. For example, miscellaneous mastitis in dairy cows has much narrower confidence limits than does TGE in either breeding swine or farrow-to-finish pigs.

In comparing results from Doane in Table 3 with results from Minnesota in Table 4, two diseases are of interest. They are mastitis in dairy cows and TGE in swine.
Table 4. Selected Results from the Minnesota Validation Study.

<table>
<thead>
<tr>
<th>Livestock Class</th>
<th>Disease Class</th>
<th>Type of Sample Validation Rate</th>
<th>Std. E.</th>
<th>Type of Sample Practitioner Rate</th>
<th>Std. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Cows</td>
<td>Lice</td>
<td>9.0%</td>
<td>5.4%</td>
<td>42.6%</td>
<td>12.5%</td>
</tr>
<tr>
<td></td>
<td>Abcesses</td>
<td>2.2</td>
<td>1.5</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Misc. Mastitis</td>
<td>18.0</td>
<td>3.7</td>
<td>39.3</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Total Mastitis</td>
<td>29.1</td>
<td></td>
<td>44.9</td>
<td></td>
</tr>
<tr>
<td>Dairy Heifers</td>
<td>Lice</td>
<td>16.3</td>
<td>9.1</td>
<td>13.7</td>
<td>6.1</td>
</tr>
<tr>
<td>Dairy Calves</td>
<td></td>
<td>1.1</td>
<td>0.8</td>
<td>9.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Breeding Swine</td>
<td>Influenza</td>
<td>0.5</td>
<td>0.6</td>
<td>13.7</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>TGE</td>
<td>32.1</td>
<td>21.1</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Total Enteritis</td>
<td>32.2</td>
<td></td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Pigs to Finish</td>
<td>Influenza</td>
<td>13.3</td>
<td>7.4</td>
<td>45.8</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>E. Coli</td>
<td>18.9</td>
<td>6.5</td>
<td>65.5</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>TGE</td>
<td>5.8</td>
<td>4.7</td>
<td>19.6</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>Dysentery</td>
<td>0.9</td>
<td>0.7</td>
<td>21.6</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>Misc. Enteritis</td>
<td>18.8</td>
<td>6.2</td>
<td>24.5</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>Total Enteritis</td>
<td>44.4</td>
<td></td>
<td>132.6</td>
<td></td>
</tr>
</tbody>
</table>

It should be pointed out that in the case of an endemic disease, average estimates over two to four years is satisfactory. This means that a smaller number of herds will suffice in providing adequate estimates than is the case when we desire rather accurate estimates of trend.

REFERENCES


CONCERNS OF THE INDUSTRY ABOUT NADDS AND THE APHIS RESPONSE

Remarks by Dr. L. J. King, USDA, APHIS, Veterinary Services, at the U.S. Animal Health Association meeting, Ft. Worth, Texas, October 25, 1984.

Any project of the Animal and Plant Health Inspection Service (APHIS) must be in the public interest, and none can truly succeed without public support. To gain and maintain that support, we seek and welcome opportunities to give an accounting. It has been stated that “To escape criticism and scrutiny—do nothing, say nothing, and be nothing.” (Elbert Hubbard) We neither intend or wish to escape the constructive criticisms and concerns of industry, nor do we intend to do nothing regarding animal health in this country.

During the past year, we have discussed the National Animal Disease Detection System (NADDS) with many groups and organizations, as well as with farmers and ranchers. We are delighted with the initial support, but we are also aware of the concerns of these people about this new activity. Today, I wish to address some of the principal concerns and issues that have been reiterated during the first year of the pilot project.

1. **Will NADDS be an activity which is conducive to “on-the-job” retirement, and will it create new regulatory programs that we don’t want?**

I can understand that a new Animal and Plant Health Inspection Service (APHIS) project might, at first, be viewed suspiciously. I believe this concern really begs the question—what will happen after brucellosis?

I am reminded of the time in the mid-19th century when colleges of veterinary medicine were first established because of the growing need to care for farm and draft animals. As the years passed, significant changes occurred, and the horse was replaced as a major source of power and transportation by the internal combustion engine. Many thought that the status of traditional veterinary medicine was threatened and surely there would not be enough work to do. The same attitude prevailed when hog cholera was brought under control and reduced to a minimal level and eventually eradicated. Yet the doomsday never arrived, and people with broad vision took advantage of those times to expand the role of veterinary and other professional services to new horizons. In a similar manner, reduction of brucellosis and the resources needed for the program presents us with new opportunities, such as NADDS, and should not be viewed as an endpoint with regard to animal health.

The future must influence the present just as much as the past. Moreover, you must learn from the past, but you cannot plan the future by the past. Modern management methods for food-producing animals necessitate that veterinary services now focus on herd health and preventive medicine. New diseases and conditions will challenge the livestock and
poultry industries as well as their ability to continue to produce safe, high-quality products. A new, complex environment has surfaced for producers of livestock and poultry. Because of these unprecedented conditions, the traditional cooperative programs of the past, as successful as they were, may no longer be applicable in this new environment. Improvement of animal health will continue to be a challenge for us all, and we will respond with innovative ideas and hard work, which is the antithesis of an “on-the-job” retirement.

NADDS represents a new role for APHIS. It cannot be judged by past regulatory experiences, because there are no laws or regulations that mandate its development. It is a volunteer activity that forces no one to participate. We rely on the abilities of the Veterinary Medical Officers (VMO's) in the field to convince farmers and ranchers of the importance of NADDS and solicit their participation.

NADDS places the VMO's in the mainstream of veterinary medicine. This entails continuous training, both didactic and self-learned, on a variety of veterinary subjects including medicine, diagnosis, survey management, economics, and interviewing skills. A familiarity of basic biostatistics is also encouraged. Because NADDS requires our VMO's to acquire and maintain competency in veterinary preventive medicine, there is neither room, nor time, for “on-the-job retirement.” This activity is certainly one of the most demanding projects, in terms of professional knowledge and ability, with which APHIS' veterinarians have been involved. By broadening the experiences and training of VMO's, especially in farm management and economics, NADDS creates more knowledgeable and more qualified personnel to interact with livestock owners and officials. This is deemed helpful in all existing program areas as well. NADDS also represents an activity to stimulate the interest of new, younger professionals, who may be considering Federal service as a career and who have excellent training in herd health management.

NADDS also creates an infrastructure of trained VMO's, which constantly allows us to have a finger on the pulse of animal health problems across the country. Because of this network, continuous feedback from the field will enable the industry, States, and APHIS to monitor the spread of an exotic disease after an incursion into this country. NADDS is not set up to detect the first case of a foreign animal disease (FAD), but because of trained personnel and diagnostic laboratory support, which is incorporated in NADDS for validation, a surveillance mode can be created to measure the extent of spread of such a disease and then serve as an alert system for its control.

Much of the livestock industry perceives itself to be over-regulated with burdensome rules. Thus, a typical reaction is that NADDS will collect data that could be used for new, unnecessary regulatory programs. However, I suggest to you that this same data may also be used to help prevent ill-advised regulatory programs in the future. NADDS will provide valid and reliable information upon which future activities will be judged. The
proper mixture of State-industry-Federal cooperation and input should then determine any future program direction or necessity. But let’s first get the essential data and proper documentation to form a basis for decision-making. Effective herd health and animal health plans can only be designed if we know how much disease is present and what the economic consequences of these problems are. It takes an active, well-trained, and motivated VMO to participate in NADDS and help answer these questions. VMO’s will also be challenged to keep up with the new knowledge and information in medicine and diagnosis which is increasing in an exponential fashion—an environment that is hardly conducive to early retirement.

Livestock and poultry producers are aware of diseases which limit marketability, but are these truly the disease problems that are reducing their efficiency of production? What about less apparent, chronic problems, that in the aggregate are insidiously eating away at profit opportunities? Who has information on these diseases? What are they costing? As good as private practitioners and extension veterinarians are in their jobs, a great information void exists. Are we focusing on diseases and conditions that truly are the most economically significant to the industry? Perhaps we should be focusing on neonatal diarrhea problems? Or perhaps subclinical immunosuppressive disorders that result in a host’s predilections for other pathogens? What about noninfectious determinants such as environmental factors and husbandry practices? No one honestly knows until a NADDS is implemented and analyzed.

So, just as the “crisis” of the automobile replacing horses and veterinarians has passed, the perceived crisis of “after brucellosis” will also pass. Veterinary Services, with much support and input, will embark on new horizons like NADDS for exchanging information with the States, universities, and industry. This has the potential to revolutionize the future direction of animal health.

2. How is the individual herd or flock owner going to gain from a NADDS?

In the past, we have directed surveillance and disease detection activities toward specific diseases and, in order to use our resources optimally, have designed detection techniques to maximize the probability of finding these diseases (e.g., brucellosis, tuberculosis, scabies). We now need a broader information basis upon which individual farmers and ranchers might base decisions that can lead to more efficient production and utilize less resources.

It is only appropriate to analyze the situation from the perspective of owners and managers. Let’s look at a hypothetical herd or flock through a window of time. This animal population is seen through the eyes of one or a group of people who visualize multiple events that occur during this timeframe; i.e., births, deaths, illness, accidents, growth, production variations, etc. Depending on the degree of contact of the observer(s) with the group of animals, only a percentage of all events will be recognized or
noticed; many will not. Based on these events and how they are perceived, the owner chooses from three main alternatives for managing his animals: 1) He may continue his current management style, where losses, much of which go unnoticed, are absorbed as part of the operation. Suboptimal production is never improved, and losses are continuously accrued. 2) He may elect to intervene on his own volition. Thus, modifying husbandry conditions, treating, culling, replacing, etc., are all weighed against the first alternative of doing nothing. Sick calls utilizing veterinarians are viewed as a necessary evil and go down in his loss column. 3) Finally, he may seek professional assistance in terms of consultation with a private practitioner or other professional assistance. At this level, diagnostic support is often utilized, and preventive medicine is considered, as opposed to focusing on individual morbidity problems. In the United States, we can find production units represented in all three of these categories in varying degrees and percentages.

How many owners and managers opt for the third alternative? Early experiences in our pilot States suggest that this group represents a small minority. Most owners choose the first or second alternative. Given that these circumstances exist, what type and how accurate has disease surveillance and detection been prior to NADDS?

First of all, regarding the first alternative, many events go unnoticed, and problems that are recognized are never defined by laboratories or professional services. Disease problems and costs are never enumerated. We believe that, in some States, perhaps half the industry may fall into this category. With regard to the second alternative (the segment of the industry which uses professional services only as a necessary evil and focuses on individual cases), a selection bias is produced which overstates incidence rates. Both situations lead to serious sampling biases, and there is no statistical validity. Past information systems (morbidity and mortality reports) have been discontinued because inaccurate and invalid inferences were made concerning disease occurrences in the two groups represented by alternatives one and two.

Some surveillance does take place at slaughter, and we acknowledge and commend the data base the Food Safety and Inspection Service (FSIS) has established. However, the population at risk is not known; there is an over-representation of certain age groups at slaughter, which does not allow for surveillance of newborn and young animals, and unless a disease produces overt post-mortem lesions, it will never be detected on the slaughter floor.

What about disease detection through State and Federal programs? The incidence of program diseases has been dramatically reduced over the years. Presently, only 3.7 herds out of every 1,000 have active brucellosis, and this is heavily regional in occurrence. Other such programs have similar limitations in terms of surveillance of the other 996 herds.

Owners and managers that opt for the third alternative fall under surveillance through diagnostic laboratories. Data from this source is
extremely valuable in detecting emerging and cyclical disease situations and is vital in FAD surveillance. The limitation is that only a small percentage of owners use these services and, because animals at risk are not known, incidence and prevalence rates cannot be evaluated, nor is there any information on costs of diseases.

Thus, a tremendous void exists, and a disturbingly large segment of the livestock industry is never involved in any disease detection or surveillance activities. The individual producer has too little information on economically significant diseases and even less on the cost-effectiveness of initiating or changing management strategies to increase efficiency of production. NADDS can fill this information void by quantifying disease problems and their economic impact and observing the effectiveness of control strategies through longitudinal studies and analyses.

Perspectives on our traditional surveillance system suggest that many errors and misinterpretations of data have resulted. Past discussions at the U.S. Animal Health Association have addressed these systems and their limitations. The NADDS strives for different objectives and utilizes stratified random sampling techniques so that inference can be made to large populations, and measurements of incidence, prevalence, trends, and the economic impact of livestock diseases will result.

The concept of NADDS is not final; concepts are formed and reformed over time. As we view NADDS over the past year, we believe its utility will lie in transferring its knowledge to studies in disease and animal health, which will have application to individual producers. We seek to achieve a scientific basis for disease control measures. What determinants are associated with the frequency of diseases and conditions? What are the characteristics of herds and flocks which determine their relative risk to disease? And how do they differ with respect to disease frequency? Effective planning and services for herd and flock owners are only plausible when valid data exists. It is a provocative idea that the health status of animal populations reflects their total life experiences, exposures, and interrelationships. A better understanding of these interactions is necessary so owners of livestock and poultry can choose proper alternatives in herd and flock health management.

3. Is the NADDS going to be cost-effective with a favorable cost-benefit ratio?

Everyone agrees that NADDS must be cost-effective. Today's budgetary realities necessitate the best use of public funds. We believe that the cost-effectiveness of NADDS is one of its selling points.

Disease control or preventive policies must have an economic basis to evaluate their rationale. An economic analysis that fails to convert most effects into dollars will be limited in its use as a decision-making aid. However, there are incommensurable, or unmeasurable, factors for which dollar conversions may not be feasible. Thus, the scope and breath of economic analysis may be limited to a strictly quantitative dimension. This is one of the difficulties confronting us in NADDS.
How can one accurately reflect in monetary terms: 1) the satisfaction of having a healthy herd; 2) environmental damage of chemicals and pesticides in treatments of external parasites; 3) the development of new biological and pharmaceutical products which are more timely and relevant to producers' needs; 4) an increased awareness of diseases and problems, which results in an earlier detection of diseases and in their earlier control; 5) the application and direction of research to more economically significant problems; 6) the creation of a stronger export market; 7) the positive attitude of consumers toward products when the industry actively supports animal health and strives to improve its products; 8) the reduction of human health risks; and 9) the reduction in animal suffering. These are benefits from a NADDS but are very difficult to accurately convert into dollars saved, even though, profoundly, this is clearly the case.

Much of NADDS will look at relevant costs, direct benefits, and measurements of the value of outputs, gains, and losses. There are techniques available to quantify the effects of animal diseases and conditions on productivity and to represent these effects in economic terms. The costs and benefits of management and interventional factors can be estimated and compared, and will provide the basis for decisions on their justification. This information then has applicability to producers. Indirect benefits, which reflect the impact of the project on the rest of the economy and society, are acknowledged as potential major benefits, but these benefits are not included in the purview of NADDS at this time.

If our estimate is accurate, livestock production is reduced each year by 15 to 20 percent due to disease. For a $90 billion industry, a small fraction of 1-percent improvement would more than pay for the cost of a national animal disease detection activity. So even without calculating sizable indirect benefits, we believe NADDS will still have a favorable return on investment. I would also like to disclose that the NADDS' budget for the pilot project in FY 1985 is $350,000, which represents a little over .001 percent of the APHIS budget. In the private sector, research and development in companies of comparable size often takes 10 to 20 percent of their budget allocations. NADDS really is a methods development project at this time. We are also working to estimate the number of herds and flocks that would be involved in a future national system and the approximate costs, should the pilot system be favorably viewed and expanded. By using proper survey techniques and randomly selected and stratified populations, only a relatively small number of U.S. herds and flocks will need to be included in a national system; yet inferences can accurately be made for larger animal populations.

I do not believe that we can ignore the necessity to undertake new activities in anticipation of future problems such as the basic processes and problems of disease and animal health. Past activities in research and animal health have led to a new, science-based animal industry. Economic pressures and opportunities have encouraged livestock and poultry owners to adopt new technologies derived from fundamental studies. At the same time, the industries serving animal health have also capitalized
on primary discoveries to develop new products and enhance productivity. This has always been in the American tradition. NADDS represents a further step in the evolution of animal health activities, albeit a step in a different direction, allowing the private sector, as well as the public sector, to take advantage of new knowledge and solve problems while increasing profit opportunities.
SERENDIPITY FINDINGS: ATTITUDES WE FIND BY WORKING CLOSELY WITH GROWERS AND PRODUCERS ON A NON-REGULATORY BASIS

Gerald L. Shook, D.V.M., Coordinator for Ohio Veterinary Services, APHIS, USDA

As you know, serendipity is a fact or happening that occurs when you are not anticipating that happening or something that happened by chance. Indeed, the first chance happening was when we were to locate our farm cooperators in Ohio, just one year ago. This occurred when State and Federal Veterinary Medical Officers (VMO’s) were securing the beef, dairy, and swine producer listings compiled by the County Agents. Some listings given to our VMO’s were incomplete or out of date and some agents did not know who all the producers were. With our help, phone calls were made, other sources utilized, the lists were finalized, and we were on our way. Next, we VMO’s had visualized finding some of these producers near our official stations, that we might know them, and all in all we had a perfect picture in mind of who our producer cooperators might be. After locating some of these producers in the far reaching corners of Ohio, or at the extreme edge of our sections, this was a second happening.

Ohio has 14 State and Federal Veterinary Medical Officers and 1 Ohio State University Veterinarian who are interviewing 60 producers. They have had very few problems in signing up a producer to be interviewed each month. It surprised our VMO’s that the farmers agreed to do it so readily, and that they were not significantly interested in the $25 being paid to each cooperator for allowing us to gather data once a month. Some of them remarked that they would have cooperated regardless of being paid. Initially most of our veterinarians found that cooperation and good attitude were universal.

I am not aware of any producer who told us we could not discuss any of the data gathering with their veterinary practitioners. However, there were producers who probably would not have been cooperative if the information we gathered was to be available to everyone by revealing the identity of the person involved. Confidentiality is definitely a factor in this project, and in our opinion should be continued to insure valid data gathering.

We soon evolved from calling this National Animal Disease Surveillance work to “NADS.” It was a unique, new program for our VMO’s and our office, and a new type of involvement rather enjoyed by all.

Last January, we had a meeting with Hyattsville staff members and Ohio VMO’s to see how everything was progressing. At that time we found producers were using veterinary practitioners only about 1/4 to 1/3 of the time. This was definitely an unexpected finding. Everyone agreed farmers were cooperating well. There was some poor data gathering primarily due to laxity or poor organization on the producers’ part. The attitude of all involved appeared to be optimistic.
This October I surveyed all our VMO's in Ohio for additional data. In grading the producer's cooperation from excellent to poor, nearly all were rated excellent; only one producer was rated poor. This is the most striking finding that some of our people found. One VMO found it hard to believe that we, as regulatory people, with government vehicles and our regulatory duties would be that well received once a month. Indeed, some of us have been so well received, we are genuinely surprised. So there is no question as to the workability of the project between the regulatory veterinarians and the producers. As a matter of fact, when our people were asked to rate any type of effect because of our being regulatory veterinarians, good or bad, the majority indicated no effect whatsoever.

When the acceptability of the farmer's records was polled, we found that only 1/4 of the producers did poorly, with two VMO's stating they had individuals who were very badly disorganized. On the whole, this speaks well for producer's records. We have always had some problems with securing the drug and labor charges of Veterinary practitioners on each farm; either the farmer doesn't get a bill, the bill is not always legible, or understandably the time lag because of practitioners getting the bills out prior to our visit. It was also noted that because of our discussions with the producer on our visits, we have achieved more uniformity in the data gathering. This could probably be further improved by some revisions in the reporting system.

Our VMO's found the producer's knowledge of disease problems good, with the few ratings of excellent equal to those rated poor in knowledge. The survey also indicated that by our data gathering system we stimulate the owner for more knowledge. Two of our VMO's stated that it is difficult to remain an information taker or data gatherer, and not to become a consultant or become involved in management decisions on the farm. We realize the obvious ethical pitfall as well as the hazard of bias in the data.

Again, I asked the VMO's how much the producers were using a veterinary practitioner. We found that after one year, 40% of the producers use a veterinary practitioner regularly; about 15% never use one; and the rest of them utilize one irregularly or seldom. This is somewhat higher than originally believed at our January meeting. We asked if the use of a veterinary practitioner correlated with the producer's knowledge of diseases and there seemed to be no relationship in this regard. We felt this would be a direct correlation. Dairy producers tend to use veterinary practitioners more than any other and swine producers use them the least; beef runs about 50/50.

Another question was whether drug sources used in the treatment and medication by the producers were from veterinary practitioners or from other sources such as feed mills, farm stores, mobile drug dispensaries, or catalog purchases. Apparently, in only 20% of our data gathering do we see the source as from a veterinarian alone, and over 50% of the medications given are apparently not supervised by a veterinarian. This surprised many of our VMO's. It surely tells us of our need to study this phenomena for possible solutions.
There are several unusual highlights that we think have occurred. One is the number of producers that actually seemed quite proud that they were selected for this program, even though they knew they were chosen by random. They are very interested in the results we have obtained and would like a report on all the data if possible. One VMO encountered a situation whereupon the producer’s veterinary practitioner actually told his clients not to cooperate with us. He apparently was worried they would get too much information about the use of drugs for medication and extra knowledge which would by-pass him. This did not occur and all of those producers are very cooperative with us. Our VMO’s have also found out the magnitude of some of our producer’s disease problems and have become aware of the overall economic problems. We have a better appreciation for his earning a livelihood. Producers have also become more aware of various cost factors in their production by accounting of labor previously overlooked, actual medication expenditures, milk losses and animal losses. One individual who was hesitant in signing the agreement, but who did enter the program, definitely had problems in his swine herd. Over a period of time it was obvious that the number of progeny produced by his sows was very poor, and losses from sickness and deaths were high. It was difficult to gather information from this individual; one had to ask the same question several ways in order to get the information needed. It became evident the farmer was embarrassed about his poor management and herd disease problems, because he also had a business of selling all types of swine handling equipment. Finally, he decided to start selling off many of his stock, and ultimately he went completely out of business. At the last monthly visit this individual apologized for his poor cooperation and felt he probably should not have entered into the agreement originally. He was sorry that our data gathering was not productive. We, too, are sorry that this occurred; because even with encouragement and empathy for his problems, this individual never used a veterinary practitioner nor would he take an animal to the State Diagnostic Laboratory. He at least faced his problem and we probably helped him resolve it.

In summation, our regulatory veterinarians have been impressed with the cooperativeness, the good attitude, and the overall receptiveness of our producers. It is better than expected, and they feel that they have definitely contributed to a new technique in disease data gathering. One veterinarian questioned the overall process. Our veterinarians enjoy meeting these producers and discussing their problems and being able to know them in a different light. Finally, they got to be the good guy going out on the farm with the white hat instead of the black hat.

G. L. Shook V.M.O.
October 25, 1984
EVALUATION OF LIST FRAMES FOR SAMPLING
CALIFORNIA SHEEP POPULATIONS-
NATIONAL ANIMAL DISEASE SURVEILLANCE PROGRAM

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SUMMARY
In a pilot study for the National Animal Disease Surveillance program,
ten list frames of Solano County sheep producers were evaluated as
sources for disease surveillance sampling. The Statistical Reporting Ser-
vice (SRS) list frame differed from the other list frames in a number of
ways. Producers on the SRS list frame accounted for 97% of the sheep and
55% of the operations, were more likely to sell animals to an abattoir, and
were less likely to keep many types of records than non-SRS producers.
The SRS list frame captured large commercial producers, but did not
contain many of the small producers with 1–9 sheep. Many producers kept
few records and had not used the services of a veterinarian during the
previous year.

INTRODUCTION
If a National Animal Disease Surveillance program (NADS) is to be
successful, effective sampling methodology for securing information on
disease prevalence, incidence, trends and economic costs must be de-
veloped (1). Under the NADS program, California was the first state to
develop a pilot program for the sampling of sheep.

One method of sampling is list frame sampling, whereby flocks may be
randomly selected from a list of the total number of sheep flocks. This
 technique provides the opportunity for telephone and mail surveys. Be-
cause creation and maintenance of list frames is time consuming and
expensive, it is logical to attempt to use existing list frames for sampling.
The Statistical Reporting Service (SRS), US Department of Agriculture,
has developed sophisticated systems to construct and maintain list frames
(3) and maintains a list frame of California sheep producers. Since this and
other list frames have not been created for the specific purpose of disease
surveillance, however, they must be evaluated in that context.

In the present study one area of California — Solano County — was
selected, and a master list frame of sheep producers was compiled by
obtaining list frames from several sources. The sheep industry of the
county and the list frames were characterized by means of a telephone
survey. The SRS list frame was compared with a composite non-SRS list
frame for suitability for livestock disease surveillance.
MATERIALS AND METHODS

Solano County was chosen for the pilot study because of its large sheep population and its proximity to the University of California, Davis. The county is located between Sacramento and San Francisco in north central California, has an area of 22000 square miles and had an estimated sheep (breeding stock and lambs) population of 67000 on January 1, 1984 (2).

A master list of potential sheep operations in Solano County was compiled from the following lists of sheep producers: Statistical Reporting Service (78 names); Wool Incentive Payment Program claimants (52 names); California Wool Growers Association membership list (33 names); Solano County Wool Growers Association membership list (78 names); 4-H sheep project registrants (86 names); California Farm Bureau (63 names); Natural Colored Wool Growers Association membership list (2 names); an abattoir list of Solano County suppliers (10 names); Solano Wool Growers Association Auxiliary (18 names); and Solano County Farm Adviser mailing list (43 names). After duplication from list overlap was eliminated, the master list frame contained 272 names.

A letter was sent to each name on the master list explaining the project, requesting cooperation, advising that contact by telephone be made, and listing the questions to be asked. The telephone questionnaire consisted of 27 questions, including characterization of operation and management, production rates, types of records kept, source of animal health information, use of veterinary and laboratory services, health problems encountered in the flock, and sheep marketing outlet. The period covered by the questionnaire was from August 1983 to August 1984. Data were collected only from people who were current sheep owners and had flocks that were maintained on pasture or feedlot in Solano County during at least part of the year. Data were then tabulated in a computer file and analyzed using the MicrostatIII(c) and SuperCalc3(c) computer programs.

RESULTS

Table 1 shows the proportion of identified sheep owners according to the list(s) on which they appeared. Because of overlap among lists, a sheep operation can appear at more than one place in this table. Of the 272 producers on the master list frame, 16 could not be contacted and 87 of the remainder were identified as current sheep owners. Among the 87 identified sheep owners, 64 (74%) responded fully to the questionnaire, 14 (16%) declined to participate, and incomplete information was obtained from 9. However, flock size data were obtained from all 14 who declined and from 4 of the 9 from whom incomplete information was obtained. Flock size information was therefore obtained from 82 of the 87 identified producers. Table 1 also gives the proportion of sheep owners that answered the questionnaire, by list frame. Response rate ranged from 67% to 83%.

The total population of breeding ewes and lambs was 53430 for the 82 operations for which size was known. The 64 completed questionnaires accounted for 49536 lambs and breeding ewes, which represented 93% of
the sheep population of the 82 operations for which size was known. Sixty of the 64 operations were classified as breeding operations, and 4 as only feeder operations. In addition, 5 of the breeding operations also were feeder operations. Only the results for the breeding operations are presented in this paper.

Overlap between SRS list and the other list frames ranged from 71% to 96%, with the exception of 32% for the 4-H list (Table 2). The SRS list contained 54/87 (62%) of the sheep operations. Of the 60 breeding operations, 33 (55%) appeared in SRS, accounting for 97% of the animals. Eight of the 9 feeder operations were included in the SRS list.

When breeding operations were stratified by size (1–9 animals, 10–50, 51–100, 101–300, 301–1000, and \( \geq 1001 \) animals), it was observed that the highest proportion of operations (37%) was in the 10–50 animals category, followed in frequency by the 1–9 animals category. This latter category was composed primarily of non-SRS producers. Few non-SRS producers appeared in the 101–300 category and none in the two highest size categories. All flocks larger than 301 sheep appeared in the SRS list (Figure 1). Median flock size was 207 breeding ewes for operations on the SRS list frame and 10 breeding ewes for operations not on the SRS list frame. For all flocks, the number of breeding ewes ranged from 2 to 6000.

Lamb crop rate (lambing rate) \[ \frac{\text{(number lambs born alive)}}{\text{(number of lambs born alive) - (number of lambs weaned)}} \] lamb death rate \[ \frac{\text{(number of lambs born alive) - (number of lambs weaned)}}{\text{(number of ewes that lambed) + (number of dry ewes)}} \] and ewe cull rate \[ \frac{\text{(number of ewes culled)}}{\text{(number of ewes that lambed)}} \] are shown in Figure 2. Median lambing rate was 1.25 for all operations combined, 1.18 for operations on the SRS list and 1.34 for operations not on the SRS list. Lamb death rates and ewe cull rates of 0.1 were reported.

Producers were asked if they kept one or more of the following types of records during the previous year: number of lambs born, number of lambs weaned, number of lambs tail docked, and data on individual ewe reproduction performance. Fifty percent of the SRS and 30% of the non-SRS producers did not keep any type of records. Number of lambs docked was the most frequently kept record, being recorded by 50% of the operations, and the individual ewe record was the least frequently kept (9% of producers on the SRS list and 41% non-SRS producers kept this record — Figure 3).

Questions were asked about health services, health management and health problems encountered on the ranch. A total of 53% of producers used the services of a veterinarian on the farm during the last year, 69% used vaccine(s), and 26% knew the cost of the purchased vaccine(s) (Figure 4). Among disease conditions reported, 67% of respondents reported snotty noses in their sheep, 48% reported pneumonia, 7% reported coccidiosis, 35% reported diarrhea in their animals, and 58% reported foot rot (Figure 4). The percentage of people that marketed their lambs and/or sheep to an abattoir was 43% — 63% of producers on the SRS list did so, compared with only 18% of producers not on the SRS list.
DISCUSSION

Selection of a list frame for animal disease surveillance depends on the target population to be sampled. The SRS list frame differed from the non-SRS list frame in a number of ways. Producers on the SRS list frame accounted for 97% of the sheep and 55% of the breeding operations, were more likely to sell animals to an abattoir, and were less likely to keep records than non-SRS producers. The SRS list frame captured the large commercial producers, but did not contain many of the small, presumably hobby producers. Some sheep operations may not have been included on any of the lists we obtained. Future studies will include in-person surveys to evaluate this possibility.

It was interesting to note how few producers kept records or used the services of a veterinarian. These facts must also be taken into account in the design of a surveillance program for sheep that requires recordkeeping by the producer.

We cannot say if the results obtained in Solano County apply to the rest of California, but some of the previously mentioned characteristics of the SRS list frame make it attractive for use in sampling sheep operations in California.

ACKNOWLEDGMENTS

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We gratefully acknowledge the assistance of M. Howard, California Wool Growers Association; R. Bosecker and G. Tucker, Statistical Reporting Service, Sacramento; and D. Wilson, M. Smargiassi and L. Lasslo, University of California, Davis.

REFERENCES

TABLE 1--Characterization of 7 list frames for Solano County, California, sheep operations by number of operations on each list frame, operations with sheep at time of interview, and operations responding to interview.
National Animal Disease Surveillance program, California, 1984.

<table>
<thead>
<tr>
<th>List framea</th>
<th>No. operations on list frame (1)</th>
<th>No. operations with sheep with sheep responding</th>
<th>% operations</th>
<th>No. responding</th>
<th>% responding</th>
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<td>69</td>
<td>36</td>
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<tr>
<td>CWGA</td>
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<td>70</td>
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<td>83</td>
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<tr>
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<td>27</td>
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<tr>
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<td>27</td>
<td>35</td>
<td>20</td>
<td>74</td>
</tr>
</tbody>
</table>

List frames: SRS = Statistical Reporting Service, CWGA = California Wool Growers Association, 4-H = 4-H, CFB = California Farm Bureau, WIPP = Wool incentive payment program, FADV = Solano County farm advisor, SCWGA = Solano County Wool Growers Association.
<table>
<thead>
<tr>
<th>List frame</th>
<th>No. respondents</th>
<th>No. operations</th>
<th>% of this list on list frame</th>
<th>% of this list that overlap with SRS list frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRS</td>
<td>36</td>
<td>N/A^d</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CWGA</td>
<td>19</td>
<td>15</td>
<td>79</td>
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</tr>
<tr>
<td>4-H</td>
<td>22</td>
<td>7</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>CFB</td>
<td>23</td>
<td>17</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>WIPP</td>
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</tr>
<tr>
<td>SCWGA</td>
<td>20</td>
<td>18</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

^a List frames: SRS = Statistical Reporting Service, CWGA = California Wool Growers Association, 4-H = 4-H, CFB = California Farm Bureau, WIPP = Wool incentive payment program, FADV = Solano County farm advisor, SCWGA = Solano County Wool Growers Association.

^b Overlap = number of sheep operations listed in both SRS list frame and the list frame in question.

^c % Overlap = (No. operations that overlap with SRS) ÷ (number of sheep operations on the specified list frame).

^d N/A = Not applicable.
Figure 1. Comparison of sheep operation list frames by flock size. Sources of lists: 4-H, Statistical Reporting Service (SRS), composite of all other than SRS (Non SRS), and all lists combined (All Lists).

EVALUATION OF SHEEP POPULATIONS

Figure 2. Comparison of list frames by production parameters. Lamb crop (CROP) = lambing rate = \[(\text{number lambs born alive}) - (\text{number breeding ewes})\] / (number of ewes that gave birth) - lamb death rate (DEATH) = \[(\text{number of lambs born alive}) - (\text{number of lambs weaned})\] / (number of lambs born alive) - cull rate (CULL) = \[(\text{number of ewes culled}) - (\text{number of ewes that gave birth} + \text{number of dry ewes})\] / number of ewes that gave birth.

Sources of lists: Statistical Reporting Service (SRS), composite of all lists other than SRS (Non SRS), and all lists combined (ALL LISTS). National Animal Disease Surveillance Program, Solano County, California, 1984.
EVALUATION OF SHEEP POPULATIONS

Figure 3. Comparison of list frames by types of records kept—given in percent of operations for the following: number lambs born alive (BIRTH), number lambs weaned (NURSE), number lambs tail docked (DOCK), and reproductive records for individual ewes (INDIV). Also shown are percent of operations for which none of these records were maintained (NO REC). Sources of lists: Statistical Reporting Service (SRS), composite of all lists other than SRS (non-SRS), and all lists combined (ALL LISTS). National Animal Disease Surveillance program, Solano County, California, 1984.
Figure 4. Comparison of list frames by use of medical services during the previous year. Services were: veterinary services (VET); and whether total vaccine costs were known (COST). Sources of lists: Statistical Reporting Service (SRS), composite of all lists other than SRS (non SRS), and all lists combined (ALL LISTS). National Animal Disease Surveillance Program, Solano County, California, 1984.

Figure 4: Comparison of list frames by use of medical services during the previous year. Services were: veterinary services (VET); and whether total vaccine costs were known (COST). Sources of lists: Statistical Reporting Service (SRS), composite of all lists other than SRS (non SRS), and all lists combined (ALL LISTS). National Animal Disease Surveillance Program, Solano County, California, 1984.
Figure 5. Comparison of list frames by diseases or conditions reported to have occurred during the previous year. (No coccidiosis or epididymitis was reported by producers on the non SRS list frame.) Sources of lists: Statistical Reporting Service (SRS), composite of all lists other than SRS, and all lists combined (ALL LISTS). National Animal Disease Surveillance program, Solano County, California, 1984.
REPORT OF COMMITTEE ON ANIMAL DISEASE SURVEILLANCE

Chairman: G. C. Poppensiek, Ithaca, NY
Vice Chairman: G. P. Combs, San Juan, PR

J. A. Acree, MD; F. J. Alderink, MD; R. K. Anderson, MN; V. C. Beal, Jr., MD; D. L. Berndt, MD; S. L. Diesch, MN; J. G. Flint, MN; C. M. Hibbs, NM; M. E. Hugh-Jones, LA; N. E. Hutton, OR; J. L. Hyde, MD; R. F. Kahrs, MO; L. J. King, MD; L. D. Mark, VA; F. B. Martin, MN; W. R. McCallon, MD; Hunt McCauley, MT; H. A. McDaniel, MD; W. R. Miller, MD; L. G. Morehouse, MO; R. S. Morris, MN; P. B. Mullenax, MD; T. G. Murnane, TX; J. C. New, TN; S. R. Nusbaum, NJ; J. C. Paige, MD; E. I. Pilchard, MD; J. C. Prucha, MD; Philip Ross, DC; L. H. Russell, TX; Vaughn A. Seaton, IA; G. H. Snoeyenbos, MA; C. D. Van Houweling, VA.

The Committee on Animal Disease Surveillance was called to order at 1:30 p.m. on Thursday, October 25, 1984. Forty-one members and guests were present at this meeting. A total of seven papers and two oral presentations were made.

Dr. John Atwell, Deputy Administrator, APHIS, Veterinary Services gave a paper titled "Vitality, Usefulness, and Receptibility of the National Animal Disease Detection Surveillance System." He indicated that the single most important function of regulatory veterinary medicine in the future may be the collection of valid and reliable data on the incidence and cost of livestock and poultry diseases. This is justified by the many users who need the information. Even though NADDS is not a traditional regulatory activity, it is very much in the interest of animal health. NADDS does not impinge on ongoing cooperative programs but serves as an important adjunct to them with an increasing and expanding role. Dr. Atwell believes that NADDS will help boost our livestock market by showing other countries how healthy and desirable our livestock are.

Dr. Billy Heron of the Bureau of Animal Health, California Department of Food and Agriculture presented a paper on "The National Animal Disease Surveillance System in California: Implementation Methodology." In his paper, he discussed the implementation of NADDS in California where it is being developed as a pilot project. California is integrating NADDS into its existing animal health programs and is developing population databases for all segments of the livestock industry. By means of a recent survey conducted in California, Dr. Heron summarized that: (1) existing databases must be improved (2) feedback is essential to the participating farmers and ranches and (3) there must be commitment and money to support the project.

Dr. H. S. Gosser, Director of the Veterinary Diagnostic Laboratory at Tifton, Georgia gave a paper on "Modern Trends in Immunodiagnosis which Facilitate and Expedite Specific Diagnosis." He reviewed new techniques being used and developed to diagnose animal diseases. He
discussed the relevance of these new proceedings toward supporting the NADDS. New technology is resulting in the development of on-farm, veterinary based, diagnostic kits. Dr. Gosser pointed out the deficiencies in these systems and the feasibility for their future use. The new technology is quite dynamic and diagnostic tests to detect environmental hazards are just around the corner. He reaffirmed the necessity of high quality laboratory diagnostic support for NADDS and the cooperation of the laboratory community in this venture.

Dr. Fred J. Alderink of USDA, APHIS, Veterinary Services presented a paper authored by Dr. Victor C. Beal on “The Nature of the Animal Disease Survey Sampling and Estimation Problem.” He highlighted the difference between disease detection and incidence rates. He pointed out that the National Animal Disease Detection System is structured to define incidence and prevalence rates. He discussed methods being used by this system for sample selection and the merits of using certain techniques in drawing valid samples from populations at risk.

Dr. Frank Mulhern, Consultant to IICA, gave an oral presentation. He commented that the information and methods being developed for NADDS will have some utility for other countries. As an IICA consultant, Dr. Mulhern envisions the eventual development of a hemispheric reporting system and stressed the importance of interchanging personnel and ideas among countries with similar interests.

Dr. Harry Goldstein, State Veterinarian of Ohio, gave an oral presentation. He indicted that he believes the National Animal Disease Detection System to be the most innovative activity that has been sponsored by the government and regulatory veterinary medicine in many years. He indicated that it is time to wear the “white” hat.

Dr. Denise Belanger of the School of Veterinary Medicine, University of California presented a paper on “Construction and Characterization of a Sampling Frame of Sheep Growers in Solano, California.” She compared nine different list frames of sheep for Solano County, California. Through questionnaires, Dr. Belanger was able to contrast and compare these sources of flock owners and infer their usefulness for the NADDS project. The small flocks were not well represented in any of the list frames and could lead to a sampling bias if not taken into consideration for selection of samples.

Dr. Lonnie J. King of Veterinary Services, APHIS, USDA, gave a paper on “Concerns of the Industry and How APHIS Will Deal with Them.” He discussed three major concerns of industry regarding the NADDS project. He also discussed the importance of being accountable to industry and the public during the development of an activity such as NADDS. APHIS is aware of industry concerns and is actively soliciting their input and help in planning, implementing and evaluating NADDS.

Dr. Gerald L. Shook, NADDS Coordinator for Ohio, gave a paper entitled “Serendipity Findings: Attitudes We’ve Found by Working Closely with Growers and Producers on a Non-regulatory Basis.” He discussed his
experiences and those of the other Ohio veterinarians. He said that producers were proud to be selected for NADDS and that they developed a heightened awareness of disease problems and their economic impact. The cooperation of the farmers has been excellent and there was an overwhelming support for NADDS by all of those participating and no one questioned its value.

This concludes the report of the Animal Disease Surveillance Committee.
REPORT ON ADVANCES IN CARRIERS AND ADJUVANTS FOR VETERINARY BIOLOGICS

M. H. Bairey and R. M. Nervig

An international conference on Advances in Carriers and Adjuvants for Veterinary Biologics, co-sponsored by the National Veterinary Services Laboratories and Iowa State University, was held in Ames, Iowa, on May 7 and 8, 1984. This report is a general summary of the papers presented at the meeting.

To obtain an immune response, a series of complex cellular and biochemical interactions occur. For example, an antigen entering the circulation encounters antigen presenting cells, principally the macrophages which have the capacity to process the antigen and present the processed antigen to the T lymphocytes, according to Dr. Harley Tse of Merck, Sharp and Dome.

It is speculated that macrophages "select" only limited regions of the antigen molecule to serve as determinants for eliciting clonal expansion of T cells and helper cell activity for antibody production. Antigen bound to macrophages is displayed on the surface membrane associated directly or indirectly with major histocompatibility complex gene products called Ia antigens. This association constitutes the basis of self versus non-self recognition. Ia is a glycoprotein coded for by genes of the major histocompatibility complex which aids in triggering T lymphocyte proliferation.

Because it has been shown that a regulatory capacity of the macrophage is mediated through the Ia molecule, it may not be possible to devise methods to manipulate the nature and expression of Ia as a means of enhancing or inhibiting immune responses.

It was explained by Dr. Merlin Kaeberle of Iowa State University that macrophages, B-cells, T-cell subsets, and soluble mediators all interact to induce an immune response. However, the presence of adjuvants and carriers may alter these interactions to enhance the immune response.

Dr. Harley Moon of the National Animal Disease Center discussed mucosal immunity and the disease process. He pointed out that in order for rational vaccine development, increased knowledge is needed of both the disease process involved and the physiology of the mucosal immune system in each species affected. This was illustrated by the difference in immunoglobulins in post colostral milk in swine versus cattle. In swine this involves the secretion of IgA which is directed against the enteric pathogen. However, in cattle the situation is different. Little IgA is secreted during the post colostral period. Ruminants appear to have developed the IgG1 system as their means of passive mucosal immunity. The causative agents of mucosal disease are often opportunistic pathogens that exist predominantly in the subclinical state on or in the mucosa. These carrier states tend to immunize the host naturally. This immunity is often not adequate to protect the animal from severe infections. He stated...
that existing vaccines for systemic infections are generally more effective than those designed for mucosal infections. Plus, the existing vaccines for mucosal infections in the neonate are more effective than for mucosal infections in the older animals.

Development of effective vaccines aimed at the mucosal diseases need to be specific for each pathogen defined and also for the appropriate antigenic determinants of the microbe. Most vaccines are more effective at preventing disease than preventing the carrier state.

Humoral protection against mucosal infections depend upon locally-secreted immunoglobulins, primarily IgA and IgM. However, most of the immunoglobulins absorbed from colostrum are IgG which provide a lesser degree of local protection to mucosal diseases. Thus it is postulated that oral vaccines that have specific antigenic determinants would be the most effective means of producing an immune response to mucosal diseases.

Adjuvants are substances that are incorporated into or injected simultaneously with an antigen and nonspecifically potentiate the immune response. The reason for using adjuvants is to produce a more durable humoral or cell-mediated immunity of a high level using either a smaller antigenic mass or fewer doses.

Dr. Edgar Ribi from Ribi ImmunoChem Research, Inc. reviewed his studies involving the adjuvant action of cell wall derived components from mycobacteria and detoxified endotoxin from gram negative bacteria. He described how the classical Freund’s emulsion was replaced by an oil-in-water emulsion, in which the oil was reduced from 50% to 1% thus minimizing sterile abscesses and persistent nodules at injection site. Eventually, whole tubercle bacilli were replaced by a combination of deproteinized cell wall skeleton and trehalose dimycolate (TMD) to avoid allergic reactions upon repeated administration. While there were no adverse effects of medicinal mineral oil in long-term studies in man, it has been replaced in many instances with the more readily digestible metabolic oils such as peanut oil or olive oil or squalene.

Endotoxin is known to be one of the more effective microbial products in enhancing the formation of antibodies to proteins. The active principle, consisting of lipopolysaccharides, is located in the cell walls of most gram negative bacteria. Bacterial endotoxins are among the most potent inducers of colony-stimulating factor generation, B-Lymphocyte mitogenic activity, and activation of peritoneal macrophages. This all lends to the adjuvanting properties of these endotoxins. Beta-hydroxy acids are typical for the endotoxins of gram-negative bacteria and adjuvant active-trehalose esters from mycobacteria. Future research regarding the structure-function relationship of bacterial adjuvants should be very rewarding.

Dr. Louis Chedid of the Pasteur Institute discussed some of the opportunities offered by using muramyl dipeptides (MDP) associated with either conventional or synthetic vaccines. Muramyl dipeptide represents the minimal adjuvant structure of the mycobacteria used in Freund’s complete
adjuvant but does not induce sensitization to tuberculin. He stated it can
be used in oily vehicles or even in saline and will enhance the immune
response even after oral administration. When this glycopeptide is admin-
istered with an antigen in Freund's incomplete adjuvant (FIA), a stronger
immune response is induced than with FIA alone. Plus, this material is
well tolerated by cattle, sheep, and swine. Dr. Chedid suggested con-
jugating small amounts of synthetic peptides and MDP to protein carriers
before adding them to FIA as a means of reducing the amount and
subsequent cost of MDP. This will also enhance the immune response even
after oral administration.

Many investigations into the mechanisms of adjuvant action have dealt
with formation of depots of antigen in tissue. The ability of emulsions
containing copolymers to retain antigen in tissue proved to be a complex
phenomenon which could, at best, explain only a portion of the observed
effects.

Dr. Robert Hunter of Emory University reported on his research on the
structural basis of the activity of surface active adjuvants. Some of these
adjuvants have active sites which react with specialized receptors on cells
of the immune system. Muramyl dipeptide is such a compound. Fatty acid
derivatives of MDP have increased adjuvant activity suggesting that
surface activity can operate synergistically with active sites of an ad-
juvant.

Copolymers which are powerful adjuvants for increasing antibody for-
formation, are the largest and least soluble preparations which have hydro-
philic chains on the ends of the molecules. These are spreading agents
which are used to spread oily materials on the surface of water. They do not
emulsify or solubilize lipids or membranes, but adhere to lipids and
influence the interaction of soluble macromolecules with them. There
appears to be a correlation between the ability of such agents to promote
retention of soluble macromolecules on the surface of oil drops and their
activity as adjuvants. Copolymers, which are strong adjuvants for anti-
body formation, form fibers, induce the release of chemotactic factors from
serum, and activate complement. Most copolymers which are not adju-
vants form optically smooth spherical masses which resemble oil drops and
are more hydrophobic.

Most compounds that cause interferon production in animals also en-
hance specific immune responses when given with antigenic stimuli. It
was also reported that interferon inducing synthetic compounds have also
been found to be immunoenhancing. Their common capacity to cause
interferon responses in animals most likely reflects induced changes in
monocyte populations that are associated with the interleukin/cytokinin
reactions present in all immune responses. Efficacy as vaccine adjuvants is
probably caused by the pharmacological actions on cell modulating sys-
tems.

The lipid amine which is now called avridine, was reported by Dr. Keith
Jensen of Pfizer Central Research to demonstrate adjuvant activity with a
wide range of viral, bacterial, protozoal and other antigens when injected into laboratory and farm animals.

The adjuvanting properties of avridine have been shown both with avridine combined with the antigen and when given separately. The data indicate some route/site dependency with equal enhancement obtained intraperitoneally when given mixed or separately. However, when mice received subcutaneous injections of antigen and avridine separately, but in an area drained by the same regional lymph nodes, there were greater responses than when the components were given together. Mice injected intravenously with avridine 48–72 hours after the antigen had a striking improvement in antibody titers over when antigen and adjuvant were injected at the same time.

It has been reported that avridine given orally or enterically yielded major increases in specific mucosal immunity. This may lead to a new era of vaccination practices for improved immunity on mucosal surfaces.

Dr. Arthur Johnson from the University of Minnesota at Duluth reported on the adjuvant action of synthetic polynucleotides whose synthesis was motivated by immunomodulating agents of microbial origin. Compounds with minimal or no toxicity have been identified which effectively modulate the diverse manifestations of the immune response. However, the timing of the administration of adjuvants in relationship to antigen administration and the age of the animal produced major differences in the adjuvant activity of three different classes of chemically-defined adjuvants, endotoxic lipopolysaccharides, muramyl dipeptides, and the polynucleotide complex [polyadenylic-polyuridylic (poly A : poly U)].

Some of the principal complications encountered with the use of adjuvants are granulomas at the site of injection, pyrogenicity, induction of adjuvant-type arthritides, and induction of acute and/or chronic uveitis. These reactions observed by Dr. Anthony Allison of Syntex Laboratories, were due to bacterial polysaccharides and their derivations, as well as muramyl dipeptides and most of their derivatives. These side effects have not been observed with the muramyl dipeptide derivative which has a threonine replacing the alanine and is a potent substitute for the tubercle bacilli in Freund's complete adjuvant.

The use of aluminum salts as adjuvants in veterinary biologics was reviewed by Dr. Thomas Bunn of the National Veterinary Services Laboratories. The most commonly used are the aqueous aluminum hydroxide gels but many toxoids are alum-precipitated.

The adjuvant effect of aluminum is manifested primarily as an increase in IgG. The adjuvanting action is as yet unknown; but may be produced by encapsulation of the antigen, trapping of lymphocytes in regional lymph nodes, production of local granulomas which are rich in macrophages and plasma cells, or the activation of complement, which in turn activates macrophages and increases their phagocytic activity. In the case of viruses and toxoids, the aluminum salts may simply create a physical complex that is more easily taken up by macrophages.
Factors which influence the absorptive properties of aluminum hydroxide to antigens are the isoelectric point and the concentration and nature of the antigen and accompanying substances, as well as the concentration of the salts and buffering ions.

The most common veterinary biologics that contain aluminum adjuvants are purified toxoid, whole culture bacterins, and inactivated viral vaccines.

These adjuvants are relatively stable at either refrigerator or room temperatures, but freezing may destroy the hydrated structure of the gel.

One problem associated with the metallic salt adjuvants is the inability to evaluate the consistency of the adjuvanting action. This can be overcome to a certain extent by determining the optimal concentration in the intended host.

Dr. Peter McKercher from the Plum Island Animal Disease Center reported on oil adjuvants and their use in veterinary biologics. He stated that the use of oil-based adjuvants dates back to the early 1900's. Freund's early studies combined paraffin oil and killed tubercle bacilli as an enhancing agent for the immunological response in animals. Later studies eliminated the killed tubercle bacilli and then the material was known as "incomplete Freund's adjuvant."

He reported that the enhancing actions of oil adjuvants can be associated with three phenomena: 1) They establish a portion of the antigen in a persistent form at the site of injection making possible a gradual and continuous release for stimulating antibody; thus, a level of response equivalent to a secondary immune response can be generated by a single injection; 2) They provide a vehicle for transport of emulsified antigen throughout the lymphatic system to distinct sites such as lymph nodes and spleen where new foci of antibody can be established and 3) They enhance the formation and accumulation of cells of the mononuclear series which are appropriate to the production of antibody at local and distant sites.

The viscosity and stability of an emulsion are important factors and depend upon the amount of emulsifying agent used, the size of the globules, the proportion of different ingredients, and the initial viscosity of the external oil phase.

Adverse reactions with oil-adjuvanted vaccines are inflammatory reactions, sterile abscesses, cyst formations and granulomas. Most adverse reactions can be overcome by the use of highly-refined standardized ingredients, reduction of the volume of inoculum by use of purified or concentrated antigens, and the selection of the route of administration.

The advantages of oil adjuvants is the increase in immune response to most antigens and in a general prolonging of the duration of immunity. Oil adjuvants are widely used in poultry biologics.

Liposomes have been put to a variety of uses in immunology since first being described in the mid 1960's according to Dr. Barry Rouse of the University of Tennessee. They are formed by hydration of bilayer-forming
lipids such as those found in natural biological membranes. The usual compositions are based on phosphatidycholines or sphingomyelins with cholesterol added to provide rigidity and often a charged lipid species included as a minor component. These lipids form bilayers with the polar head groups placed as a boundary between the aqueous medium and the hydrophobic core of fatty acid chains. It is this bilayer arrangement of the lipid molecules which enables the membrane to be a continuous sheet, impermeable to water soluble molecules but providing the liposome with the capacity of trapping molecules within its internal compartments.

In order to be effective most antiviral vaccines contain virions which may give rise to undesirable reactions. To minimize these reactions, components of the virions called “subunits” are being researched as well as synthetic peptides which represent the immunogenic and functional domains of the viral proteins. However, while inactivated vaccines are less immunogenic than attenuated strains, subunits make even weaker antigens.

Liposomes may become useful in enhancing the responses to weak antigens such as “subunit” vaccines by either attachment to the bilayers by noncovalent bonds or entrapment within the aqueous phase of the liposome. It has been found that liposome-bound antigens are more effectively trapped by macrophages and provide a better immunogenic stimulus.

Interest has been increasing in a controlled release system for macromolecules. Therapeutic agents such as insulin and growth hormones which cannot be taken orally because they are degraded in the digestive tract are excellent candidates for implantable polymer-based sustained release dosage forms.

The sustained release of macromolecules from ethylene-vinyl acetate copolymer matrices has been studied by Dr. Larry Brown of the Massachusetts Institute of Technology. Powdered protein was added to a solution of the copolymer dissolved in methylene chloride and then cast at low temperatures in a glass mold. Release of the protein in buffered solution was sustained for over 6 months. Adjustment of drug particle size, loading, and matrix geometry were used to vary release rates over a 2000-fold range. Microstructural studies showed that the incorporation of powdered macromolecules during casting created a series of interconnecting channels within the matrix through which the dissolved drug could diffuse. Constant release rates were achieved by laminating the matrix with an impermeable coating, except for a small cavity in the center face.

Prolonged immunization of mice was attained with the delivery of gama-globulin from a 0.1 ml polymer pellet with antibody formation still evident 25 weeks after implantation. The polymer material alone did not induce any inflammatory or antigenic response. The practical application in immunology of such a controlled release system suggests many possibilities.
Dr. Dennis Kleid of Genentech discussed the difficulties encountered in the biosynthesis of viral antigenic proteins or peptides in bacterial systems, such as *E. coli*. The problems most often described include rapid degradation of the antigenic protein within the bacteria, extremely slow growth of the bacteria when the antigen is expressed, or lack of significant immunogenic activity of the recovered protein. In the biosynthesis of the antigenic sequences of Foot-and-Mouth Disease virus, these problems have been overcome by the use of a protein carrier derived from the *E. coli* tryptophan operon. The gene coding for the carrier protein was incorporated into a bacterial plasmid vector and linked, in the same reading frame, to a gene coding for an important immunogenic determinant of the FMD virus. A fusion protein made from sequence, derived from both the *E. coli* protein and the FMD viral surface protein VP1 was shown to be produced in high yields in *E. coli*. The protein was stable in the cell because it precipitated in the form of an intracellular inclusion body. Upon recovery, purification, and formulation into a vaccine, the protein was demonstrated to be immunogenic and to protect cattle from viral challenge.

Dr. James Bittle of Scripps Clinic and Research Foundation explained that vaccines containing antigens which are specific for protection but are devoid of nucleic acids and other nonessential components have not been widely used because the separation and purification techniques are impractical to use on a large scale. The exceptions are the toxoids, and more recently pili, antigens of *E. coli* which have high specificity and are practical to produce.

Two limitations prevented early development of chemically synthesized viral antigens as vaccines: 1) the difficulty in accurately determining the amino acid sequence of surface proteins and 2) the dilemma of chemically synthesizing these antigens with the assumed necessary configuration. The problem was overcome by the development of recombinant DNA techniques which made possible the cloning of genes that code for specific proteins, including surface antigens.

Small chemically-synthesized peptides are weakly antigenic and stimulate antibody that is short lived. Coupling these peptides to carrier proteins and emulsifying them with adjuvants increases their antigenicity. Chemically-synthesized peptides mimic the antigenic site found on the native antigen.

Adjuvants and carriers added to veterinary biological products are considered to be ingredients and must comply with the standards of purity and quality as required by the Virus-Serum-Toxin Act of 1913 according to Dr. David Espeseth of the Veterinary Biologics Staff, Animal and Plant Health Inspection Service, United States Department of Agriculture. They also shall be non-toxic to the recipient and shall not denature specific substances in the product throughout the dating period.

Adjuvants and carriers in products recommended for use in meat-producing animals must also be evaluated to assure that adequate withholding periods have been established and that no harmful residues will
result in meat from vaccinated animals. This clearance is obtained from USDA's Food and Safety Inspection Service (FSIS).

The approval of new adjuvants and carriers in veterinary biological products involves procedures that do not restrict development, yet provide assurance of safety for animals and protection of the public health interest.

Dr. David Reed of Molecular Genetics, Inc. summarized the symposium by stating that the future of carriers and adjuvants in veterinary biologics is promising, but contains barriers which may deter progress.

Certain synthetic adjuvants are being developed that aid antigen processing by macrophages and clonal expansion of T and B cells.

Safe and effective adjuvants will allow killed virus vaccines to replace live-attenuated vaccines. However, live virus vaccines which are genetically manipulated to delete virulence might replace the killed vaccines.

Dr. Reid also stated that the advances in recombinant DNA technology and synthetic antigen technology will lead to the purest, safest, and ultimately the most efficacious vaccines we have known. As knowledge increases, more control over the immune response by selection of the optimal adjuvants, along with the appropriate immunogenic sites of antigens will increase this efficacy.

Novel delivery systems such as implanting a multivalent vaccine in a slow-release polymer will be a part of the future.

One of the barriers to be faced is our basic research deficiencies. We have only begun to identify immunogenic determinants on synthetic or biosynthetic proteins. With many microbes of veterinary importance, we have not identified the immunogenic proteins let alone determinant sites. In many diseases, like the bovine respiratory disease, we have not even determined which microbes are the pathogens and which are the opportunists.

Other barriers are the regulatory and safety issues which must be resolved prior to the use of a new adjuvant or carrier. Also patent and proprietary restrictions will delay wide-spread usage and increase the cost.

These barriers can and will be overcome, but it will take the cooperative effort of all of us that develop, produce, regulate, and use these advancing technology veterinary biological products.
REPORT OF THE COMMITTEE ON BIOLOGICS

Chairman: R. W. Loan, College Station, TX
Vice Chairman: Majon Huff, Denver, CO

J. B. Addison, MO; D. C. Alexander, Canada; M. H. Bairey, IA; W. H. Beckenhauer, NE; Neal Black, MN; D. E. Bordt, IL; A. C. Braemer, CA; M. L. Crandall, MD; D. A. Espeseth, MD; John Finnell, IL; J. S. Gloyd, IL; R. F. Hall, GA; B. B. Hancock, IA; L. E. Hanson, IL; D. W. Johnson, MN; G. L. Johnson, KS; D. E. Kahn, NJ; R. F. Kahrs, MO; L. L. Lauerman, Jr., AL; Vincent Marshall, NE; J. P. Newman, MI; Duane Pankratz, SD; R. J. Price, MD; D. C. Randall, CO; R. C. Stewart, KS; W. C. Steward, IA; O. H. Timm, CA; J. D. Todd, KS; P. R. Turner, TX; C. D. Van Houweling, VA; G. B. E. West, CA; R. D. Williams, IN.

Forty committee members and other interested persons attended the Biologics Committee meeting held on Tuesday, October 23, 1984. The chairman announced that the present committee members would be polled to determine their continuing interest in serving on the committee. Other persons wishing to be appointed to the committee should contact the chairman. Appointments will be recommended with regard to balance in committee membership between industry, producer, government and scientific groups.

Dr. David Espeseth, Senior Staff Veterinarian, Biologics Staff, USDA, presented the status of the proposed Animal Biologics Product Act, an update on new regulations, current issues and proposed directions. The proposed biologics product act was not cleared by the Office of Management and Budget because of an apparent disagreement over the definition of "animal biological products." This definition is important for defining future responsibilities for the regulation of biologics resulting from new biotechnology. Dr. Espeseth further reported on the licensing of experimental products for sale, use of provisional licenses, split manufacture of products, and new standards for embryonating eggs used in vaccine manufacture.

Dr. Espeseth reported that 60 biological firms are currently licensed and that 160 new product licenses were issued during the past year. Twenty-one of these new products were never available before and 14 of these later were diagnostic tests. A total of 1,295 biological products are currently licensed for use in animals.

Dr. Marty Vanier, Science Coordinator for the Animal Health Institute, reported on industry perspectives for the regulation of biologics. She discussed her organizations attempts to have introduced and passed by Congress an amendment to the Virus, Serum, Toxin Act of 1913. She further reported that none of these attempts were successful, but that further attempts will be made during the next session of Congress.

Dr. Albert Brown, Director of Molecular Microbiology, Norden Laboratories, reported on subunit vaccines. Three methods of subunit manufacture were described: subunit purification from conventional sources,
REPORT OF THE COMMITTEE

subunit genetic engineering and biosynthesis, and chemical subunit synthesis. In animal health, cost is an important consideration. The ineffectiveness of some highly purified antigens was noted.

Dr. Miles Bairey reviewed presentations made at a recent symposium on Adjuvants and Carriers for Veterinary Biologics. Three hundred scientists from 13 countries attended the conference. A proceedings book from this conference will be published within the next few months. Basic immune mechanisms were reviewed. Methods discussed for enhancing the immune response included aluminum hydroxide adjuvants, oil adjuvants, lysosomes and controlled release matrices. The use of effective adjuvants assumes high priority as biologics resulting from new biotechnology are developed. It is anticipated that these new genetically engineered products will require effective adjuvants to be highly efficacious.

Dr. Hancock reported on killed versus live vaccines. He reviewed concerns about reversion of attenuated vaccines to virulence. He also indicated that in some instances killed vaccines were as immunogenic as attenuated vaccines. While recognizing the usefulness of many attenuated vaccines, he described the ideal vaccine as a “non-living” entity without the potential for reversion to virulence or the potential to cause disease.

The meeting concluded with a discussion of the scientific presentations and future legislative strategies for the regulation of animal biologics. The following resolution was passed unanimously by the Committee:

WHEREAS, the Biologics Committee continues to support previously adopted resolutions that uniform regulations, throughout the United States, assuring the safety and efficacy of all veterinary biologicals are necessary, and

WHEREAS, it is most beneficial to the livestock industry that this authority be vested in the U. S. Department of Agriculture.

Therefore, BE IT RESOLVED that USAHA strongly support the general concept of uniform regulation of veterinary biologicals with specific comments to come upon the introduction of legislation.

The Committee adjourned by 5:00 p.m.
EXPERIMENTAL BLUETONGUE VIRUS VACCINE INACTIVATED BY GAMMA IRRADIATION

T. L. Barber, DVM, PhD and C. H. Campbell, PhD
U.S. Department of Agriculture, Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory, P.O. Box 25327, Denver, CO 80225 (TLB) and Plum Island Animal Disease Center, P.O. Box 848, Greenport, NY 11944 (CHC)

SUMMARY

The immunogenicity of bluetongue virus (BTV) inactivated by gamma irradiation was tested in sheep. The first preparations of BTV were exposed to a 6 megarad dose of irradiation but, because 1 of 3 preparations of BTV serotype 11 contained residual live virus, the dose was increased to 10 megarads for subsequent preparations. Except for this 1 preparation, tests in cell culture, embryonated chicken eggs and sheep revealed no evidence of residual live virus in irradiated preparations. When inoculated into sheep, the inactivated preparations of BTV elicited humoral antibodies and protective immunity thus indicating that gamma irradiation might be useful as a means of preparing inactivated BTV vaccine. Further studies will determine a) the optimal dose of gamma irradiation for virus inactivation, b) stability and optimal storage conditions for inactivated preparations, c) the optimal dose and adjuvant for immunizing sheep and d) duration of immunity in vaccinated sheep.

INTRODUCTION

Experimental inactivated bluetongue (BT) virus (BTV) vaccines have been described.1,2,3 These however, have not led to the development of a commercially feasible inactivated BTV vaccine for sheep, cattle or other ruminants. The inactivation of BTV with gamma irradiation at ambient temperatures has been reported.4 This report describes preliminary results of the inactivation of frozen, buffered BTV by gamma irradiation and the immunogenicity of the experimental inactivated BTV vaccine in sheep.

MATERIALS AND METHODS

Virus – The US prototype strains for BTV serotypes 11 and 17 were previously described.5,6 The strains used for experimental inactivated vaccines were adapted to hamster kidney (BHK-21) cells or mouse brain; strains used for immunity challenge had been passed in sheep and were used as blood in oxylate-phenol-glycerin, an anticoagulant and preservative.7 Virus suspensions were combined 1:1 with buffered lactose peptone (pH 7.2)6 and were frozen at the temperature of dry ice before and during inactivation.

Virus assay – Heparinized blood samples from sheep were assayed by intravascular inoculation of embryonated chicken eggs (ECE) and sub-
culture on BHK 21 cells. Virus for immunity challenge was assayed by titration in ECE.

**Serology** – Assays for precipitating (P) and neutralizing (N) antibody were described previously.

**Experimental Design** – In experiment 1 the BTV, serotype 17 preparations were inactivated with 6 megarads of gamma irradiation. The virus suspensions and their preinactivation titers were as follows: mouse brain virus, 10^7.8 plaque forming units (PFU) per ml; polyethylene glycol (PEG) precipitated BHK cell infected fluids, 10^8.1 PFU per ml; infected BHK cells extracted in 0.002 M tris buffer, pH 8.8, 10^8.9 PFU per ml. There were 4 sheep in each of 4 treatment groups. Group 1-A was given the mouse brain inactivated vaccine, group 1-B the PEG precipitated inactivated vaccine and group 1-C the tris extracted inactivated vaccine. All vaccines were 1:1 in oil adjuvant that was similar to incomplete Freund’s adjuvant. Group 1-D was given oil adjuvant with saline instead of vaccine. All sheep were given 1 dose on day 0 and a second dose on day 21; vaccines in all experiments were given subcutaneously in the lateral cervical area.

For experiments 2 and 3 a single lot of BTV, serotype 11 was inactivated with 10 megarads of gamma irradiation. The virus suspension was derived by sonication of infected BHK cells in 0.002 M tris buffer at pH 8.8. The preinactivation titer was 10^{10.9} cell culture infectious doses (50%) per ml. In experiment 2, two adjuvants were compared. There were 2 sheep in each of 3 treatment groups. Group 2-A was given the vaccine in incomplete Freund’s adjuvant (Difco Laboratories, Detroit, MI) and group 2-B was given the vaccine in aluminum hydroxide (Rehsorptar II, Armour Pharmaceutical Co., Kankakee, IL). Each sheep was given 3.0 ml subcutaneously on day 0 and 21. Group 2-C was unvaccinated and served as a control for the immunity challenge. In experiments 1 and 2 immunity challenge was given on day 42 (3 weeks after 2nd dose of vaccine) and sheep were observed for 4 weeks after challenge.

In experiment 3, varying doses of vaccine prepared with the aluminum hydroxide adjuvant were compared. There were 3 sheep in each of 5 treatment groups. Group 3-A was given 3.0 ml on 0 and 21 days; on day 0 group 3-B was given 3.0 ml, group 3-C was given 4.0 ml and group 3-D was given 6.0 ml. Group 3-E sheep were unvaccinated controls but were given 3.0 ml of adjuvant on day 0. Immunity challenge was given on day 56 of the experiment and sheep were observed for 4 weeks after challenge.

In experiments 1 and 2 rectal temperatures were determined twice daily from vaccination (day 0) through 28 days after immunity challenge. In Experiment 3, rectal temperatures were determined only on the 28 days after immunity challenge. Blood samples for serum were collected from all sheep (experiments 1, 2 and 3) once each week. Heparinized blood samples were collected on days 0, 5, 7, 9, 11, 14 and weekly until after immunity challenge when they were collected on days 5, 7, 9, 11, 14, 17, 21 and 28. The serum samples were assayed for P and N antibodies; heparinized blood samples were assayed for BTV.
RESULTS

Experiment 1 – The inactivated BTV preparations did not yield residual live virus when assayed in cell culture and ECE. However, the vaccine prepared from 1 of the cell culture preparations yielded residual live virus after inoculation of sheep; see group 1-C following.

The 4 sheep in group 1-A had detectable P antibodies 7 days after vaccination (DAV) (1st dose); they had detectable N antibodies 7 DAV (2nd dose) (Fig. 1). The sheep had no febrile response after vaccination; virus was not detected in blood samples collected during the 6 weeks before immunity challenge. After immunity challenge, there were no clinical signs of illness, no leukopenia and minimal febrile responses in the sheep but viremia of brief duration was detected in all 4 sheep (Table 1); there was not an anamnestic N antibody response to immunity challenge (Fig. 1).

The 4 sheep in group 1-B had detectable P antibodies 7 DAV (1st dose); they had detectable N antibodies 21 DAV (1st dose) (Fig. 1). The sheep had no febrile response after vaccination; virus was not detected in blood samples collected during the 6 weeks before immunity challenge. After immunity challenge there were no clinical signs of illness, no fever and no leukopenia in the sheep but viremia of minimal duration was detected in 3 of the 4 sheep (Table 1); there was not an anamnestic N antibody response after immunity challenge (Fig. 1).

All 4 sheep in group 1-C had detectable viremia between 5 and 9 DAV (1st dose). The group was removed from the experiment without immunity challenge.

The unvaccinated control sheep, group 1-D, had no P or N antibody to BTV and no febrile response or clinical signs of illness until after immunity challenge (Fig. 1). The sheep all had clinical signs of illness, febrile responses and leukopenia typical of BTV infection of susceptible sheep (Table 1). Viremia was detected in sheep in group 1-D from the 5th through 17th days after immunity challenge.

All vaccinated and control sheep in experiment 1 had swellings in the lateral cervical areas at the sites of inoculation. The swellings hardened and at necropsy appeared to be sterile abscesses that may have formed in response to the oil adjuvant.

Experiment 2 – The inactivated BTV that was utilized in vaccine preparations for experiments 2 and 3 did not yield residual live virus in any assays including the vaccination of sheep described hereafter. The 2 sheep (group 2-A) that were given the inactivated vaccine in incomplete Freund's adjuvant had detectable P antibodies 7 DAV (1st dose); they had detectable N antibodies at 14 or 21 DAV (1st dose) (Fig. 2). The 2 sheep (group 2-B) that were given the inactivated vaccine in aluminum hydroxide adjuvant had P antibodies 7 or 14 DAV (1st dose) and N antibodies 21 DAV (1st dose) (Fig. 2). Live virus was not recovered from the blood of any of the sheep (groups 2-A and 2-B) after vaccination and there were no clinical signs of illness, and no leukopenia. The only febrile response was
in 1 of 2 sheep in group 2-A and it occurred on the 1st day after the 2nd dose of vaccine was given. After immunity challenge, the viremias and febrile responses in groups 2-A and 2-B were minimal in comparison to group 2-C (Table 2). The sheep in group 2-C had clinical signs of illness, febrile responses and leukopenia typical of BTV infection of susceptible sheep. Sheep that were given the vaccine in oil adjuvant had less severe sterile abscesses than sheep in experiment 1; sheep given vaccine in aluminum hydroxide adjuvant had small lumps at inoculation sites which disappeared by the time of immunity challenge.

**Experiment 3** – The 3 sheep in group 3-A had P antibodies by 14 DAV (1st dose) and N antibodies by either 14 or 21 DAV (1st dose). The sheep in group 3-A all had detectable P and N antibodies for the remainder of the experiment (Fig 3).

The 3 sheep in group 3-B had P antibody 14 DAV but in all 3 sheep the P antibody became undetectable before immunity challenge was given. Two of the 3 sheep had detectable N antibodies at 14 DAV and the 3rd sheep was positive by 21 DAV. The sheep had detectable N antibodies for the remainder of the experiment (Fig 3).

The 3 sheep in group 3-C had P antibodies by 7 or 14 DAV; the P antibodies persisted for the remainder of the experiment. The sheep had N antibodies beginning 14 or 21 DAV and these persisted for the remainder of the experiment at levels that exceeded those of group 3-B (Fig 3).

The 3 sheep in group 3-D had P antibodies by 7 or 14 DAV but the P antibodies became undetectable in 2 of 3 sheep before immunity challenge. Two of the 3 sheep had P antibodies 21 DAV but both were negative or at undetectable levels before immunity challenge. The 3rd sheep had no detectable N antibodies until after immunity challenge.

After vaccination, the sheep in groups 3-A, 3-B, 3-C and 3-D had no clinical signs of illness and BTV was not obtained from the heparinized blood samples that were collected.

The unvaccinated control sheep (group E) had no detectable P or N antibodies until 14 days after immunity challenge (Fig 3). Sheep in all groups, after immunity challenge had viremia, leukopenia and febrile responses for varying periods of time (Table 3). The sheep with the highest N antibody titers and the most protective immunity were in group 3-A but sheep in all vaccinated groups had an anamnestic N antibody response after immunity challenge (Fig 3).

**DISCUSSION**

The experimental BTV vaccines that were gamma irradiated are believed to have been inactivated by first order kinetics. Exposure of BTV serotype 17 to various levels of irradiation showed that a dose of approximately 0.6 megarad was required to reduce the virus titer by one log or 90%. It was further shown that the experimental inactivated vaccine elicited both neutralizing antibodies and a cell mediated immune response. This is in agreement with another study in which a cell mediated
immune response was shown to play a role in protective immunity.\textsuperscript{12}

In the present study, the sheep in group 2-B were given the same vaccine in the same dose and adjuvant as the sheep in group 3-A. The vaccine however, had been held for 5 months at 5 C between the 2 experiments. The poor immune responses of sheep in group 3-A indicated the possibility of deterioration of the experimental vaccine at the 5 C temperature. The poor response of sheep in group 3-D to the single large dose (3.0 ml of vaccine with 3.0 ml of adjuvant) showed that it is possible to overwhelm the immune system of the sheep. The presence of BHK cell source non-viral antigens probably contributed to the poor response to the BTV antigens in the vaccine.

It was concluded that protection is feasible with inactivated BTV vaccines but that viremias of brief duration may occur on immunity challenge which, by furnishing further antigenic stimulation, may be beneficial. Further studies are needed to determine the lowest dose of gamma irradiation that can be used for BTV inactivation, stability and proper storage conditions for the vaccine and duration of immunity in vaccinated sheep.

ACKNOWLEDGMENTS

The authors thank Mr. Lee Thompson and Mr. Donald Card for technical assistance and Mrs. Helen Britton for word processing and editing.

REFERENCES


Table 1. Experiment 1: Response of sheep vaccinated with bluetongue virus, serotype 17 inactivated with 6 megarads of gamma irradiation to immunity challenge

<table>
<thead>
<tr>
<th>Treatment group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rectal temperature&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Viremia detected days 5-28 after challenge&lt;sup&gt;c&lt;/sup&gt;</th>
<th>White blood cell count &lt;sup&gt;d&lt;/sup&gt; (Avg. 4 sheep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-A</td>
<td>0.25</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>1-B</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>1-D</td>
<td>4.0</td>
<td>6.0</td>
<td>3.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sheep were vaccinated 3 and 6 weeks before challenge; a 2.0 ml dose of vaccine was given on 0 and 21 days; the dose included 1.0 ml of oil adjuvant. Group 1-A was given vaccine derived from mouse brain virus; group 1-B was given vaccine derived from BHK cell culture. Group 1-D were unvaccinated control sheep.

<sup>b</sup> Recorded twice daily for 28 days after immunity challenge.

<sup>c</sup> Heparinized blood was collected for virus assay on days 5, 7, 9, 11, 14, 17, 21 and 28 after immunity challenge.

<sup>d</sup> Total white blood cell counts were determined from the day of challenge through 14 days after challenge.
Table 2. Experiment 2: Response of sheep vaccinated with bluetongue virus, serotype 11 inactivated with 10 megarads of gamma irradiation to immunity challenge

<table>
<thead>
<tr>
<th>Treatment group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rectal temperature&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Viremia detected days&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 103.8°F</td>
<td>5-28 after challenge</td>
</tr>
<tr>
<td>(Avg. 2 sheep)</td>
<td>(Avg. 2 sheep)</td>
<td></td>
</tr>
<tr>
<td>2-A</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>2-B</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>2-C</td>
<td>4</td>
<td>4.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sheep were vaccinated 3 and 6 weeks before challenge. Group 2-A sheep were given a 3.0 ml dose of vaccine in incomplete Freund's adjuvant on days 0 and 21; group 2-B sheep were given the same treatment except the vaccine was in aluminum hydroxide adjuvant. Group 2-C sheep were unvaccinated controls.

<sup>b</sup> Recorded twice daily for 28 days after immunity challenge.

<sup>c</sup> Heparinized blood was collected for virus assay on days 5, 7, 9, 11, 14, 17, 21 and 28 after immunity challenge.
Table 3. Experiment 3: Response of sheep vaccinated with bluetongue virus, serotype 11 inactivated with 10 megarads of gamma irradiation to immunity challenge

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Rectal temperature&lt;sup&gt;b&lt;/sup&gt; (°F)</th>
<th>Viremia detected days</th>
<th>White blood cell count (Avg. 3 sheep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-A</td>
<td>&gt; 103.8</td>
<td>5-28</td>
<td>&lt; 3,200</td>
</tr>
<tr>
<td>3-B</td>
<td>3.3</td>
<td>3.0</td>
<td>0.6</td>
</tr>
<tr>
<td>3-C</td>
<td>3.3</td>
<td>3.6</td>
<td>1.3</td>
</tr>
<tr>
<td>3-D</td>
<td>2.0</td>
<td>4.0</td>
<td>1.6</td>
</tr>
<tr>
<td>3-E</td>
<td>5.0</td>
<td>5.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> The vaccine was combined 1:1 with aluminum hydroxide adjuvant. Group 3-A was given 3.0 ml on 0 and 21 days; on day 0, group 3-B was given 3.0 ml, group 3-C was given 4.0 ml and group 3-D was given 6.0 ml. Group 3-E were unvaccinated control sheep.

<sup>b</sup> Recorded twice daily for 28 days after immunity challenge.

<sup>c</sup> Heparinized blood was collected for virus assay on days 5, 7, 9, 11, 14, 17, 21 and 28 after immunity challenge.

<sup>d</sup> Total white blood cell counts were determined from the day of challenge through 14 days after challenge.
Figure 1 — Experiment 1: mean neutralizing antibody titers (log_{10}) of sheep vaccinated with inactivated mouse brain origin BTV (open circles) group 1-A; cell culture origin BTV (closed squares) group 1-B; and unvaccinated control sheep (closed circles) group 1-D. Bluetongue virus, serotype 17 (homologous) immunity challenge was given to all sheep at 6 weeks.
Figure 2 — Experiment 2: mean neutralizing antibody titers (log$_{10}$) of sheep vaccinated with inactivated cell culture origin BTV in incomplete Freund’s adjuvant, group A; in aluminum hydroxide adjuvant, group B; and unvaccinated control sheep, group C. Bluetongue virus, serotype 11 (homologous) immunity challenge was given at 6 weeks.
Figure 3 — Experiment 3: mean neutralizing antibody titers (log$_{10}$) of sheep vaccinated with inactivated cell culture origin BTV in aluminum hydroxide adjuvant. Group A was given 2 doses, 3.0 ml each; group B, 1 dose, 3.0 ml; group C, 1 dose, 4.0 ml; group D, 1 dose, 6.0 ml. Bluetongue virus, serotype 11 (homologous) immunity challenge was given at 8 weeks.
EXCESSIVE CULLING PREDICTED FOR DAIRY COWS WITH ANTIBODIES TO BOVINE LEUKEMIA VIRUS

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Veterinary Medicine Teaching and Research Center
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Tulare, CA 93274

SUMMARY

Regression equations describing age-specific cull rates in cows with and without antibodies to the gp-51 antigen of bovine leukemia virus (BLV) were used to predict the number of cows culled per year due to an association with BLV infection. Based on the age distribution for BLV antibody-positive cows on a 184-cow dairy, which experienced a BLV-antibody prevalence rate of 45 percent, 10 antibody-positive cows were predicted to be culled in excess of the expected number computed from cull rates for BLV-negative cows.

INTRODUCTION

Livestock producers, particularly those with registered dairy cattle, are becoming more interested in controlling and eradicating bovine leukemia virus (BLV) from their herds. Motivation behind this interest lies mainly in economics of restricted foreign sales of cattle with BLV infection.1 Another important consideration in assessing benefits and costs of eradication is the cost associated with condemnation of cattle with malignant lymphoma.2,3 Recently, a study has found evidence for an additional cost of BLV infection associated with rates of culling.4 The purpose of this report is to present an application of results of that study to prediction of a cost of BLV infection based on excessive rates of culling.

MATERIALS AND METHODS

Transformed age-specific cull rates for cows with and for cows without antibodies to the gp-51 antigen of BLV were predicted using regression equations of cull rates computed in a previous study.4 Those equations were Arcsin $\sqrt{\text{cull rate}} = 0.013 + 0.14 \text{ age}$ and Arcsin $\sqrt{\text{cull rate}} = 0.597 - 0.015 \text{ age}$ for antibody-positive and antibody-negative cows, respectively, where age was expressed in years. Rates were computed as a percent by multiplying transformed rates (Arcsin $\sqrt{\text{cull rate}}$) by $\sin^2$. Predicted rates were computed for cows in each of the 4 one-year age increments from 3 to $\geq 6$ years in a 184-cow dairy in which 45 percent of cows had antibodies to BLV. Presence of antibodies to the gp-51 antigen of BLV in these cattle was measured by agar-gel immunodiffusion, as previously described.3 The herd consisted of registered Guernsey cows (33%), registered Holstein cows (33%) and unregistered Holstein cows (33%).
The excessive number of cows culled annually in this herd that was associated with presence of BLV antibodies was computed by summing age-specific differences between predicted number of antibody-positive cows culled at the rate for BLV-positive cows and the predicted number of antibody-positive cows culled at the rate for BLV-negative cows. The total number predicted to be culled was adjusted to conform to a more realistic overall cull rate of 25 percent for this herd, rather than the rate of 37 percent that would have been imposed using rates predicted from data from another herd. The predicted number culled was multiplied by the factor \(25 \div 37\).

**RESULTS**

Predicted age-specific cull rates for BLV antibody-positive cows are presented in Table 1. Rates crossed at approximately 3.3 years of age. Rates beyond 6 years of age were extrapolated because the study from which rates were derived examined only cows less than 6.5 years of age.

The number of BLV antibody-positive cows in each age group and the predicted number of cows culled by the rates for BLV-positive cows and BLV-negative cows for the 184-cow dairy are presented in Table 1. Ten cows were predicted to be culled annually due to an association with the presence of antibodies to BLV \((15.19 \times 25 \div 37)\).

**DISCUSSION**

As more livestock producers become interested in eradicating BLV from their herds, it will become increasingly important to evaluate all costs and benefits of such an undertaking. The application presented here offers an approach to estimating a cost of BLV that may accrue in addition to loss of sales in foreign markets and loss of salvage value due to condemnation of cows with lymphoma. For the 184-cow dairy studied, the 10 cows predicted to be culled would be expected to incur an annual cost of \$6,000 (10(\$1,000 - \$400)), if replacement cost was $1,000 per head and salvage value was $400 per head. Such cost may justify building segregation pens, pasteurizing colostrum and milk for calves and other management changes necessary to reduce transmission of BLV infection.

The excessive number of culled cows predicted here may represent a conservative estimate for other herds for the following reasons: 1) The overall cull rate of 25 percent per year is probably lower than for many herds, particularly in California. A typical rate would be about 29 percent\(^5\). 2) In order to avoid extrapolation of rates beyond 6 years of age, antibody-positive cows older than 6 years of age were assumed to be culled at the same rate as were 6 year old cows. It is likely that antibody-positive cows older than 6 years would be culled at a higher rate, as suggested by extrapolated rates. 3) Rates were computed for cows between 3 and 3.5 years of age which was an age at which cows were not found to differ in survival based on presence or absence of BLV antibodies.\(^4\) Exclusion of cows between 3 and 3.5 years of age from calculations would have allowed for a prediction of a greater difference between numbers of cows culled.
In order to test the validity of this approach in predicting costs of BLV infection, more studies are needed to ascertain if the finding of differing cull rates is consistent among herds. It also is not known if there is a cause-and-effect relationship between BLV infection and culling or if culling is related in an independent way to BLV infection only through a shared factor which itself predisposes to culling and BLV infection.

REFERENCES


Table 1. Predicted number of cows culled in a 184-cow dairy due to an association with antibodies to bovine leukemia virus (BLV).

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of Cows</th>
<th>Predicted BLV Antibody-positive Cows (%)</th>
<th>Cull Rate</th>
<th>Predicted Excessive Culling Number</th>
<th>Predicted Excessive Culling Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>17</td>
<td>17.6</td>
<td>2.99</td>
<td>27.5</td>
<td>4.67</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>29.4</td>
<td>2.65</td>
<td>26.2</td>
<td>2.36</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>42.8</td>
<td>6.42</td>
<td>24.9</td>
<td>3.74</td>
</tr>
<tr>
<td>&gt;6</td>
<td>42</td>
<td>56.7</td>
<td>23.81</td>
<td>23.6</td>
<td>9.91</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td></td>
<td>(43%)*</td>
<td></td>
<td>(25%)*</td>
</tr>
</tbody>
</table>

*Percent of 83 BLV antibody-positive cows.
REPORT OF THE COMMITTEE ON BLUETONGUE—BOVINE LEUKOSIS

Chairperson: Bennie I. Osburn, Davis, California
Co-Chairperson: Lyle Miller, Ames, Iowa
Members Present: Don A. Abt, PA; Joan Arnoldi, WI; T. L. Barber, CO; David Bartlett, WI; Chris Groocock, NY; Michelle Howard, CA; Michael Jochim, CO; Lester Larson, WI; Albert Luedke, CO; Maurice Mix, VT; Don Monke, OH; John Nehay, CA; James Pearson, IA; Jack Pitcher, MD; Dean Smith, WA; Jeff Stott, CA; Mark Thurmond, CA; Olin Timm, CA; Percey Turner, TX.

The Bluetongue—Bovine Leukosis Committee met at 1:30 p.m. on Thursday, October 25, 1984. There were 20 members and 38 guests, a total of 58 in attendance.

The Committee met as requested by the president of USAHA to consider the business of the committee and submit the following report. Dr. Lyle Miller conducted the discussion on bovine leukemia.

Bovine leukemia virus (BLV) infection continues to cause economic losses from slaughter condemnations of lymphosarcoma, from loss of export sales of live animals that are seropositive and from loss of sales of semen from seropositive bulls.

Various aspects of BLV infections were addressed in papers presented to the committee.

Dr. Gary Cockerell reported that sheep infected with the virus developed precipitating antibodies within four weeks but have not developed detectable immunologic aberrations in humoral or cell mediated immunity during the first 32 weeks of a continuing study.

Dr. Mark Thurmond presented a statistical model to predict annual cull rates for seropositive and seronegative cows in a dairy herd. Increased costs are associated with a higher culling rate for BLV seropositive cattle. These studies are ongoing in an area with a high prevalence of BLV infection and where carcass condemnations from lymphosarcoma exceed the national average.

Dr. Lyle Miller reported that small differences in infectivity of whole blood from seropositive cattle were detectable, however, inoculation of minute quantities of blood (1 to 10 microliters) will infect susceptible calves.

Dr. Harris Lewin presented data from studies using monoclonal antibodies to T and B lymphocytes. Alternations in the T/B cell ratio occur in some cattle with persistent lymphocytosis and these changes do not correlate directly with BLV infection or BLV precipitating antibody. Dr. Lewin indicated that the bovine major histocompatibility complex plays a role in resistance to BLV.

Dr. Donald Monke reported a retrospective study on artificial in-
semination in a dairy herd free of BLV antibodies for five years. Approximately 50% of 2,110 units of semen purchased during the five year period were from seropositive bulls, providing further evidence that BLV is not transmitted via semen.

Dr. James Pearson reported that a BLV immunodiffusion proficiency test was sent to 48 laboratories in 1983 and to 51 laboratories in September 1984. Results for 1984 are not yet available. He indicated that 3.6% of 682 samples submitted for import/export were serologically positive.

Dr. Jack Pitcher led discussion on the "Uniform Methods and rules for the Establishment and Maintenance of Designated Bovine Leukosis Virus-Free Herds" as approved previously by committee resolution.

Dr. Richard Edling, Redmond, Washington, reported that economic losses in 1983, caused by bovine leukosis and bluetongue, to a major U.S. artificial unit was conservatively estimated at $610,000.

A motion to proceed with a voluntary certification program sanctioned by APHIS-VS was approved by the committee.

Bluetongue

Dr. Osburn conducted the discussion on bluetongue.

Mr. Olin Timm, California, discussed the proposed moving of the Arthropod-Borne Animal Disease Research Laboratory (ABADRL) from Denver, Colorado to Ames, Iowa. Mr. Timm indicated that the reasons given for moving the laboratory were inadequate facilities to carry out research and the need for additional space by the Government Services Administration. Mr. Timm quoted from a National Academy of Sciences Report on Foreign Animal Disease Research which stated that the ABADRL has a vigorous program with well planned collaborative research being performed in undesirable facilities. The report recommended that the nucleus for the program needs to be expanded and moved to modern well equipped facilities. Mr. Timm further indicated that there is a real need for better diagnostic tests as bluetongue (BT) is the single most serious limitation to exportation of ruminants and germplasm from the United States. These tests are needed for importation of livestock as well. Sheep continue to die of bluetongue virus (BTV) infection where vaccines are not available. Movement of the ABADRL from Denver to Ames means relocation of infectious viruses into a highly populated livestock area. Mr. Timm indicated that this move is unwise since containment is not perfect. The movement will destroy the research facility as it now exists and it will require at least five years for reestablishment of an effective laboratory if moved.

Dr. D. Hare from Agriculture Canada, Nepean, Ontario, reported on the effects of bovine leukosis virus (BLV) and bluetongue virus (BTV) on bovine and ovine embryos. Embryo transfer presents a potential means to control and eradicate disease. Approaches taken in the study of disease transmission by embryos was by "in vitro-in vitro; in vivo-in vitro; in vitro-in vivo; and in vivo-in vivo" studies. Unfertilized zona pel-
The results of these disease transmission studies, and what is known from other studies about the transmission of BLV indicates that zona pel-lucida-intact embryos can be removed from BLV-infected donors and transferred to BLV-uninfected recipients or resulting progeny. In the BT studies, results from 30 BT viremic donors whose embryos were transferred to 81 recipients, indicated that there was not evidence of viral transmission. The number of studies is minimal; however, the results are encouraging. Dr. Hare further emphasized the need to properly wash embryos following collection and before other handling, including transfer, is to be carried out. This is necessary because it has been demonstrated that BRV can be transmitted by intrauterine inoculation of virus.

Dr. David Bartlett, Madison, Wisconsin, offered viewpoints on bluetongue diagnostic testing for the Artificial Insemination (AI) industry. Dr. Bartlett indicated that the AI industry is aware of the technical limitations and deficiencies of the bluetongue immunodiffusion test (BTID). On the economic and practical management side of the coin, it is critical that such a very high proportion of BTID positive animals are ones from whom the virus has departed but the incriminating antibody lingers on. Many non-infectious cattle must carry the bluetongue brand. Correlation between the BTID test and the presence or absence of BTV is inadequate for the purposes employed and result in a negative consequence for the cattle industry. Dr. Bartlett challenged the bluetongue researchers to develop new diagnostic techniques to identify the presence or absence of BTV in individual cattle. He encouraged the use of the serum neutralization test as a recognized supplement to the BTID test. Dr. Bartlett further encouraged the development of a meaningful certification system based on virus rather than antibody.

Dr. R. Miller, Veterinary Biologics, discussed the proposed changes in the regulation of BTV vaccines as published in the June 11, 1984 Federal Register. He reviewed the need for changes in safety, efficacy and potency testing of modified live virus vaccines. Dr. Miller indicated that Veterinary Services has adopted a position of licensure which 1) include caution before licensure of multistrain modified live virus vaccine, 2) will probably license monovalent vaccine but restrict licensure for use in specific species.

Dr. Jack Pitcher, USDA/APHIS, discussed the protocol for importation of semen and possibly embryos from Brazil. He stated that three consultants met with APHIS staff to develop reasonable guidelines for semen and embryos from serologically positive cattle. The committee recommended that both semen and blood samples will have to be inoculated into sheep and embryonating chicken eggs to test for the presence of virus.

Dr. James Pearson, National Veterinary Services Laboratory reported that two bluetongue virus isolates were made out of 1,100 semen submissions this last year. Both samples were from California, in December and March. In addition, BTV has been isolated from a sheep from Montana and two pronghorn antelope from Wyoming. Of 3,114 serum samples tested by
immunodiffusion for import/export 5.4% were positive. Dr. Pearson also indicated that a serological survey to certify 17 northeastern states as free of BTV infection indicated that of 11,339 samples tested, 99 were BTID positive. These states did not qualify as free of BT. Another survey was recently started.

Dr. Lyn Barber of the Arthropod-borne Animal Disease Research Laboratory, Denver, gave a report on bluetongue research at that location. A study on the effects of BTV on pregnant heifers has been initiated. Dr. Al Luedke found that the most frequent tissues infected in fetuses were the liver, blood, spleen and femoral bone marrow. In the dams, ovaries and liver were most likely to yield BTV at necropsy. In a study of isolation methods for BTV from infected bulls, semen was better than blood and embryonated chicken eggs were better than cell culture for primary isolation. The laboratory has completed reference antisera stocks to exotic serotypes of BTV 1, 3, 4, and 5 produced in sheep. Highly serotype-specific reference antiserum stocks to serotype 1, 3, 4, 5, 6, and 20 made in rabbits are also completed.

Dr. Fred Holbrook's pilot test program for Integrated Control of Blue-tongue in an enzootic area revealed a low antibody prevalence and fewer BTV isolations in the area where vector control has been applied. A unique BTV 11 observed in the area since 1981 shifted slightly in 1983 but otherwise remains relatively stable. The virus causes subclinical infections in sheep, cattle and goats. Dr. Ellen Collioson's research on a diagnostic probe has resulted in insertion of BTV genome copies into E. coli and expression of the material. These, along with a probe from Dr. Polly Roy's laboratory, are being examined for application in improved diagnostic procedures.

Vaccine work has included Dr. Mike Jochim supplying Colorado Serum Company with attenuated strains of BTV 11, 13, and 17. Dr. Charles Campbell of Plum Island Animal Disease Center along with Dr. Barber are testing gamma irradiated inactivated vaccine.

A sentinel herd of cattle at Ona, Florida yielded BTV 2 in 1982, a sentinel herd in Bellglade yielded BTV 2 in 1983; BTV 2 and 13 were isolated from cattle near Brookville in 1984. All infections are subclinical in nature. Serotype 2 is not readily transmitted by C. varispennis from sheep to sheep. C. insignis is probably the most important vector in Florida. The movement of cattle from Florida to other parts of the U.S. may result in dissemination of BTV 2. Dr. Barber expressed concern about movement of livestock from the Caribbean to the Mainland.

Dr. Michael Jochim deposited patented monoclonal antibody producing cells in the American Type Culture Collection. These monoclonals are to 1) define and characterize the important surface polypeptides of BTV, 2) to locate the neutralizing epitopes on polypeptide 2 of BTV serotype 10; 3) conduct peptide mapping of BTV proteins, and 4) to identify BTV in peripheral blood cells from infected sheep.

Dr. Jeff Stott, Davis, California, reported on research at the University
of California. He reported on improved diagnostic tests with the emphasis being placed upon identification of viral proteins and/or viral genetic material in cells or tissues of animals. Monoclonal antibodies are used for identification of BTV in formalin fixed tissues by the peroxidase antiperoxidase method and an antigen capture detection system for identifying virus in blood and tissue. In addition, Western blotting procedures have been used to identify bluetongue virus infected cell cultures. Gene probes have been and are being developed to identify virus in tissues by hybridization techniques. Epidemiological studies include a report on the isolation of serotypes 10, 11, and 13 from cattle in Mexico. Prevalence of BTID antibodies was relatively high in Mexican sheep and cattle. Blood samples collected from cattle originating in Florida and now in southern California feedyards showed serologic evidence of BTV-2 infection. Virus isolates from these animals are being serotyped at this time.

In a study on a dairy it was found that cattle which seroconverted to BTV or were serologically positive at the time of breeding had 3.8 services per conception whereas cattle that remained serologically negative had 2 services per conception. The conclusion is that BTV may cause early embryonic deaths.

Three strains of BTV 11 have been studied for evidence of virulence. Preliminary results indicate that 2 strains are neurovirulent for newborn mice while the third isolate is avirulent. Additional studies are underway to determine the molecular basis of neurovirulence.

There were 2 resolutions passed by the committee. These resolutions addressed the industry’s possible participation in the decision regarding the possible relocation of the Denver ABADR Laboratory and restricting movement of ruminants from U.S. possessions in the Caribbean to the U.S. Mainland.
A LYSED, CHARCOAL-ADSORBED VACCINE FOR BRUCELLOSIS
Terry L. Foster, Ph.D. and John H. Brewer, Ph.D.
Hardin-Simmons University, Abilene, Texas 79601

INTRODUCTION
Attempts to control bovine brucellosis by vaccination began as early as 1906 when Bang\(^9\) attempted to induce immunity in cattle by inoculation of living virulent cultures. This work led to numerous attempts to use other virulent strains as the vaccination to produce greater protection. Many of these attempts were unsuccessful because they resulted in infection in a high percentage of previously uninfected cattle, with excretion of the brucella, and consequent risk to man and animals\(^9,10,11\). Using guinea pigs as an animal model, Cotton, Buck, and Smith\(^4,5,6,7\) demonstrated that \textit{Brucella abortus} strain 19 produced fewer infections from vaccination yet possessed good immunizing capability. As a result of this and other work, the United States officially approved the use of strain 19 vaccine in its brucellosis program in 1939\(^1\). In 1950, after continued evaluation of this vaccine, calfhood vaccination with strain 19 was recommended as the best method for reducing the level of infection in cattle. Its use declined in the 1960's and early 1970's\(^8\), but calfhood vaccination is strongly recommended in the United States at present\(^2\).

Our interest in brucellosis was sparked by the threat of potential quarantine of Texas cattle in late 1975 and early 1976. The objective of our research was to develop a safe effective vaccine for bovine brucellosis. The research progressed until 1980 and resumed again in late 1982, just prior to a second threatened quarantine of Texas cattle. A summary of this project is depicted in Figure 1.

PROCEDURES AND RESULTS
Challenge studies to evaluate the killed vaccine as compared to regular strain 19 vaccine were performed in guinea pigs. An outline of the general protocol is presented in Figure 2. Blood samples were obtained from all guinea pigs 50 days after vaccination and 40 days after challenge. They were assayed for antibrucella antibodies by the card test, tube agglutination test, and rivanol tests.

Results of the challenge studies are shown in Tables 1 and 2. The study revealed in Table 1 is based upon dosage calculation of regular dose strain 19; whereas that of Table 2 is based upon reduced dose of strain 19. The studies were conducted at different times using different vaccine and challenge lots.

Serological results of the guinea pig study revealed virtually identical responses for the card and standard tube agglutination tests following the two different vaccine protocols. The rivanol test, however, demonstrated much high titers in guinea pigs vaccinated with live strain 19. Figure 3 reveals that 90% of the animals vaccinated with live strain 19 had rivanol
titers of 200 or greater; whereas, almost 85% of the animals vaccinated with killed strain 19 had rivanol titers of 50 or less.

To address the question of persistent antibody titer following strain 19 vaccination, five heifer calves were vaccinated with killed strain 19 and 3 with live strain 19. Blood samples were obtained periodically and assayed as shown in Figure 4. Serological results are shown in Figures 5 and 6 and demonstrate that titers from the killed vaccine regress at least as fast as those from live vaccine. Indeed the rivanol titers are longer lasting with live vaccine.

Two of the killed vaccine calves were boosted with a second injection of killed vaccine. The results in Figures 7, 8, and 9 demonstrate titers increase appreciably, but still diminish in an acceptable time period (120 days) with the possible exception of the ELISA. Time did not permit continued evaluation of these animals to determine when the ELISA titer would decrease.

**CONCLUSIONS**

The killed strain 19 vaccine is prepared by sonically disrupting *B. abortus* strain 19 and adsorbing onto finely-divided, activated charcoal. Some of the advantages of this vaccine are shown in Figure 10. In addition to the results given in this paper, we have also demonstrated that significantly increasing the dosage of the killed vaccine does not cause immune paralysis. We have vaccinated guinea pigs and rabbits with the equivalent of \( 1 \times 10^{15} \) cells of killed, charcoal-adsorbed *B. abortus* strain 19 and elicited serological responses similar to those given in this paper. Not only does the killed vaccine offer the advantages similar to those given in Figure 10, it also provides a mechanism by which to drastically increase vaccine dose without danger of producing infection in the vaccinated animal. Such an increase in dosage might provide a corresponding increase in protection against field infection. Results of serological studies also suggest that rivanol titer may be used to differentiate vaccinated from infected animals when the killed vaccine is used.

While these results are encouraging it is understood that they are experimental and need to be evaluated in more detail. The vaccine needs to be evaluated in cattle, both as to serological response and protection against infection. It is anticipated that this vaccine will be a benefit in adult bovine vaccination programs as well as calfhood vaccination.
Strain 19 Disadvantages

1. Causes Infection in Some Animals
2. Pathogenic for Human—Biological Hazard
3. Must be Refrigerated
4. Persistent Antibody Titers

Research Objective:

To develop a safe, effective vaccine for bovine brucellosis

Rationale:

Use killed organisms with some agent providing an adjuvant effect.

Choice of Carrier Agent:

Finely-divided, activated charcoal with strong adsorptive capability.

Purpose:

1. To provide slow release of antigen to yield long-term booster effect.
2. Permanently mark the animal as indication of vaccination.
VACCINE FOR BRUCELLOSIS

FIGURE 2

Method of Preparation:

1. Titer *B. abortus* strain 19 to appropriate body weight dose.

2. Split vaccine into two samples.

3. Use one sample as standard vaccine.

4. Sonically disrupt the other sample and adsorb onto 1.0 to 2.0 micron, activated charcoal (0.1% final charcoal concentration).

Test Method--Guinea Pig Challenge Studies:

1. Vaccinate test animals with 0.25 ml subcutaneous injection of standard or experimental vaccine.

2. Hold animals for 60 days.

3. Challenge animals via intraperitoneal injection of 0.1 ml of viable *B. abortus* strain 2308 containing a total of $7.5 \times 10^5$ cells.

4. Hold animals for 40 days.

5. Sacrifice animals.

6. Isolate and aseptically culture homogenized spleen and mesenteric lymph nodes.

7. Identify all isolates and verify as strain 2308 or strain 19.
### TABLE 1

**RESULTS:**

**Experiment Number 1**

**Regular Dose Strain 19**

<table>
<thead>
<tr>
<th>Vaccinated</th>
<th>Challenged</th>
<th>Total Number</th>
<th>Infected** (\text{w/2308} (%))</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 19</td>
<td>Yes</td>
<td>51</td>
<td>31 (61%)</td>
<td>39%</td>
</tr>
<tr>
<td>LCA*</td>
<td>Yes</td>
<td>107</td>
<td>22 (21%)</td>
<td>79%</td>
</tr>
<tr>
<td>Strain 19</td>
<td>No</td>
<td>6</td>
<td>0 (0%)</td>
<td>100%</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>Yes</td>
<td>50</td>
<td>49 (98%)</td>
<td>2%</td>
</tr>
</tbody>
</table>

*Lysed, Charcoal-Adsorbed Strain 19

**None were infected with Strain 19**

### TABLE 2

**RESULTS:**

**Experiment Number 2**

**Reduced Dose Strain 19**

<table>
<thead>
<tr>
<th>Vaccinated</th>
<th>Challenged</th>
<th>Total Number</th>
<th>Infected** (\text{w/2308} (%))</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 19</td>
<td>Yes</td>
<td>44</td>
<td>6 (13.6%)</td>
<td>86.4%</td>
</tr>
<tr>
<td>LCA*</td>
<td>Yes</td>
<td>45</td>
<td>3 (6.7%)</td>
<td>93.3%</td>
</tr>
<tr>
<td>Strain 19</td>
<td>No</td>
<td>7</td>
<td>0 (0%)</td>
<td>100%</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>Yes</td>
<td>55</td>
<td>55 (100%)</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Lysed, Charcoal-Adsorbed Strain 19

**None were infected with Strain 19**
FIGURE 3. Guinea Pig Serology, 50 Days Postvaccination (45 Animals Per Group)

SRC vs USDA VACCINE

RIVANOL TEST

PERCENT POS

DILUTION (1:xxx)
FIGURE 4
Test Method--Bovine Serological Studies:

1. Vaccinate one set of cows with standard strain 19 vaccine (reduced dose).

2. Vaccinate one set of cows with killed, charcoal-adsorbed vaccine.

3. Collect blood samples periodically and perform serological tests.
   a. Card Test
   b. Standard Tube Agglutination Test
   c. Rivanol Test
   d. Complement Fixation Test*,
   e. ELISA*,
      *Performed by the USDA Laboratory, Austin, Texas.

4. Give booster vaccination to experimental cows after titers subside.

5. Collect blood samples and perform serological tests.
VACCINE FOR BRUCELLOSIS

FIGURE 5
Bovine Serology. Five Calves Vaccinated with Killed Strain 19; Three with Live Strain 19.

SRC vs USDA VACCINE
CARD TEST

SRC vs USDA VACCINE
RIVAROL TEST

DAYS POST-VACCINATION

DAYS POST-VACCINATION

SRC vs USDA VACCINE
TUBE AGG. TEST

SRC vs USDA VACCINE
ELISA

DAYS POST-VACCINATION

DAYS POST-VACCINATION
FIGURE 6.
Bovine Serology. Five Calves Vaccinated with Killed Strain 19; Three with Live Strain 19.
FIGURE 7. Serological Results of Two Calves Boosted with Killed Strain 19.
FIGURE 8. Serological Results of Two Calves Boosted with Killed Strain 19.

SRC BOOSTER STUDY

TUBE AGG.

SRC BOOSTER STUDY

ELISA

DAYS POST-BOOSTER
VACCINE FOR BRUCELLOSIS

FIGURE 9. Serological Results of Two Calves Boosted with Killed Strain 19.

FIGURE 10
ADVANTAGES OF KILLED VACCINE:

1. At least as effective as strain 19 in laboratory animals.

2. No danger of inducing bovine infection.


4. In limited bovine studies, it produces a similar serological response to that produced by strain 19.


6. Provides a permanent marking of the animal
REFERENCES


THE USE OF ELISA AND COMPLEMENT FIXATION TESTS IN MANAGING FIELD OUTBREAKS OF *BRUCELLA ABORTUS*  
PART II  
Scott L. Reynolds, DVM, MSPH  
TEXAS ANIMAL HEALTH COMMISSION  
Austin, Texas

INTRODUCTION

In an earlier report, the enzyme-linked immunosorbent assay (ELISA) was shown to be more sensitive than the automated complement fixation test (ACF) in detecting antibody activity in adult vaccinated animals infected with Field Strain *Brucella abortus*. Twelve cattle negative to the ELISA on initial tests showed positive ELISA titers 30 to 120 days prior to positive ACF tests. Five animals tested positive by the ELISA and ACF tests during the same test period. Furthermore, six animals that were ELISA positive 30 to 120 days prior to slaughter were either negative or suspect to the ACF test at time of slaughter. Of these 28 animals, 23 were bacteriologically cultured and showed positive assays to Field Strain *B. abortus*.¹

These earlier studies were designed to compare the effectiveness of ELISA and ACF tests in predicting infection commencing 120 days post-adult vaccination. Cattle, vaccinated as adults, are not normally retested for four to six months. During this time, some cows fail to develop protective immune responses prior to exposure. Consequently, susceptible animals may be exposed to *Brucella* contagious cows. This study addresses this problem and reports on the use of the ELISA and ACF tests to predict low grade infections prior to and after adult vaccination with *B. abortus* vaccine.

STUDY DESIGN

Animals

Four herds consisting of adult cattle were used for this study. Herd A was a commercial beef herd containing 263 animals. The herd experienced an abortion storm involving 27 animals 90 days prior to adult vaccination. During this time period, ten of these aborters were removed from the herd as reactors. Seventy-two reactors, of which 17 were the remaining aborters, were removed from the herd at time of adult vaccination. Herd B was a commercial beef herd containing 271 cattle and had no previous history of brucellosis. One hundred-thirteen reactors were removed at the time of adult vaccination. Herd C was a commercial beef herd containing 112 cattle and had no previous history of brucellosis. Forty-two reactors were removed at the time of adult vaccination. Herd D was a commercial beef herd containing 47 cattle and had no known history of brucellosis. Eighteen reactors were removed from the herd at the time of adult vaccination.
Animal Management

All cows were inoculated subcutaneously with $0.5 \times 10^9$ Strain 19 *Brucella abortus* organisms. Serum from all cattle was studied by the ELISA, ACF and card test at each test period. The rivanol test was performed only on card positive animals. All animals were retested 120 days after vaccination and 60 to 90 day intervals thereafter. Figure 1 is a block diagram depicting the management of animals tested for *Brucella abortus*. Thus, ACF positive animals were removed to slaughter at time of vaccination. Animals showing suspect titers only on the ELISA were isolated and those animals subsequently exhibiting reactors level titers by ACF tests were removed to slaughter. The main herd consisting of those animals originally seronegative on all tests was retested in 120 days and those animals exhibiting reactors level titers were removed to slaughter. Those animals subsequently becoming suspect on the ELISA test were isolated. All animals contained in the “ELISA suspect” herds were retested three times after the 120 day postvaccination retest. Animals in suspect herds remained isolated until 120 days after the last reactor was removed to slaughter. When available, lymph nodes were harvested on slaughter animals and bacteriologically assayed for *B. abortus*.

Lymph Nodes

The suprapharyngeal, mandibular, supramammary and internal iliac lymph nodes were removed. All tissues were packed in whirlpacks and stored immediately in ice until received at the laboratory. Lymph nodes were removed from surrounding fat, dipped in alcohol, flamed, and sliced. The cut surface was minced and rubbed over the surface of the medium. All tissue was assayed according to the procedures utilized by the National Veterinary Services Laboratory in Ames, Iowa, except Farrel’s media was substituted for W media.

CONVENTIONAL SEROLOGICAL TESTS

The card and rivanol tests were performed by the State/Federal Diagnostic Laboratory, Austin, Texas and interpreted as prescribed in the Brucellosis Eradication Uniform Methods and Rules (UM&R).

AUTOMATED COMPLEMENT FIXATION TEST

The Technicon Auto Analyzer II, located at the State/Federal Diagnostic Laboratory, Austin, Texas was used for automated complement fixation tests (ACF). A detailed description of reagents may be found in the Technico Instruction Manual on “Automated Complement Fixation Testing.” Diagrams of equipment and flow of serum and reagents have been pictured in detail. ACF results were interpreted according to the following standard: $20^+$ or greater = reactor, $10^+$ = suspect, and $5^+$ or less = negative.
USE OF ELISA AND FIXATION TESTS

ENZYME-LINKED IMMUNOSORBENT ASSAY

The enzyme-linked immunosorbent assay (ELISA) was performed at the State/Federal Diagnostic Laboratory, Austin, Texas. Equipment utilized for these studies was manufactured by Dynatech, Alexandria, Virginia. This equipment consisted of the following:

Dynatech MR 600 microplate reader, Dynatech Microshaker, Dynawasher II, dynadrops dispenser and Immulon II microtiter plates. Standards for the ELISA are as follows: Spectrophotometric absorbance values (SAV 0.000 to 0.599 = negative, SAV 0.60 to 0.999 = suspect and SAV 1.00 or greater = positive). For this study, all suspect and positive animals were classified as suspect. A detailed description of reagents and procedures are described by Heck.²³

RESULTS

Culture positive isolates from all four herds were identified as Biotype I.

Figure 2 summarizes the use of ELISA and ACF serodiagnostic tests in the management of brucellosis in Herd A. The herd consisted of 263 cows at the time of vaccination. Of these animals, 72 (27.4%) exhibited reactor level titers on all serodiagnostic tests and were removed to slaughter. Of the remaining 191 ACF negative animals, 46 were suspect on the ELISA. One hundred forty-five animals were negative on all serodiagnostic tests. At the 120 days postvaccination test period, 13 (28.3%) ELISA suspect animals exhibited reactor level titers on the ACF test. Conversely, only four animals from the previously serodiagnostic negative main herd showed reactor level titers. These four animals were the last reactors removed from the main herd. Not shown in Figure 2 is one reactor removed from the ELISA suspect herd seven months postvaccination. This animal was detected and isolated at the 120 day test period. It is of special interest that two animals aborted their calves while in the suspect herd. No abortions were experienced by cattle remaining in the ELISA negative main herd.

Figure 3 summarizes the use of the ELISA and ACF serodiagnostic tests in the management of brucellosis in Herd B. The herd contained 271 cows at the time of test and vaccination. Of these animals, 113 (41.7%) showed reactors level titers on all serodiagnostic tests and were removed to slaughter. The remaining herd containing 158 ACF negative cows had 16 ELISA suspects removed to isolation. One hundred forty-two animals were negative on all serodiagnostic tests. One reactor each was removed from the previously ACF negative and ELISA suspect herds at the 120 day postvaccination test. These two animals were the last reactors removed from either herd.

Figure 4 summarizes the use of the ELISA and ACF tests in the management of brucellosis in Herd C. The herd consisted of 112 animals at the time of vaccination. Of these animals, 42 (37.5%) exhibited reactor level titers on all serodiagnostic tests and were removed to slaughter.
Seven of the remaining 70 ACF negative animals were suspect on the ELISA. Sixty-three animals were negative on all serodiagnostic tests. One animal in the ELISA suspect herd aborted while isolated and was removed to slaughter as a reactor. The remaining seronegative main herd experienced the loss of one reactor animal at the 120 days postvaccination test period. No other reactors were removed from either herd.

Figure 5 summarizes Herd D and the use of the ELISA and ACF tests in the management of brucellosis. The herd contained 47 animals at the time of vaccination. Of these animals, 18 (38.3%) exhibited reactor level titers on all serodiagnostic tests and were removed to slaughter. The remaining herd containing 29 ACF negative animals had four animals isolated as ELISA suspects. Twenty-five animals were negative on all tests. One animal each in the suspect and negative herds exhibited reactor level titers. These two animals were the last reactors removed from Herd D.

A total of three animals in the ELISA suspect herds aborted their calves. No animals experienced abortion that remained in the seronegative herds. In addition, 245 reactors were removed from four herds prior to adult vaccination. Conversely, only 23 animals exhibited reactor level titers after these herds were vaccinated and the ELISA suspects isolated.

DISCUSSION

Table 1 summarizes the pooled results from all four herds comparing the efficiency of ACF and ELISA tests in predicting *Brucella abortus* infection 120 days prior to retest. At time of adult vaccination, 693 animals were tested and 245 removed from the herd as reactors. These animals exhibited reactor level titers on ACF as well as suspect level titers on ELISA tests. Seventy-three additional animals that were negative on ACF tests showed suspect titers on the ELISA. Sixteen of these ELISA suspects exhibited reactor level titers 120 days postvaccination. Only seven animals previously negative on all serodiagnostic tests showed reactor level titers at the 120 day postvaccination retest. At time of adult vaccination, the ACF test showed a 91.4% effectiveness in predicting brucellosis 120 days prior to retests. Conversely, when the ELISA was added to the ACF test, a 97.4% effectiveness was achieved.

These data suggest that the use of the ELISA and the subsequent isolation of suspects from the main herd reduced further exposure to animals that may not have experienced a protective immune response during the 120 days prior to retest. These results are further reinforced by the fact that no other reactors were removed from the ELISA negative herds after the 120 day retest. Furthermore, ELISA negative herds showed no reactors throughout a 12 month period after the last reactor was removed. It is of special interest that three animals in the suspect herds experienced abortions during a seven month postvaccination period. No animals in the ELISA negative herds had a history of abortion.

Those seven reactors noted above exhibiting negative ELISA titers were not discernible due to the lack of detectible antibody activity. This may be
explained either by a lack of individual responsiveness or a very low level of infection at time of test.

In addition, the ELISA showed similar effectiveness in four small herds not previously described. Two of these herds showed no ELISA suspects and exhibited no reactors during any postvaccination test period. One herd showed a single ELISA suspect at the time of vaccination. The other herd showed two ELISA suspects at the time of vaccination. Both herds experienced one reactor at the 120 day postvaccination test period.

This study shows that the use of the ELISA as an early indication of brucellosis, when used in conjunction with adult vaccination and the isolation of ELISA suspects, is a valuable serodiagnostic tool in the reduction of exposure to *Brucella abortus* infection.

Studies are currently in progress to investigate the efficacy of the ELISA test in latent calfhood infection. In addition, a 1,500 cow dairy herd is being utilized to further investigate the predictability of the ELISA and ACF tests prior to and after adult vaccination.

ACKNOWLEDGEMENTS

The author is grateful to Mary Menn, Roger Brasfield, Deanna Krawczyk, and Rick Nabors, State/Federal Diagnostic Laboratory, Austin, Texas for their support.

The author extends a special acknowledgement to Dr. James L. Lindstrom, Texas Animal Health Commission, for his invaluable field support in this study.

REFERENCES


POOLED RESULTS OF FOUR HERDS COMPARING THE ELISA AND ACF TEST IN PREDICTING BRUCELLA ABORTUS INFECTED ANIMALS PRIOR TO A 120 DAY RETEST

NUMBER OF CATTLE - 693

NUMBER OF ACF REACTORS AND ELISA SUSPECTS - 245

NUMBER OF ELISA SUSPECTS AND ACF NEGATIVE ANIMALS - 73

REACTORS AMONG ELISA SUSPECTS - 16

REACTORS AMONG SERONEGATIVE ANIMALS - 7

ACF TEST $\frac{245}{268} \times 100\% = 91.4\%$ EFFECTIVE

ACF + ELISA TEST $\frac{261}{268} \times 100\% = 97.4\%$ EFFECTIVE
FIGURE 1

A BLOCK DIAGRAM DEPICTING THE
MANAGEMENT OF CATTLE TESTED FOR BRUCELLA ABORTUS

HERD

NEGATIVE

ACF POSITIVE
ELISA SUSPECTS

ACF NEGATIVE
ELISA SUSPECTS

RETEST

SLAUGHTER

ACF POSITIVE
ELISA SUSPECTS

ACF NEGATIVE
ELISA SUSPECTS

SLAUGHTER

RETEST

RETEST X 3

RETEST X 3
FIGURE 2
A BLOCK DIAGRAM SUMMARIZING THE RESULTS OF TESTING HERD A THROUGH 120 DAYS POST ADULT VACCINATION

HERD A

263 CATTLE

MAIN HERD
191 ACF NEGATIVE

145 ELISA NEGATIVE

4 REACTORS
120 DAYS POST AV

46 ELISA SUSPECTS

13 REACTORS
120 DAYS POST AV

72 REACTORS

REMOVE AT TIME OF AV
FIGURE 3

A BLOCK DIAGRAM SUMMARIZING THE RESULTS OF TESTING HERD B THROUGH 120 DAYS POST ADULT VACCINATION

HERD B

271 CATTLE

MAIN HERD 158 ACF NEGATIVE

113 REACTORS

REMOVED AT TIME OF AV

142 ELISA NEGATIVE

16 ELISA SUSPECTS

1 REACTOR 120 DAYS POST AV

1 REACTOR 120 DAYS POST AV
FIGURE 4

A BLOCK DIAGRAM SUMMARIZING THE RESULTS OF TESTING HERD C THROUGH 120 DAYS POST ADULT VACCINATION

HERD C

112 CATTLE

MAIN HERD 70 ACF NEGATIVE

42 REACTORS

REMOVED AT TIME OF AV

63 ELISA NEGATIVE

7 ELISA SUSPECTS

1 REACTOR 120 DAYS POST AV

1 REACTOR (ABORTER) 120 DAYS POST AV
A BLOCK DIAGRAM SUMMARIZING THE RESULTS OF TESTING HERD D THROUGH 120 DAYS POST ADULT VACCINATION

HERD D

47 CATTLE

MAIN HERD
29 ACF NEGATIVE

18 REACTORS

REMOVED AT TIME OF AV

25 ELISA NEGATIVE

4 ELISA SUSPECTS

1 REACTOR
120 DAYS POST AV

1 REACTOR
120 DAYS POST AV
STATUS REPORT — 1984
COOPERATIVE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM

G. H. Frye, D.V.M.
Hyattsville, MD

Nineteen hundred and eighty-four has been a year of continuing progress in the Brucellosis Eradication Program. The traditional indicators by which progress is measured — newly infected herds, herds under quarantine, and market cattle reactors rates — have all declined during the year. The number of calves vaccinated increased for the seventh consecutive year and is now 230 percent higher than 1977, the year that the 30-year low in official vaccinations was recorded. It is also notable that each year since 1980 the number of calves vaccinated has exceeded the 7.2 million that were vaccinated in the previous peak year of 1964.

Thirty-three states had Brucellosis Information System (BIS) equipment at the end of the year and were either utilizing the system or were in the process of obtaining necessary personnel or training. Computerization of program records has been accelerated in many states, causing rapid expansion of the useable information in their data bases. These States are now experiencing a few of the benefits BIS will provide the brucellosis program when it is fully operational.

The initial plan to complete the development of Release II for Brucellosis Ring Test (BRT) and vaccination records and for programs currently in Release I by April 1 and for the rest of the system (epidemiology, indemnity, and certificates) by October 1984 has been delayed. The current goal is to initiate tests and acceptance of four of the seven programs in Release II in November of this year with implementation by April 1985. Test and acceptance of the remaining three programs will start in the spring with implementation by August 1, 1985.

A major change was made in the Uniform Methods and Rules (UM&R) during the year to require female dairy cattle born after January 1, 1984, that are 4 months of age and over to be official vaccinates if moved into or out of Class B States or areas and all female cattle born after January 1, 1984, that are 4 months of age to be official vaccinates if moved into or out of Class C States or areas.

The information on the following visuals is estimated due to the unavailability of data for the final month of the fiscal year.

Slide 1

At the end of Fiscal Year 1984, 18 States plus the Virgin Islands held Class Free status, 17 States plus Puerto Rico were Class A, 8 States were Class B, and 3 States Class C. In addition, four States have been approved for two-area classifications, Wyoming and Montana for Class Free and Class A areas, and Texas and Florida for Class B and Class C areas.
North Carolina, South Carolina, and Wisconsin qualified for Class Free status during the year, and Wyoming advanced all but two of its remaining Class A counties into Class Free.

Slide 2

There were 8,473 infected herds found in Fiscal Year 1984, a 14 percent drop from the 9,862 found last year. The majority of this decline occurred in the Class C States where 5,539 infected herds were found this fiscal year, 773 fewer than during the previous year. Class B States had 2,613 infected herds for a decline of 486, and Class A States had 319 for a reduction of 123. Twelve reactor herds were found in the Class Free States, only one of which was determined to have actual infection.

Slide 3

In Fiscal Year 1984, 88 percent of the infected herds were located in 8 States and 12 percent in the remainder of the country. Thirty-four States, each with fewer than 30 infected herds, accounted for 2.1 percent of the total. Eight States, having between 30 and 300 infected herds each, made up 9.9 percent, and 7 States with between 300 and 1,000 accounted for 54.3 percent of the infected herds found. One State, Texas, had 2,852 infected herds, 33.6 percent of the total for Fiscal Year 1984.

Slide 4

Infection was found in 195 dairy herds as a result of testing BRT suspicious herds. There were 2,369 suspicious ring tests of which 1,879 herds were blood tested. One hundred and sixty-one of the infected herds were found in Class B and C States while 34 were found in Class A States.

Slide 5

There were 14.6 million cattle tested under the Market Cattle Identification (MCI) program in Fiscal Year 1984 for an increase of 1.5 million over the previous fiscal year. Of these, 45.9 percent were tested at packing plants and 54.1 percent were tested at livestock markets and other places. The increase in MCI tests this year is due in part to drought conditions in certain areas of the country which caused greater than normal culling of herds located there.

Slide 6

The total number of cattle tested in 1984 was 20.1 million with 5.5 million of these tested on farm or ranch and 14.6 million tested under the MCI program. Despite increased testing, 30,000 fewer reactors were found than during Fiscal Year 1983 (124,000 vs 154,000).

Slide 7

The number of calves vaccinated reached a new high of 8.8 million in Fiscal Year 1984 for an increase of 700,000 over last year. Although this increase is gratifying, it is still less than might be expected considering the new movement requirements in the UM&R and recent industry emphasis on this aspect of the program.
Slide 8

The number of swine tested for brucellosis in Fiscal Year 1984 was 2.8 million, slightly higher than the number tested in Fiscal Year 1983. This total included 2.4 million tested at slaughter under the MST program and 447,000 tested on farms.

Slide 9

The reactor rate on all tests conducted declined from 0.058 percent in Fiscal Year 1983 to 0.039 percent this year. The rate on farm tests dropped from 0.12 percent to 0.057 percent and on MST tests from 0.039 percent to 0.036 percent during the year. This marks the first year that the reactor rate has been below the 0.1 percent level in each category.

Slide 10

One State, Illinois, attained Validated Brucellosis-Free Area status — Stage III — during the year, bringing to 26 the number of States that have achieved this goal. Over 60 percent of the nation's swine are now located in States that are free of swine brucellosis.


Eight states, Alabama, Arkansas, Connecticut, Georgia, Hawaii, Louisiana, New York, and Virginia were in Stage II. Eleven states, Florida, Kansas, Kentucky, Massachusetts, Michigan, Nebraska, New Jersey, North Carolina, Ohio, Oklahoma, and South Carolina were in Stage I. Seven states, Mississippi, Missouri, New Mexico, Oregon, Tennessee, Texas, and West Virginia remained in the “No Program” classification at the end of the year.

Slide 11

There was a substantial drop in the number of Validated Brucellosis-Free herds from 4,558 at the end of Fiscal Year 1983 to 3,171 at the end of this year. Much of this decrease can be attributed to a change in program methods in certain States where individual herd testing conducted for area validation purposes has helped maintain a high level of validated herds in the past.
Cattle Brucellosis

State Classifications

Free
Class A
Class B
Class C

September 1984
Brucellosis Eradication

Number of Infected Herds Found (According to State Classification)

<table>
<thead>
<tr>
<th>State Classification</th>
<th>New State Classification (Effective May 1, 1982)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certified-Free</td>
</tr>
<tr>
<td></td>
<td>Modified Certified</td>
</tr>
<tr>
<td></td>
<td>Noncertified</td>
</tr>
</tbody>
</table>

Thousands

20

15

10

5

0

1977 28 22 0
1978 27 23 0
1979 30 20 0
1980 31 19 0
1981 32 18 0
1982 32 18 0

Number of States in Each Classification (before May 1982)

Fiscal Year | Certified Free | Modified Certified | Non-Certified |
-------------|----------------|--------------------|---------------|
1977         | 28             | 22                 | 0             |
1978         | 27             | 23                 | 0             |
1979         | 30             | 20                 | 0             |
1980         | 31             | 19                 | 0             |
1981         | 32             | 18                 | 0             |
1982         | 32             | 18                 | 0             |

Number of States in Each Classification (Effective May 1, 1982)

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Class Free</th>
<th>Class A</th>
<th>Class B</th>
<th>Class C</th>
</tr>
</thead>
</table>
1983*        | 16         | 22      | 10      | 5       |
1984+        | 20         | 19      | 10      | 5       |

*States with dual status
Wyoming—Class Free and Class A
Texas and Florida—Class B and Class C
Montana—Class Free and Class A

*Estimated.
Brucellosis Eradication

Percent of Total Reactor Herds Found

*Fiscal Year 1984
Total Herds: 8,483

33.6%
States: 1
Herds: > 1,000
Total Reactor Herds = 2,852

2.1%
States: 34
Herds: < 30
Total Reactor Herds = 178

9.9%
States: 8
Herds: 30 < 300
Total Reactor Herds = 843

54.3%
States: 7
Herds: 300 < 1,000
Total Reactor Herds = 4,610

*Estimated.
Brucellosis Eradication

Milk Ring Test Results (BRT)

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

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Total Suspicious BRT Tests</th>
<th>Follow-up Herd Blood Tests</th>
<th>Infected Herds Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1971</td>
<td>2,840</td>
<td>801</td>
<td>801</td>
</tr>
<tr>
<td>1973</td>
<td>2,179</td>
<td>592</td>
<td>592</td>
</tr>
<tr>
<td>1975</td>
<td>2,450</td>
<td>411</td>
<td>411</td>
</tr>
<tr>
<td>1977</td>
<td>2,018</td>
<td>1,629</td>
<td>1,629</td>
</tr>
<tr>
<td>1978</td>
<td>2,586</td>
<td>435</td>
<td>435</td>
</tr>
<tr>
<td>1979</td>
<td>2,177</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>1980</td>
<td>3,091</td>
<td>317</td>
<td>317</td>
</tr>
<tr>
<td>1981</td>
<td>4,771</td>
<td>353</td>
<td>353</td>
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<td>1982</td>
<td>3,607</td>
<td>281</td>
<td>281</td>
</tr>
<tr>
<td>1983</td>
<td>3,519</td>
<td>243</td>
<td>243</td>
</tr>
<tr>
<td>1984*</td>
<td>2,369</td>
<td>1,875</td>
<td>1,875</td>
</tr>
</tbody>
</table>

*Estimated.
### Brucellosis Eradication

#### Market Cattle Testing Program

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>At Packing Plants</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974</td>
<td>60.6%</td>
<td>39.4%</td>
</tr>
<tr>
<td>1975</td>
<td>70.0%</td>
<td>30.0%</td>
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<tr>
<td>1976</td>
<td>69.6%</td>
<td>30.4%</td>
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<tr>
<td>1977</td>
<td>67.5%</td>
<td>32.5%</td>
</tr>
<tr>
<td>1978</td>
<td>62.2%</td>
<td>37.8%</td>
</tr>
<tr>
<td>1979</td>
<td>54.8%</td>
<td>45.2%</td>
</tr>
<tr>
<td>1980</td>
<td>41.7%</td>
<td>58.3%</td>
</tr>
<tr>
<td>1981</td>
<td>42.0%</td>
<td>58.0%</td>
</tr>
<tr>
<td>1982</td>
<td>44.4%</td>
<td>55.6%</td>
</tr>
<tr>
<td>1983</td>
<td>43.3%</td>
<td>56.7%</td>
</tr>
<tr>
<td>1984*</td>
<td>45.9%</td>
<td>54.1%</td>
</tr>
</tbody>
</table>

*Estimated

**Millions of Cows Blood Tested**

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
<th>8</th>
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<td></td>
</tr>
</tbody>
</table>

*Estimated
Brucellosis Eradication

Blood Testing: Cattle

Fiscal Year

*Estimated.
Brucellosis Eradication

Calves Vaccinated

Millions
8
6
4
2
0
1956 '58 '62 '66 '70 '74 '78 '81 '82 '83 '84

Fiscal Year

* Estimated.
Swine Brucellosis

Animals Blood Tested

Thous. Animals

5,500
5,000
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0


*Estimated. Fiscal Year
Swine Brucellosis
Infection Rate

Percent
0.5

Total Tests
On Farm
MST

Fiscal Year

*Estimated.
Swine Brucellosis
Program Stages—Sept. 30, 1984

Stage 3
(Validated-Free)

Stage 1

Stage 2

No Program
Swine Brucellosis

Validated Herds FY 1984*

*Validated
State or Territory

- None
- 1-25
- 26-100
- Over 100

Total Herds: 3171

*Estimated.
REPORT OF THE COMMITTEE ON BRUCELLOSIS

Minutes — Monday, October 22, 1984

(numerals on left indicate place on agenda)

Issues were discussed Monday and acted on at the Tuesday meeting.

Chairman: J. B. Armstrong, Kingsville, TX
Vice Chairman: John A. Cobb, Atlanta, GA

1. The meeting was called to order at 1:30 p.m. by Chairman Armstrong. He welcomed the group to Texas, outlined procedures for the meeting and introduced members of the subcommittees and the Scientific Advisory Committee. He noted that members of the Scientific Advisory Committee are now listed as members of the Brucellosis Committee but are non-voting members, at their request.

2. Dr. Billy Johnson reported on the National Brucellosis Eradication Program. The complete status report will be presented at the General Session, Thursday, Oct. 25. Highlights of his report are:

   There are now 18 Class Free states, 17 Class A, 8 Class B and 3 Class C. Two states have dual Free and A status and 2 have dual B and C status.

   The MCI rate has continued the downward trend with a .29% rate for FY 84, down from .37% in FY 83. The change in the UM&R to remove reactors from herd sell-outs and reclassified reactors from the totals may have caused some of the drop. The impact of reduced dosage Strain 19 vaccination cannot be measured but may also have contributed to the drop.

   The number of herds under quarantine on 8/31/84 was 6,120,
down from 6,817 on 8/31/83. This reduction is consistent throughout the country, with a few exceptions. One notable exception is Arkansas, where a task force is now in operation which should lead to reduced infection in the future.

The total number of newly infected herds fell from 6,782 in 1983 to 5,783 in 1984. The distribution of infection is as follows: Class Free — 0.1%; A — 4%; B — 31%; and C — 65%.

The estimated number of calves vaccinated during the year is 8.8 million, up about 800,000 from FY 83.

The FY 85 budget has not been finalized and they are now operating under a continuing resolution at the level of $73.4 million. The appropriation language specifies the following goals be met by the end of FY 85:

1. Not less than 5 states move from Class A to Free.
2. Not less than 3 states move from B to A.
3. Not less than 1 state move from C to B.
4. No state will move down in classification.

The UM&R has been reprinted in loose-leaf form so revised pages can be added easily in the future. A private firm has been contracted to rewrite the UM&R to make it easier to understand.

3. Dr. John Cobb reported for the panel that reviewed the MCI program standards for state or area classification as contained in the UM&R. The panel recommended:

1. A return to the gross MCI rate for classifying states and that all non-vaccinated animals positive to the present card test, and vaccinated animals with titers of +25 or greater to the rivanol test, or the equivalent reduced sensitivity card test, be counted as MCI reactors. Out-of-state reactors and identified reactors from known infected herds would not be counted in determining the gross MCI rate.
2. A study be made to determine if a need exists to change the infected herd rate classification standards to reflect the change in accounting methods of counting sold-out herds as infected herds.
3. Special consideration be allowed when a change in state classification is affected by unusual or extreme marketing conditions.

The panel felt that, in view of the language in the appropriations bill, these changes should be considered, possibly at a later date.

4. Dr. John Holcombe described the Texas Animal Health Commission Brucellosis program. He illustrated how the recent UM&R changes concerning the Adjusted MCI Reactor rate had affected the infected herd rate and the MCI reactor rate. He recommended a return to the 1983 method of calculating the herd infection rate and the adjusted
MCI reactor rate for state or area classification. The subcommittee that reviewed that consisted of John Cobb, Claude Barton, Robert Hartin, and John Holcombe.

5. Dr. John Hudelson reported for the subcommittee of Jack Dahl, Hudelson and W. D. Prichard. They suggested the following changes in the definition of the Official Eartag:

"An Official Eartag is USDA approved identification eartag (metal, plastic, or other) conforming to the nine character alpha numeric National Uniform Eartagging System and of the appropriate color (e.g. — orange for vaccination). It provides unique identification for each individual animal."

He showed examples of plastic tags and listed several companies that manufacture them. He listed the following advantages of the plastic tags over the metal tags:

— Fewer are lost.
— Can be read accurately with less restraint.
— Meat inspectors can cut them out easily.
— Owners and markets can read them easier (should discourage double-tagging).
— Many producers already use them.

Both plastic and metal are tamper-resistant, although neither are tamper-proof. The only disadvantage to the plastic, he said, was the cost (which varies with company and quantity ordered).

6. Dr. David Walker reported for the subcommittee of Walker, John Cargile, J.O. Pearce, and John O’Harra concerning changes in the official health certificates. The subcommittee recommended the following changes:

1. The name be changed to “Certificate of Veterinary Inspection”.
2. It include a veterinary certification statement signed by the accredited veterinarian: “I certify, as an accredited veterinarian, that the above described animals have been inspected by me and that they are not showing signs of infectious, contagious, and/or communicable disease, (except where noted). The vaccinations and results of tests are as indicated on the certificate. To the best of my knowledge, the animals listed on this certificate meet the state of destination and federal interstate requirements. No further warranty is made or implied.”

2. The following Owner/Agent statement be used where applicable and be signed by the Owner/Agent: “The animals in this shipment are as certified to and listed on this certificate.”

7. Dr. R. L. Hartin discussed concerns held by market veterinarians about their liability in the brucellosis program. He introduced Dr. Van Ricketts of the Springfield, Missouri Livestock Auction. Dr. Ricketts recommended the following addition to the CFR:
Part 78.25 (d) "... The specifically approved stockyard shall not dismiss that selected accredited veterinarian from his position at that stockyard or replace him with another accredited veterinarian without an informal hearing being conducted. The informal hearing shall be held in a convenient place to all parties and shall be attended by the Veterinarian in Charge and the State Veterinarian or State Animal Health Official, the stockyard management, the selected accredited veterinarian, and if determined necessary by the Veterinarian in Charge, any other state representative or veterinary service representative. If at the conclusion of such hearing it is determined by both the Veterinarian in Charge and the State Animal Health Official that the selected accredited veterinarian should be removed from his position or replaced they shall approve the selection of a new selected accredited veterinarian who meets with the approval of the stockyard management. If at any time a selected accredited veterinarian by his own volition and without any duress from any party wishes to be replaced by another accredited veterinarian he shall notify the stockyard management and the Veterinarian in Charge of his intentions sixty days prior to his expected termination date."

8. Dr. J. Lee Alley reported for the subcommittee of George Hall, Alley, and Taylor Wood. Part 71.18 of the CFR requires an owner to carry a statement when he transports livestock from his premises to a market in another state. The subcommittee felt there is no real benefit to this requirement and the Brucellosis committee should recommend it be removed from the CFR.

9. Dr. J. D. Kopec reported for Dr. G. H. Frye on the proposal to change vaccination requirements for imports from abroad (Title 9, Part 78 of CFR). He offered the following options:

1. Propose import regulation amendment consistent with the proposed interstate movement regulations under 9 CFR 78.
2. Require calfhood or adult vaccination and post-entry quarantine and testing upon entry into the United States for certain cattle imported into Class B and C states.
3. Amend import regulations to require that all female cattle be vaccinated prior to entry.
4. Incorporate vaccination exemption under 9 CFR 78 with the consent of the state veterinarian for cattle imported into the United States.

10. Report by Dr. Ragan was deleted from the agenda.

Dr. Garry Adams, Chairman of the Scientific Advisory Committee, reported for the Committee on the following concerns (Agenda items 11–14). More detailed answers to the concerns were distributed to Committee members.

11. Restriction on calves less than 6 months old from infected herds.

The Scientific Advisory Committee recommends that all heifer calves
from infected herds should be spayed or permanently identified in an 
unmistakable manner which is unrelated to vaccination status. Inter-
state movement of permanently identified heifers shall be governed 
by the regulations of the individual states.

12. **Requirement in UM&R that negative cream shall be used in all milk 
samples when conducting the BRT.**

The Scientific Advisory Committee recommends that negative 
gravity-separated cream continue to be added to all milk samples as 
given on page 36, Part 2, Section X of the UM&R to maintain a 
stabilized standard sensitivity and to enhance the ease of reading the 
BRT test. With regard to increasing the effectiveness of the BRT for 
detecting herd infection, the committee recommends that, as a state 
option, it is preferable to increase the frequency of BRT testing 
although adjusting the herd compensation rate to 60 cows per 1 cc of 
milk sample is also acceptable.

13. **Embryo transfers from reactor cows.**

The Scientific Advisory Committee recommends that as a state 
option, embryo transfer from brucellosis reactor cows be allowed as a 
pilot study. The herd, including the reactor donors, isolated recipients, 
and progeny must be maintained under quarantine conditions by the 
regulatory officials and collaborating scientists. Based upon pre-
viously published data, the committee recommends that the following 
guidelines be considered to reduce the likelihood of *Brucella abortus* 
third:  
1. Use only zona pellucida intact embryos.  
2. Wash the embryo through 10 high dilution serial washes.  
3. Use antibiotic supplemented flushing and collection media.  
4. Collect embryos only after 4 expressed estrous cycles post-
    parturition.  
5. Use recipients from certified brucellosis-free herds.  
6. Strictly isolate recipients following embryo transfer.  
7. Monitor both the recipients and the embryo transfer progeny until 
    2 months after each have calved.  
8. Identify and protect progeny from environmental exposure.

The Committee also recommended that funding be provided to sup-
port these definitive research efforts.

14. **Quarantine of a herd without testing.**

The Scientific Advisory Committee proposed the following mea-

ures: These would apply only in Class C areas and with approval of 
each individual herd plan by the State/Federal regulatory authorities. 
For a defined period (maximum 2 years) all animals that can be 
assembled would be adult and calfhood vaccinated. The frequency of 
gathering and the percentage of the herd to be assembled should be 
the maximum possible within limits imposed by the nature of the
ranching operation. Over the two year period all animals should be vaccinated. In order to reduce the rate of transmission while herd immunity is being developed through vaccination, on-site serological testing and reactor culling, with appropriate indemnity payments, shall be undertaken. Quarantine and other measures to prevent and detect spread of infection to adjacent herds will be taken. Cattle would leave the herd only for slaughter (S branded). After the initial two years, calfhood vaccination would continue along with serological testing and removal of reactors, if necessary.

Chairman Armstrong urged the Committee members to attend the General Session Thursday morning to hear reports on a Brucellosis vaccine by Drs. Brewer and Foster and the use of Elisa and CF tests by Dr. Reynolds.

Chairman Armstrong informed the Committee that the recommendations of the Scientific Advisory Committee will be first on the agenda at Tuesday's meeting (1:30 p.m.).

16. Dr. F. M. Enright presented information on the control of Brucellosis in the marshes of Louisiana — an alternative to conventional control.

(Dr. Enright gave me the following summary: The study was supported by the LA Dept. of Ag. It is now in the 4th year of a 5 year project. The major conclusion so far is that there is no difference in the number of new cases of Brucellosis in the Plan B herds (those with retained reactors) and the Plan A herds (with reactors removed). The herds were adult vaccinated and tested—reactors removed from Plan A herds and left in Plan B herds. Cost effectiveness: Advantages — 1) less frequent testing (savings in manpower to producer and regulatory agency); 2) Producers were able to maintain their breeding age cattle; 3) High degree of producer acceptance and cooperation. Disadvantages — 1) loss of market options (cattle are only moved to slaughter); 2) increased risk of spread to adjacent herds, however, the adjacent herds were already infected in this project. Dr. Enright stressed that this project was specifically designed for this area and would not be appropriate everywhere.)

15. Dr. Joe Hendricks presented a paper on four decades of Brucellosis eradication in Puerto Rico — some advantages of adult vaccination.

17. Report from Dr. Deyoe was deleted from the agenda.

18. One year ago, an ad hoc committee was appointed to review the Complement Fixation test procedures. Dr. Wynn Ray was chairman and the committee became non-functional upon his retirement. Dr. G. M. Brown, NVSL, Ames, IA, was recently appointed chairman. The committee will have a report at next year's meeting.

19. Dr. Brian Espe presented a paper on the socio-economics in Brucellosis control — results of a survey conducted in Oklahoma and
Arkansas by the Kerr Foundation, Winrock International and the University of Arkansas.

20. Dr. F. J. Drazek presented an overview of the national Brucellosis program.

The committee was adjourned until 1:30 p.m., Tuesday October 23, 1984.

USAHA BRUCELLOSIS COMMITTEE

Minutes — Tuesday, October 23, 1984

The meeting was called to order at 1:30 p.m. by Chairman John B. Armstrong.

The proposals and recommendations presented at Monday’s meeting were discussed and acted upon. (In this report, the numbers on the left indicate the agenda item and the place in the minutes of Monday’s meeting.)

Dr. Paul Doby reported for the Swine Brucellosis Subcommittee. The report included a motion, which was passed by the subcommittee, that if domestic swine in a state are free of brucellosis under program standards and there is no direct evidence of association between feral and domestic swine, a state may be validated, regardless of the brucellosis status of the feral swine in that state.

The subcommittee’s report was approved and accepted by the committee. The complete report appears at Attachment #1 to these minutes.

The recommendations of the Scientific Advisory Committee were discussed next.

11. The recommendation to spay or permanently identify all heifer calves from infected herds:

The motion to accept that recommendation was amended by the subcommittee. That amendment failed.

A substitute to the original recommendation was moved. The substitute motion stated that this issue be assigned to a subcommittee to be reviewed further and an economic impact statement be prepared on this subject.

The substitute motion passed.

12. The recommendation concerning negative cream use in milk samples when conducting the BRT:

It was moved and seconded to accept the recommendation as presented Monday. The motion passed.

13. The recommendation about embryo transfers from reactor cows:

The Scientific Advisory Committee clarified the guidelines presented Monday. They amended guideline #7. That guideline now reads as follows: “7. Monitor both the recipients and the embryo transfer progeny until 2 months after each have calved or two years of age for bulls.”
It was moved and seconded to accept the recommendation as amended. Motion passed.

14. After much discussion, the Scientific Advisory Committee withdrew the recommendation regarding the “quarantine of a herd without testing”. They stated that the current UM&R allows adequate flexibility to provide an individual herd plan adaptable to practically all circumstances. The proposed approach of “quarantine of a herd without testing”, that is, without surveillance and vaccination, does not provide an epidemiological program for eliminating infection, improving herd immunity, and protecting neighbors.

3-4. Dr. John Cobb reported for the subcommittee that dealt with the two proposals concerning the MCI reactor rate and the effect on state or area classification. He presented the following report:

These proposals combine the recommendations of the panel convened on August 8, 1984, to study the MCI program standards for state or area classification and those of the subcommittee appointed to consider the proposal to return to the 1983 method of calculating the herd infection rate and adjusted MCI reactor rate for state or area classification. The proposals are:

1. MCI reactors for purposes of state or area classification are defined as nonvaccinated animals positive to the present card test and vaccinated animals with titers of +25 or greater to the rivanol test or the equivalent reduced sensitivity card test.

2. Sold out MCI reactors will not be counted in the accumulative infected herd rate for classification of states or areas. The reactors from those herds will be included in the adjusted MCI reactor rate. However, for this procedure to apply, the following conditions must be satisfied.
   a) A complete epidemiological investigation must be done on all sold out MCI reactor herds, including adjacent herd testing where necessary.
   b) Sold out MCI reactor herds will be reported in the remarks section of the Monthly Report of Brucellosis Activities (VS Form 4-33D). These herds will be summarized and reported in the quarterly MCI report prepared by the APHIS, VS staff as well as the annual brucellosis statistical tables.

3. Special considerations will be allowed when a change in state or area classification is affected by unusual or extreme marketing conditions.

It was moved and seconded to accept the recommendations above. Motion passed.

Dr. Cobb presented the following recommendation from the same subcommittee: Interest has been expressed in returning to a gross MCI rate as a classification standard. The subcommittee recommends a panel be appointed to evaluate the gross MCI reactor rate for all
REPORT OF THE COMMITTEE

states and develop the levels that would be appropriate for each classification category.

It was moved and seconded to accept the recommendation. Motion passed.

5. Jack Dahl reported for the subcommittee of Dahl, Hudelson and Prichard. That subcommittee recommended that the UM&R be changed to include the following language for the definition of an official eartag:

"An Official Eartag is a USDA approved identification eartag (metal, plastic, or other) conforming to the nine character alpha numeric National Uniform Eartagging System and of the appropriate color (e.g. — orange for vaccination). It provides unique identification for each individual animal.

It was moved and seconded to accept the recommendation. Motion passed.

Dr. Billy Johnson presented the following recommendation to resolve the problem in some states of running out of combinations when using the V prefix for vaccination tags:

"States which use a complete series of vaccination tags from "VAA" thru "VZZ" in less than 15 years will use the state prefix and a letter "T" or "S" followed by two letters and four numbers. After 15 years states will reuse the "V" series of tags. Series of letters beginning with these two letters would be restricted for use on vaccination tags only."

It was moved and seconded to accept the recommendation. Motion passed.

6. Dr. David Walker recommended adoption of the wording for Health Certificates that was presented Monday, with the following change in the Owner/Agent Statement.

   The Owner/Agent Statement would read: "The animals in this shipment are those certified to and listed on this certificate."

   It was moved and seconded to accept the recommendation as presented on Monday with the above change. Motion passed.

7. Dr. Walker reported for the subcommittee that studied the proposal to amend Title 9, CFR, part 78.25 (liability of market veterinarians).

   "The subcommittee recommends against adoption of the proposal to amend Title 9, CFR, part 78.25 at this time. While merit may be found for investigation by state and federal officials in individual situations where undue pressure is applied against accredited veterinarians, the matter seems best handled through the market approval process. Furthermore, this committee is of the opinion that there is ample basis in law to prosecute for intimidation of those individuals carrying out regulatory duties."

   It was moved and seconded to accept the recommendation above. Motion passed.
8. The subcommittee that reviewed the Owner/Shipper statement agreed with the report given Monday. They proposed that the language in Part 71.18 of the CFR (requiring an owner to carry a statement when he transports livestock to a market in another state) is not needed and should be removed from the CFR.

   It was moved and seconded to accept that recommendation. Motion passed.

9. Dr. G. H. Frye reported on the proposal to change vaccination requirements for imported cattle.

   Problem — Certain cattle moving into or out of Class B and C states must be vaccinated. Current import regulations do not require brucellosis vaccination for cattle entering the United States.

   Recommendation — “It is recommended that appropriate portions of 9 CFR be amended to provide an exemption to allow importation of non-vaccinated cattle with the concurrence of the state veterinarian of the state of destination. This prior concurrence would be included as a condition on the importation permit.”

   It was moved and seconded to accept the recommendation as amended (amendment language is underlined). Motion passed.

Dr. Clint Jewitt presented the report of the Education Subcommittee. The complete report and the motion the subcommittee passed appears as attachment #2 in these minutes. It was moved and seconded to accept the report as presented. Motion passed.

Point of clarification — Use of plastic or metal eartags would not affect the use of the nine character numbering system. There would be no duplicate numbers between the plastic and metal tags. All committee members agreed.

Dr. John O’Hara presented the following resolution:

   “Be it Resolved: The United States Animal Health Association expresses its appreciation to John B. Armstrong for his untiring effort to gain acceptance of the industry for use of the reduced dosage of Brucella vaccine in the Brucellosis program in Texas. This vaccination program has proved to be of great benefit in the control and eradication of Brucellosis in this state.”

   The resolution passed unanimously.

   There being no further business, the committee adjourned at 3:30 p.m.

**REPORT OF THE SUBCOMMITTEE ON SWINE BRUCELLOSIS**

Dr. Paul B. Doby, Chairman

The meeting was called to order at 1:30 p.m., October 22, with all members of the Subcommittee, or their representatives, present during at least a portion of the discussions.
Chairman Doby introduced new members and recognized the service of the late B. D. Spahr to the Subcommittee.

Dr. Victor F. Nettles of the Southeastern Cooperative Wildlife Disease Study, Athens, Georgia, reviewed the situation with regard to brucellosis in feral swine (copy of paper attached). He pointed out that the feral swine population in the U.S. is estimated at about one million hogs in 18 states, plus Puerto Rico and the Virgin Islands; with Florida, Texas, Hawaii and California having the largest populations. A total of 1,366 samples from feral swine in a number of those areas indicates a prevalence of 11.8 percent positive for brucellosis. He posed two questions for the Subcommittee: (1) Does enzootic swine brucellosis in wild swine pose a threat to the success of the eradication program in domestic animals?, and (2) If a threat exists, what can be done to eliminate the disease risk?

He noted that no positives have been found in some feral swine populations and urged that steps be taken to avoid infecting them. He also stated it is possible to eliminate a population of feral swine but extremely difficult to do so, especially to eliminate the last remaining vestiges of the population.

He urged that controls be intensified on movements of such swine from one area to another.

Chairman Doby asked for Nettles’ assistance in contacting fish and game organizations in furtherance of the objective of better controls on movements of feral swine. Dr. Nettles volunteered to be of assistance to the Subcommittee.

Dr. Nettles suggested a study be made on whether reduction of the density of a feral swine population would be effective in eradicating a disease in such a population.

Dr. Granville H. Frye of APHIS, USDA, reported on program status. He noted the total number of swine tested during 1984 increased slightly from the previous year to a total of 2.8 million tested, including 2.4 million at slaughter and 477,000 on farms.

The reactor rate, which has declined since 1976, dropped to .039 in 1984 from .058 in 1983.

Illinois has been added to the list of validated states, bringing the total to 24, plus Puerto Rico and the Virgin Islands, with the validated states containing 60 percent of the nation’s hogs.

The number of validated herds in the country declined from 4,558 in 1983 to 3,171. Dr. Frye indicated this is a result of apparent discontinuance of herd validations in some states, especially in the northeast where there is very little or no brucellosis.

The number of infected herds by state is as follows: Alabama — 1, Florida — 4, Georgia — 28, Hawaii — 7, Maine — 1, Louisiana — 1, Massachusetts — 6; a total of 48.

Frye reported that of the 783 MST reactors disclosed during the year,
371 were identified and 412 were not identified. A total of 308 reactors were successfully traced.

Frye said Dr. Mitchell Essey has been assigned to the swine brucellosis program by APHIS, VS.

Chairman Doby called for discussion of the Subcommittee's recommendation with regard to validating states which have feral swine populations which may be infected with brucellosis. The precedence of the cattle brucellosis program of ignoring infection in the wild bison herd in Yellowstone Park was noted, along with the decreased intermingling of domestic swine with feral swine as a result of current confinement swine production systems.

Dr. Merle Lang moved that if domestic swine in a state are free of brucellosis under program standards and there is no direct evidence of association between feral and domestic swine, a state may be validated regardless of the brucellosis status of the feral swine in that state. The motion was seconded and carried. There was one dissenting vote.

It was pointed out, in clarification of that recommended policy, that in cases where feral swine are trapped and fed out as domestic swine, they would then be considered domestic swine for purposes of the program and would be required to meet test regulations under program standards.

Dr. John A. Cobb, Georgia State Veterinarian, reported on the status of the program in that state. He said state funding has been proposed in the amount of $65,000 the first year, and half that amount the next two years. "I think we can get rid of swine brucellosis with a program funded in that amount, except for infection in feral swine." He said the backtag being used in the state program is working well. "We have a good surveillance program and are finding infection, the problem is what we do with it after we find it," he said, referring to inadequate indemnities — a problem which would be resolved with the funding that he anticipates.

The following responses were made by Frye to recommendations of the Subcommittee in 1983:

Recommendation for amending CFR Part 78 to eliminate the option of the slap tattoo for identification of boars because of the difficulty of enforcement: No action was taken pending the study on boar identification. Fry indicated there was a misunderstanding of the intent of the recommendation and that action could be taken if the recommendation was renewed.

Effectiveness of the boar as a surveillance device: The data acquired from infected herds continue to indicate that about half the boars are infected at the time such herds are disclosed.

Discontinuation of testing of sows as a monitoring device, with substitution of testing of boars only in validated states: APHIS did not feel it could drop the required identification of sows in view of other impending programs such as pseudorabies, but has included an alternate method of revalidation of validated states which would allow
REPORT OF THE COMMITTEE

revalidation but no initial validation on the basis of testing of 90 percent of boars and tracing of 90 percent of reactors.

Increase in indemnity payments: Funds are not available for increasing the amount of indemnity paid per animal in the program. Frye estimated annual federal indemnity expenditures in the swine brucellosis program at $3,000 to $4,000.

Frye pointed out that APHIS hopes to accelerate the program in states with high prevalence, using first point testing with an emphasis on traceback and better identification, as well as in states with a very low prevalence.

Relaxation of testing requirements in validated states: Change of ownership testing is not required by the states if revalidation can be achieved by other testing methods. States now have the option to accept swine from free states without a test, so the option to begin relaxing requirements in validated states which have had no positives for a number of years already exists.

An Ohio proposal regarding a suggested change in Brucellosis Eradication Uniform Methods and Rules, Chapter 2, Part I B, was resolved by interpretation by Frye. The question involved movement of a group of hogs from a single herd in which a card test positive was disclosed, and which was negative on a rivanol test. Frye indicated that the Uniform Methods and Rules requires the herd of hogs be quarantined only until the rivanol test is completed and if it is negative, all hogs except the card test positive-rivanol negative swine may be released from quarantine, with no further samples and testing being required.

There being no further business, the meeting was adjourned.

REPORT OF THE EDUCATION SUBCOMMITTEE

Dr. Clint Jewett, Chairman

The committee met at the Hyatt Regency Hotel at 7:00 a.m. in Room “Texas ‘B’”.

Members Present: Clint Jewett, Chairman; Sid Moore, Co-Chairman; Tom Cook; Bill Alexander; Neal Black; Joyce Mitteness; Jim Horne; John Cargile; Larry Marck; Don Nielson; and Clark Bolt.

1. Jim Horne reported on The Kerr Foundation involvement in the Brucellosis Program.
   The foundation produces a number of AV aids and bulletins which are being used in the field.
   They send bulletins to 300 DVM’s, 250 lending agencies and 1700 producers.
   They have produced hand out materials that would be of benefit to any program.

2. Jim Horne reported on a survey of 100 producers in Arkansas and 100 producers in Oklahoma.
78% are part time farmers with an average herd size of 28 in Oklahoma; and 19 in Arkansas.
60% of these farmers derive less than 25% of their income from cattle. In these areas, the Progressive Farmer magazine was the predominant source of agriculture information. Second on the list was the DVM practitioner. Down the list was the county agents, Vo-Ag teachers and regulatory employees. It is recommended that the committee try to receive wider coverage through agricultural periodicals.
60% of these farmers do not have cattle handling facilities and 15% or more do no vaccination at all. Sale barn operators and Vo-Ag teachers were not important sources of information.

3. Sid Moore reported on the radio announcements.

4. The state information offices' program needs to be initiated again and importance placed on its value.

5. Extensive effort needs to be placed on information being disseminated through the FFA chapters and Vo-Ag programs. There are 8300 chapters with 540,000 students in the United States.

6. John Cargile made a motion and it was accepted to seek active support by the Extension Services to fulfill the obligation included in the explanation for the motion.

7. Sid Moore is leaving the Education Committee and I am pleased to announce that Jim Horne of the Kerr Foundation will replace Sid.

8. The committee acknowledges Sid Moore for the outstanding work that he has provided for this committee and to the Brucellosis Committee.

Motion #1

Producer ignorance of brucellosis and the brucellosis eradication program is a major reason for the lack of participation and success of the program. Success of the program is directly related to the cooperation and understanding of the producer.

The extension service, particularly in rural areas where county agents, beef and dairy specialists are present, should be an ideal vehicle for disseminating information and educating cattle producers.

County agents have been slow to conduct producer educational programs because of the controversy of the brucellosis eradication program.

BRUCELLOSIS IN WILD SWINE

Victor F. Nettles
Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine
The University of Georgia
Athens, Georgia

Wild swine are present in 18 states, Puerto Rico, and the U.S. Virgin Islands.
Islands. The nationwide population inventory probably is about 1 million hogs with Florida, Texas, Hawaii, and California being the leading states. Several independent studies have disclosed that wild swine can harbor important domestic swine diseases, including swine brucellosis.

In 1970–71, 268 wild swine in Hawaii were tested serologically and 21 (8%) were found positive. *Brucella suis* of unspecified biotype was recovered from 3 of 4 animals cultured. An 18 percent reactor rate (46 of 255 animals) was found in a South Carolina wild swine population in 1974–75. Culture attempts yielded a *B. suis* biotype 1. An additional infected population was identified in Glades County, Florida, in 1977–78. Here, 50 of 95 (55%) animals were seropositive and *B. suis* biotype 1 was isolated from 9 animals.

Beginning in January 1979, the Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, initiated a region-wide survey of diseases in wild swine. Eight wild swine populations in four states (FL, GA, LA, and SC) had seropositive animals. *Brucella suis* biotype 1 was isolated from 2 areas in Florida and 1 area in Louisiana.

California wild swine were recently surveyed by Dr. David Jessup of the California Fish and Game Department. Seven counties were studied and serologic positives were found in two areas. *Brucella suis* biotype 3 was recovered from some animals. Swine in Hawaii were resurveyed in 1983 and 12 of 113 animals tested were seropositive. A small sample of 10 pigs was taken from the only wild swine population in Arizona and all animals were found negative.

The total number of serum samples taken for all of the aforementioned studies was 1366, and 161 (11.8%) were considered positive. Although these data do not constitute a random sample of the Nation's wild swine, there is little doubt that swine brucellosis is enzootic in these animals in many places. In consideration of the State-Federal Brucellosis Eradication Program in domestic swine, two questions need answers. First, does enzootic swine brucellosis in wild swine pose a threat to the success of the eradication program in domestic animals? Second, if a threat exists, what can be done to eliminate the disease risk? These questions are not posed to express pessimism in current programs. Instead, this information was provided to the Swine Brucellosis Subcommittee of the USAHA Committee on Brucellosis to apprise its members of a potential area of concern.
LEPTOSPIROSIS: A NEW APPROACH TO A CONTINUING PUBLIC HEALTH PROBLEM

Presentation by Ernest T. Takafuji, M.D., at the USAHA Meeting, Fort Worth, Texas, October 26, 1984.

Leptospirosis continues to be a public health problem throughout the world. High incidence rates associated with significant morbidity have been reported in populations particularly in tropical regions, with domestic animals and wildlife being reservoirs of infection. With the deployment of military personnel to such tropical areas, the risk to the soldier of acquisition of infection when exposed to contaminated environments is especially great. Recent experiences in Panama have clearly identified leptospirosis as a common cause of febrile illness and a significant health threat to U.S. forces undergoing jungle training at Fort Sherman in the Republic of Panama. Environmental studies of the training environment by military medical authorities in Panama have confirmed the high prevalence of infection among wildlife and have documented contamination of the training environment with the same leptospiral serovars infecting soldiers.

Recent medical research studies conducted by the U.S. Army at the Walter Reed Army Institute of Research, Washington, D.C. demonstrated the efficacy of doxycycline against leptospirosis when administered in a regimen of 200 mg. orally on a weekly basis during and immediately following exposure. This regimen has been shown to be associated with few side effects and has now been adopted into a health policy for trainees at Fort Sherman in Panama. The incidence of disease has now been significantly reduced among jungle warfare trainees. These findings provide a new approach to the prevention of leptospirosis, and other high risk occupational groups with well-defined exposure could benefit from this type of prophylaxis.

Medical research on leptospirosis has also been conducted in the areas of early diagnosis and therapy. A medical study conducted by the U.S. Army Medical Department has indicated that Doxycycline is also effective in reducing the morbidity of disease if administered within the first two days of infection. A rapid diagnostic dot-ELISA test developed at the Walter Reed Army Institute of Research directed at confirmation of infection during early illness has been developed and will soon be field-tested. Additional chemoprophylaxis and chemotherapeutic studies in other geographical areas are contemplated to address other issues of concern.

AN EFFICACY TRIAL OF DOXYCYCLINE CHEMOPROPHYLAXIS AGAINST LEPTOSPIROSIS

Ernest T. Takafuji, M.D., James W. Kirkpatrick, M.D., Richard N. Miller, M.D., Jerome J. Karwacki, M.D., Patrick W. Kelley, M.D., Michael R. Gray, M.S., K. Mills McNeill, M.D., Harold L. Timboe, M.D., Robert E. Kane, M.D., and Jose L. Sanchez, M.D.
Abstract

Because leptospirosis has been an important cause of morbidity in U.S. soldiers training in the Republic of Panama, we conducted a randomized, double-blind, placebo-controlled field trial during the fall of 1982 to determine whether doxycycline was an effective chemoprophylactic agent against this infection. Doxycycline (200 mg) or placebo was administered orally on a weekly basis and at the completion of training to 940 volunteers from two U.S. Army units deployed in Panama for approximately three weeks of jungle training. Twenty cases of leptospirosis occurred in the placebo group (an attack rate of 4.2 per cent), as compared with only one case in the doxycycline group (attack rate, 0.2 per cent, P<0.001), yielding an efficacy of 95.0 per cent. This study demonstrated the value of doxycycline as a prophylactic drug against leptospirosis. (N Engl J Med 1984; 310:497-500.)

For many years leptospirosis has been recognized as an occupational hazard of U.S. soldiers training in the Republic of Panama. In 1981 and 1982, several large outbreaks of this disease occurred among soldiers training during the fall. Attack rates ranging from 2 to 8 per cent in military units were documented (Takafuji ET: unpublished data).

Preventive measures against leptospirosis have been unsatisfactory. Vaccines directed against prevalent serovars (serovarieties of *Leptospira interrogans*) are currently used in domestic animals, but serovar-specific vaccines have limited usefulness in tropical environments with numerous serovars. Environmental control measures and the wearing of protective clothing have not been practical. Field trials conducted in the 1950s among rice-field workers suggested that those taking penicillin daily had a reduced risk of contracting leptospirosis. In 1957, however, Broom and Norris reported that penicillin was not effective in preventing infection; they suggested that oxytetracycline might be capable of modifying the course of illness.

Recent field studies with volunteers demonstrated that 200 mg of doxycycline administered weekly was effective in the prevention of scrub typhus, a rickettsial disease with an incubation period similar to that of leptospirosis. The drug has also been used in the prophylaxis of travelers' diarrhea. In this study we report on the efficacy of doxycycline in the prevention of leptospirosis.

METHODS

Study Location and Population

The study was conducted at the Jungle Operations Training Center at Fort Sherman, a military installation on the Atlantic side of the Panama Canal region. Two military groups were studied during and after their 2½ to 3 weeks of jungle training in the fall of 1982. The first group (Deployment I) consisted of 790 men assigned to the 82nd Airborne Division stationed at Fort Bragg, North Carolina. They were deployed at the training center from October 22 to November 12, 1982. The second group
(Deployment II) consisted of 464 men assigned and attached to the Ranger battalion stationed at Fort Lewis, Washington. They trained at the center from November 28 to December 16, 1982.

Both units underwent similar training that included instruction in jungle survival, combat techniques, waterborne operations, and land navigation. The course provided ample opportunity for exposure to surface water and mud potentially contaminated with leptospiral organisms.

**Study Design**

This study was a double-blind placebo-controlled trial to determine the protective efficacy of doxycycline against naturally acquired leptospirosis. Volunteers were randomly assigned to one of two groups; a computer-generated coded list of drug and placebo assignments blocked in groups of six was used to achieve approximate parity of drug and placebo assignments within the smallest training unit possible. Volunteers were assigned to study groups as they were enrolled in the study. Signed informed-consent statements were obtained from all volunteers. Soldiers giving a history of allergy to tetracyclines or receiving other antibiotics at the beginning of the study were ineligible for participation. Volunteers who were subsequently placed on antibiotics for unrelated medical problems were withdrawn from the study. All soldiers in the deployments, however, were followed for clinical and laboratory evidence of leptospirosis.

At the beginning of the first week of training, two 100-mg doxycycline or placebo capsules were individually distributed to study participants and swallowed under medical supervision at the time of enrollment in the study. The same dose was taken at the beginning of each subsequent week of training and at the completion of the exercise immediately before departure from Panama. The medication used by doxycycline hyclate (100-mg capsules), lot ED-G-182-98, obtained directly from Pfizer Laboratories. Doxycycline and identical-appearing placebo capsules (lot ED-G-189-981) were obtained through the assistance of Dr. Ira Weinstein at Pfizer Laboratories.

At the beginning of each week's training, questionnaires were completed by everyone in the deployment. Soldiers were queried for specific symptoms — including fever, chills, headache, neck stiffness, dizziness, back pain, muscle aches, joint pain, tiredness, nausea, vomiting, abdominal pain, diarrhea, eye redness or pain, photophobia, rash, cough, and nasal congestion — that might have occurred during the previous week. A record was also made of individual activities during the previous week. Approximately four to five weeks after completion of training, a final questionnaire was administered to identify any late illnesses.

Soldiers presenting to military medical-treatment facilities with fever (temperature ≥38.33°C) during training or in the period immediately after deployment were hospitalized for evaluation. Soldiers on leave status were given letters of instruction to present to military or civilian providers of medical care. The letters included information on the signs and symptoms
of leptospirosis and the need to notify one of the investigators of any suspicious illnesses.

Chi-square tests with correction for continuity were used to analyze the data.

Laboratory Studies

Serum samples were collected from soldiers deployed to Panama approximately one week before travel to Panama, within a week after their return to the United States, and approximately four to six weeks later. Additional specimens were collected from soldiers with symptoms suggestive of leptospirosis. Samples were screened for evidence of leptospiral antibody by the microscopic agglutination technique with live leptospiral antigens.\textsuperscript{12,13} Sixteen antigens were used in the screening process; the screening battery included prevalent serovars identified in prior outbreaks of leptospirosis at the training center and included serovars \textit{L. kobbe}, \textit{L. cynopteri}, \textit{L. djasiman}, \textit{L. grippotyphosa}, \textit{L. borincana}, \textit{L. maru}, \textit{L. mankarso}, \textit{L. Javanica}, \textit{L. panama}, \textit{L. proechimys}, \textit{L. alexi}, \textit{L. pyrogenes}, \textit{L. shermani}, \textit{L. bravo}, \textit{L. chagres}, and \textit{L. gatuni}. These serovars represented 12 different serogroups.\textsuperscript{2} Microscopic agglutination tests were performed by the Veterinary Laboratory Service at Brooke Army Medical Center, Fort Sam Houston, Texas.

Blood and urine samples from soldiers with symptoms suggestive of leptospirosis were cultured with protein-supplemented semisolid Ellinghausen-McCullough-Johnson-Harris media.\textsuperscript{14,15} Cultures were maintained at 28°C under aerobic conditions and read weekly for six weeks, using darkfield illumination. Isolates were typed by Dr. Katherine Sulzer at the Centers for Disease Control.

Case Definition

An infection was confirmed in the presence of at least a fourfold seroconversion to a leptosprial serovar on the microscopic agglutination test or a positive leptospiral culture or both. A case was confirmed if a soldier met the criteria for the presence of a confirmed infection and had symptoms of fever, chills, myalgia, or headache accompanied by neck stiffness during training or in the three-week period immediately afterward.

RESULTS

Study Participation

Of the 1254 eligible soldiers deployed in Panama, 1047 volunteered for the study — a volunteer rate of 83.5 per cent. Of these, 107 were removed from the study before completion of the trial, primarily because they received other antibiotics for unrelated medical problems during training. After these 107 volunteers were excluded, 469 doxycycline recipients and 471 placebo recipients remained in the study (Table I). There were no statistically significant differences in age, rank, ethnic background, military occupational specialty, or history of travel to Panama between the two groups.
One hundred eighty-eight soldiers (23.7 per cent) in Deployment I and 200 (43.1 per cent) in Deployment II reported having been to Panama previously. Thirteen per cent of all these men had leptospiral titers in their predeployment serum samples that were consistent with prior infection. This prevalence was not statistically different from the prevalence among the soldiers who were going to Panama for the first time.

Table 1. Incidence of Leptospirosis in the Subjects Given Doxycycline or Placebo and among Nonvolunteers.

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<tr>
<th>Deployment</th>
<th>No. of Cases/Total No. of Soldiers</th>
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<tr>
<td></td>
<td>DOXACYCLINE</td>
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<tr>
<td>I</td>
<td>0/280</td>
</tr>
<tr>
<td>II</td>
<td>1/189†</td>
</tr>
<tr>
<td>Total</td>
<td>1/469†</td>
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</tbody>
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*Volunteers were excluded if they did not complete the study because they were receiving antibiotics for unrelated medical problems or if they decided on their own not to continue.

†The only affected subject in the doxycycline group received his last dose six days late and at least one week after probable exposure. Chi-square (Yates' correction) = 15.70, P<0.001.

**Incidence of Leptospirosis**

Twenty leptospirosis infections occurred in the placebo group (an attack rate of 4.2 per cent), as compared with only one in the doxycycline group (an attack rate of 0.2 per cent). This represented a protective efficacy of 95.0 per cent. (P<0.001). Four additional infections were identified among nonvolunteers (Table 1).

Twenty-two of the 24 infections seen among placebo recipients and nonvolunteers were associated with fever. One of the soldiers without fever described a syndrome of headache, neck stiffness, and myalgia; the other soldier was completely asymptomatic. Therefore, the ratio of apparent to inapparent infection observed in subjects not taking doxycycline was 23:1, suggesting that leptospirosis in adults is primarily a symptomatic infection. The major symptoms reported by affected subjects were fever, headache, myalgia, chills, back pain, joint pain, and neck stiffness. All subjects describing neck stiffness also had headache. Almost half the infections were associated with eye problems, such as photophobia or eye redness. The only affected subject among the doxycycline recipients had symptoms of fever, headache, myalgia, chills, back pain, joint pain, and neck stiffness. He received his last dose of medication six days late and approximately one week after the time of probable exposure. This soldier was also the last person in his deployment group to have fever, suggesting a delayed development of symptoms. His symptoms and clinical course were not otherwise different from those in the placebo and nonvolunteer cases. A positive leptospiral isolate was obtained from this soldier. Twenty-one of the affected subjects were hospitalized, but no serious
complications were observed in any of the participants. Symptoms usually persisted for four to seven days, and no relapses were documented.

Seventeen of the 25 subjects who had confirmed infection with leptospirosis submitted blood and urine specimens for culture within three days of the onset of fever. Leptospiral isolates were obtained from blood or urine specimens (or both) from 16 of these 17 soldiers.

Serologic results by the agglutination test were consistent with the specific isolates identified from the cases. Isolates recovered from ill soldiers included *L. swajizak* (hebdomadis group), *L. alexi* (pyrogenes group), *L. shermani* (shermani group), *L. canalzonae* (grippotyphosa group), and *L. gatuni* (tarrasovi group). On the basis of isolate identification and antibody patterns during convalescence, the serogroups identified in the outbreaks included hebdomadis, pyrogenes, shermani, grippotyphosa, bataviae, tarrasovi, djasiman, and cynopteri. Convalescence titers against at least one serovar ranged from 1:400 to higher than 1:12,800. No soldiers who acquired leptospirosis had predeployment titers indicating previous infection.

One side effect that was thought to be related to doxycycline administration was noted during the first deployment. The last dose of doxycycline was administered five to seven hours after the last meal, unlike previous doses, which were given to participants within two hours of meals. Thirteen soldiers (4.6 per cent) who received doxycycline reported vomiting after taking the capsule, as compared with only one soldier who had received placebo (*P*<0.01). The vomiting was therefore assumed to be drug-related, and emphasized the need to administer doxycycline with meals whenever possible. No other adverse reactions were noted during either of the deployments.

**Epidemiologic Information**

Figure 1 shows the date of onset of symptoms of fever or neck stiffness in the cases over time. In deployment I, cases of leptospirosis were evenly distributed among the four training companies, with no unit appearing to be particularly affected. In Deployment II, however, all eight cases of leptospirosis occurred in A Company, and symptoms tended to occur later in the deployment cycle than those reported for Deployment I. This finding was probably related to differences in environmental conditions and exposure. Deployment I was associated with very wet training conditions characterized by daily rainfall throughout the exercise. In contrast, Deployment II was associated with much drier conditions in the jungle, except for the third week, when A Company conducted a two-day exercise in a swamp on Ft. Sherman. No other company in either of the deployments conducted any exercises in this swamp.

The soldier with the earliest onset of symptoms in Deployment II became ill early during the third week and probably had an exposure unrelated to the swamp. With this soldier excluded and the second day in the swamp used as the presumed time of exposure, the median incubation period was
Figure 1. Distribution of 24 Cases of Leptospirosis According to Date of Onset of Symptoms. Letters in boxes designate training companies. The last case in Company A during the fifth week in Deployment II was the only case in a subject who received doxycycline.
determined to be 13 days, with a range of 11 to 18 days. This calculated incubation period is consistent with that reported in previous outbreaks.\textsuperscript{1,2}

**DISCUSSION**

Leptospirosis in U.S. troops training in Panama has not been associated with the high rates of complications such as renal failure and death reported from other areas of the Caribbean.\textsuperscript{16} This may be due to the healthier and younger population of U.S. soldiers in Panama or perhaps to the relative scarcity of particular strains in Panama that are more commonly associated with Weil's syndrome. Another explanation for the difference may be the more intense surveillance effort that is conducted on jungle trainees, in which attempts are made to identify every ill soldier. The complicated infections reported in the Caribbean may represent only the most serious portion of a spectrum of infections in the population, with the vast majority being unrecognized milder cases.

The regimen used in this study was designed for short-term exposure. The feasibility of chemoprophylaxis on a more prolonged basis still needs to be evaluated. In situations in which repeated exposure will occur over protracted periods or a very limited number of leptospiral serovars are present in the environment, active or passive immunization may still have promise. Until vaccines for human beings are available, and under specific circumstances, doxycycline appears to be an effective alternative.

We are indebted to the officers and men of the Jungle Operations Training Center at Fort Sherman, the 1/325 Infantry Battalion, the 2/75 Infantry (Ranger) Battalion, and the Surgeon's Office of the 82nd Airborne Division; to Drs. Katherine R. Sulzer, James W. Higbee, John B. McLain, and Shannon Harrison; and to Arthur Peters, Albert L. MacFarland, James P. Brueggemeyer, George L. Rockenbaugh, L. Charlene Breeden, and the medical staffs at the U.S. Army Medical Department Activity, Ft. Bragg, N.C., Madigan Army Medical Center, Ft. Lewis, Wash., and the U.S. Army Medical Department Activity in Panama.

**REFERENCES**


From the Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, D.C.; Preventive Medicine Activity, U.S. Army Medical Department Activity, and 82nd Airborne Division, Ft. Bragg, N.C.; the 2/75 Infantry (Ranger) Battalion, Ft. Lewis, Wash.; and the Veterinary Laboratory Service, Brooke Army Medical Center, Ft. Sam Houston, Tex. Address reprint requests to Dr. Takafuji at the Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC 20307.

The opinions or assertions contained herein are the private views of the authors and are not necessarily to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

I. Introduction

This publication on the serologic characterization of leptospires is the third in a series prepared by the Leptospirosis committee of the U.S. Animal Health Association. This trilogy, “Laboratory Diagnosis of Leptospirosis of Domestic Animals,” has been compiled to provide uniformity among the various laboratories. Part I described the microscopic agglutination microtiter technique (MAMT), which is the reference serologic test, and was published in the Proceedings of the 83rd Annual Meeting, USAHA, 1979. Part II discussed Isolation Procedures and appeared in the Proceedings of the 85th Annual meeting, USAHA, 1981.

The primary purpose of serologic characterization is for aiding interpretation of MAMT results in order to determine the recency of leptospiral exposure and the identity of the infecting serovar. Part III describes three methods used for the serologic characterization of leptospires. These methods are 1) Determination of antibody class (IgM or IgG), 2) Agglutinin absorption to identify the infecting serovar if only serum is available and 3) Serotyping of isolates by agglutinin absorption using specific hyperimmune serum.

II. Determination of antibody class: IgM or IgG.

The MAMT measures both IgM and IgG. Knowing the antibody class (IgM or IgG) will help to determine how recent the infection was since IgM antibodies are produced within the first seven days after exposure, and thus indicate recent or persistent infection. IgG appears within 7-10 days after the beginning of IgM and persists longer.

A. Differentiation between IgM and IgG.

To determine whether the antibody detected by the MAMT is IgM or IgG, sera are tested both before and after treatment with 2-mercaptoethanol (2-ME) or dithioerythritol (DTE). The purpose of treating serum with 2-ME or DTE is to inactivate early leptospiral agglutinins of the IgM antibody class. The mode of action of these chemicals is the reduction of disulfide bonds in the IgM fraction. The stage of the infection can be estimated by the effect of 2-ME on the serum IgM level. The MAMT titer of the serum sample should be reduced to near negative if the sample was taken during the first few days after infection.

Another effect of 2-ME and DTE is to eliminate cross-agglutination to
heterologous antigens. This approach is especially helpful in working with sera reacting to members of the Hebdomadis serogroup.

1. Methods

A. 2-Mercaptoethanol (2-ME) — See appendix for source. 2-ME serum treatment can be performed in tubes or microtiter plates.

**Tube Test**

1. Prepare a 0.2 M solution of 2-ME by adding 0.14 ml 2-ME to 9.86 ml phosphate buffered saline (PBS), pH 7.2.
2. Dilute test serum 1:5 in PBS.
3. In a tube (13 x 100 mm), add equal volumes of the 2-ME and the 1:5 serum dilution, e.g., 1 ml 0.2 M 2-ME plus 1 ml 1:5 serum dilution (1:10 dilution). Stopper tube and mix by swirling.
4. Incubate at 37°C for 2 hr.
5. After the incubation period, the treated and untreated sera are tested by the MAMT.

**Microtiter Test**

1. Alternatively, the 2-ME treatment can be performed by adding 0.025 ml of a 1:5 serum dilution to a well in Row H of a microtiter plate and then adding 0.025 ml of 0.2M 2-ME to this same well (1:10 dilution).
2. Mix by gently tapping, cover the plate, and incubate at 37°C for 2 hr.
3. After the incubation period, the treated and untreated sera are tested by the MAMT.

**Note:** Do not add, 0.2 M 2-ME directly into undilute bovine serum; it will coagulate.

B. Dithioerythritol (DTE) — See appendix for source.

1. Prepare 0.2 M solution of DTE by dissolving 308.6 mg DTE in 10 ml PBS.
2. The test is then performed as described above for 2-ME, beginning at section 1, A, 2.

As a control reagent for working with serum treated with 2-ME and DTE, serum from hyperimmune rabbits bled at 7, 14, and 21 days post injection should be treated and run as controls. Examples of reduction of agglutinin titers of such sera after treatment with 2-ME are shown in Table 1.

**B. Interpretation of Results.**

Serologic interpretation is based on a comparison of the titers of the untreated and treated sera. For example, if an untreated serum sample with a titer of 1:640 is negative after treatment with 2-ME or DTE, only antibodies of the IgM class are present. A serum sample showing the same
Table 1. Reduction of agglutinin titer of rabbit hyperimmune sera by treatment with 2-ME (0.1M final concentration).

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or higher titer after treatment indicates that IgG is predominant. Partial reduction in titer would likely mean that the IgG response has begun.

III. Determination of specific infecting serovar by agglutinin absorption.

Leptospirosis can be detected by either serologic or cultural methods. Because of the time involved for culture, the serologic method is most frequently relied upon to establish a diagnosis of leptospirosis.

Although the serotype of an infecting leptospira can be determined with certainty only by isolation followed by cross-agglutinin absorption studies, absorption studies on serum from an infected animal can indicate the most probable infecting serotype. A tentative identification of the serogroup should be made by cross-agglutination using the MAMT. The serum is then absorbed with individual serovars within the group to determine the identity. If a known serovar absorbs all agglutinins from a test serum, it is likely to be the serovar responsible for the agglutinins.

A. Test. The serum to be used is first tested by the MAMT to determine the antibody titer.
B. Medium. A liquid albumin-polysorbate medium as described in the appendix.
C. Antigens. Serovars used in the MAMT must be obtained from the WHO Leptospirosis Reference Laboratory, CDC, Atlanta, GA 30333, or the National Leptospirosis Reference Laboratory, NADC, Ames, IA 50010.

CAUTION: Extreme care must be exercised and all safety requirements followed when working with leptospiral cultures.

1. The antigens for the agglutinin absorption procedure are 4-8 day cultures grown in liquid medium at 29°C.
2. The need for live cultures requires subculturing in liquid medium. Subcultures are prepared by adding 5 ml of culture to 50 ml
of medium and incubating 4-8 days at 29°C.

3. Transfer the subculture (about 50 ml) to centrifuge tubes.
4. Centrifuge at 5000 x g for 25 min.
5. Discard supernatant.
6. Resuspend cells with PBS to a MacFarland No 10, or 6% T at 400 nm on a Bausch and Lomb Spectronic 20 or equivalent.

D. Serum Absorption.
If the titer as determined in IIIA above is ≥ 6400, treat serum as follows:
1. Prepare tubes for all serovars to be tested by placing 1 drop (50 μl) of serum into each 13 x 100 mm or 12 x 75 mm test tube. Add 24 drops (50 μl each) of each standardized antigen as described below (*note that this will equal a 1:25 dilution*).
   a. Add 10 drops (50 μl each) antigen; mix, wait 10 min.
   b. Add 10 drops (50 μl each) antigen; mix, wait 10 min.
   c. Add 4 drops (50 μl each) antigen; mix, wait 90 min. at room temperature.
   d. Control — 1 drop of sera + 24 drops (50 μl each) of PBS.
2. If the titer is 1600 or 3200, follow the procedure in a, b and c substituting PBS for the last 4 drops of antigen.
3. If the titer is 800, add only 10 drops of antigen, as above, and substitute PBS for the last 14 drops of antigen. Do not run the test if the titer is less than 800, since there must be at least a 5-step reduction in titer for the test to be of value.
4. Remove antigen by centrifugation at 5000 x g for 25 min.
5. If a preservative such as glycerine is added to the serum, add 2 drops of the test sera to a total of 23 drops of the antigen or PBS. Note that glycerine may be added up to 50% of the total volume.

E. Serologic Procedures.
After the absorption procedure has been completed, the absorbed and non-absorbed sera are tested by the MAMT. A control using hyperimmune sera (absorbed and nonabsorbed) must be run against each antigen tested.

The following example illustrates the testing of 1 absorbed and non-absorbed serum sample against a single leptospiral serovar, e.g. *pomona*.
1. Add the sera to the wells in the plate in the following order: (rows on the standard microtiters plates are labeled A through H, as shown in Figure 1.).
   a. Row H, column 1 – 0.1 ml *pomona* hyperimmune serum (non-absorbed).
   b. Row G, column 1 – 0.1 ml Test (Diagnostic) serum (non-absorbed).
   c. Row F, column 1 – 0.1 ml *pomona* hyperimmune serum (absorbed).
d. Row E, column 1 – 0.1 ml Test (Diagnostic) serum (absorbed).

2. Add 0.05 ml PBS to each well in the plate except for the wells in column 1.

3. Using the 0.05 ml microdiluters, mix the serum dilutions in column 1 by turning the diluters 10-15 times.

4. Transfer the diluters to the next column (2) and mix.

5. Repeat step 4 through column 11.
   Do not transfer the diluters into column 12, which will be the antigen control.

6. Add 0.05 ml *pomona* antigen (standardized to a MacFarland No. 0.5 or an equivalent method) to each well in the plate.

7. 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 min.

8. Using the darkfield microscope, examine each well for agglutination and/or clearing of the antigen. Report the end point as the highest dilution in which at least 50% (2+) of the leptospires are agglutinated.

9. A chart similar to the one provided (Figure 2) should be used for recording results.

When testing is done to obtain the identity of the infecting serovar within a serogroup, all of the serovars in the group are used. In this case all combinations of antigen, test sera, and hyperimmune sera must be included. If the hyperimmune antisera has been previously tested against all antigens in the group it needs to be run only against its homologous antigen.

**F. Serotyping of Isolates**

For cross agglutination and agglutinin absorption studies to definitively identify an isolate, good quality hyperimmune serum must be available. These sera must be produced against all serovars which might be isolated. The antisera against the known antigens can be obtained from the National Leptospirosis Reference Laboratory, Ames, IA, from the WHO Leptospirosis Reference Laboratory, Centers for Disease Control, Atlanta, GA, or by hyperimmunization of rabbits (see Appendix).

The procedure for serotyping an isolate is:

1. The unidentified leptospiral isolate should be cultivated in liquid polysorbate 80 medium and standardized as described for performance of the MAMT.

2. The antisera used in the cross agglutination screening test should include representatives of the predominant serogroups prevalent in the geographic area.

3. Agglutinin absorption testing on the isolate should be performed using the same procedure as that described for sera, except that in this case the antigen is unknown.
G. Interpretation of Results.

If agglutination has been eliminated and the MAMT reaction is negative after absorption with an antigen, it is presumed that the serovar is the same as that of the antigen used for absorption. There must be at least a 5 dilution reduction in titer; e.g., reduction from a titer of 6400 to at least 200, for the test to be valid. Another method for expressing this reduction would be to attempt to achieve a 10% post absorption titer limit; e.g. reduction from 6400 to 640. An immune serum with a titer of 12,800 or 25,600 is considered optimum for use in an agglutinin absorption test for purposes of classification.

Appendix

REAGENTS

A. 2-Mercaptoethanol-(2-ME)
   Eastman Kodak
   Rochester, NY 14650
   or
   Bio-Rad Labs. #161-0700
   Bio-Rad
   32nd and Griffin
   Richmond, CA 94804
   or
   Sigma Chemical Co.
   P.O. Box 14508
   St. Louis, MO 63178

B. Dithioerythritol (DTE)
   Calbiochem – Behring Corp.
   LaJolla, CA 92112
   or
   Sigma Chemical Co.
   P.O. Box 14508
   St. Louis, MO 63178

MEDIA

A. Ellinghausen-McCullough Bovine Serum Albumin Polysorbate 80 Medium.

Note: All solutions must be made with distilled or deionized water. Deionized water can be a source of saprophytic leptospires if only filtration sterilization procedures are employed. Deionized water, if used, must be heat sterilized.

1. Ingredients:
   Keep all stock solutions refrigerated.
   a. Phosphate buffer (25X)  
      16.6g Na₂HPO₄
      2.172g KH₂PO₄
      per liter H₂O
b. Salts (20X)
   - 38.5g NaCl
   - 5.35g NH₄Cl
   - 3.81g MgCl₂6H₂O
   per liter H₂O

c. CuSO₄·5H₂O
   - 30mg/10 ml H₂O

d. ZnSO₄·7H₂O
   - 80mg/200 ml H₂O

e. FeSO₄·7H₂O
   - 500mg/200 ml H₂O

f. Vitamin B₁₂ (Concentrate)
   - 10 mg/100 ml H₂O

   Vitamin B₁₂ (Working solution)
   - 10 ml Concentrate (f)
   plus 90 ml H₂O

  
g. Thiamine HCl
   - 200 mg/100 ml H₂O

h. Polysorbate 80 Solution
   - Dissolve 10 ml of polysorbate in 70 ml H₂O at 60°C. Dilute to 1000 ml. Solution should be stored frozen at -60°C and thawed when needed.

i. Bovine Albumin Fraction V (BSA)
   - Dissolve 5 gm/100 ml of single strength phosphate buffer (dilute 40 ml Solution a/1000 ml H₂O). Sterilize by filtration. To sterilize the 5% BSA, it is recommended that a 0.8 micron pore size membrane filter be used as a clarifier followed by filtration through a 0.45 and finally a 0.22 micron pore size membrane filter.

2. Preparation:
   To 700 ml H₂O add and mix the following:
   1. 40 ml 25X phosphate buffer (solution a)
   2. 50 ml 20X Salts (solution b)
   3. 1 ml Copper solution (c)
   4. 10 ml Zinc solution (d)
   5. 20 ml Iron solution (e)
   6. Stir with magnetic stirrer for at least 10 minutes, allowing the precipitable iron-phosphate complex to form.
   7. Add 200 mg L-cystine, stir for additional 10 minutes.
      Note: Not all of the cystine will dissolve.
   8. Filter through triple Whatman #1 filters to obtain a clear solution.
   9. To the filtrate add 20 ml of the Vitamin B₁₂ working solution and 0.1 ml of Thiamine HCl(g).
10. Add 120 ml of the 1% polysorbate 80 solution (h).
11. Adjust volume of basal medium to 1000 ml.
12. Autoclave basal medium to sterilize (121°C. 15 min. 15 lbs.)
13. Allow solution to cool and add 2 ml of the 5% Bovine Albumin solution (i) to each 8 ml of basal medium. Dispense in 10 ml amounts into sterile screw-cap test tubes and check for sterility. As an alternative, 8 ml amounts of the basal medium can be dispensed into screw-cap test tubes prior to sterilization. After sterilization, cool and aseptically add 2 ml of the 5% BSA to each tube of sterile basal medium.

B. Commercially available media.
1. Albumin-polysorbate 80 media or individual solutions are available commercially from Difco Labs., P.O. Box 1058A, Detroit, MI 48732, Phoenix Laboratories, 1614 N. 74th St., Omaha, NE 68114, Reheis Chemical Co., Armour Pharmaceutical Co., 303 South Broadway, Tarrytown, NY 10591, and Scientific Protein Laboratories, Inc., P.O. Box 158, Waunakee, WI 53597.
2. The Difco product (EMJH medium) uses a dehydrated basal medium 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 solutions, Reheis offers a sterile 30% non-preserved albumin solution for those who elect to make EMJH medium.
3. Semi-solid agar (0.2%) is prepared by adding 2.0 gm of agar per liter (1000 ml) of complete medium (basal + enrichment).

HYPERIMMUNIZATION OF RABBITS

A. Preparation of hyperimmune sera.
1. Young normal New Zealand white rabbits weighing about 8 lbs. are bled from the ear and tested by the MAMT to determine any pre-existing titers to leptospires.
2. Prior to bleeding, the ear is rubbed with xylene to dilate the blood vessels. A beam of light is placed behind the ear so that the vessels are more readily visible.
3. A 22 g – 1 1/2 inch needle is placed into the artery located along the middle of the ear. Approximately 5–6 ml of blood is directed into a clean 13 X 100 mm tube, allowed to stand for 4–5 hours, and the serum collected for testing.
4. For hyperimmunization, the rabbits are injected intravenously in the marginal ear vein with a 7-day culture of live antigen (Dinger-zone) according to the following schedule:
   Day 0 – 1 ml; Day 7 – 2 ml; Day 14 – 4 ml; and Day 21 – 6 ml.
5. Fourteen to 21 days after the last injection, a small amount of blood is collected and the serum tested for antibodies. If the titer is at least
12,800, the rabbits are exsanguinated by obtaining as much blood as possible by bleeding from the ear, and then by cardiac puncture after administration of anaesthesia.

6. The serum is harvested and stored at 4°C, frozen, or lyophilized. An equal volume of glycerine may be added as a preservative if desired.

Suggested References


Acknowledgement

The assistance of Mr. A. R. Pursell and Mrs. C. R. Sulzer in preparation of this manuscript is appreciated.
Figure 1. Agglutinin-Absorption Procedure

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*Row H - *pomona* hyperimmune sera; nonabsorbed

G - Diagnostic sera; nonabsorbed

F - *pomona* hyperimmune sera; absorbed

E - Diagnostic sera; absorbed
Figure 2. LEPTOSPIRA TYPING RECORD

Lab. No. __________

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REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

Chairman: H. L. Rubin, Kissimmee, FL
Vice Chairman: J. R. Cole, Jr., Tifton, GA

B. O. Blackburn, Ames, IA; J. J. Cecil, Charles City, IA; S. L. Diesch, St. Paul, MN; John Finnell, White Hall, IL; J. W. Glosser, Alexandria, VA; R. F. Hall, Tifton, GA; L. E. Hanson, Urbana, IL; C. A. Kirkbride, Brookings, SD; M. L. Main, Keldon, SD; R. L. Morter, Lafayette, IN; R. M. Nervig, IA; J. G. Songer, Tucson, AZ; A. B. Thiermann, Ames, IA; D. H. Tripathy, Urbana, IL; F. H. White, Gainesville, FL; J. M. Williams, St. Joseph, MO; C. M. Hibbs, Albuquerque, NM.

The USAHA Leptospirosis Committee met on Thursday, October 25, 1984.

Dr. Thiermann, IA, presented a brief report on the European Economic Community Veterinary Research meeting on the present state of leptospirosis; diagnosis and control which was held September, 1984 in Belfast, Northern Ireland. The papers presented at this meeting pertained to current work on leptospirosis in farm animals with emphasis on laboratory diagnosis by the European community.

Dr. Thiermann briefed the committee on his work on restriction endonuclease analysis which he presented at the EEC meeting.

Col. E. T. Takafuji, M. C., USA, briefed the committee on the U. S. Army’s leptospirosis prophylaxis program and emphasized the importance of leptospirosis as a major zoonotic disease, one which is inadequately recognized by the American Medical Community. The committee again wishes to point out the need for renewed efforts, through appropriate media, to inform veterinarians, animal producers, dairymen and other individuals involved in animal production of the potential dangers of leptospiral infection.

The committee recommends that funding of the National Leptospirosis Reference Center be increased to allow for strengthening of both service and internal research programs. Further, funds should be made available for extramural research on priority items. A subcommittee consisting of J. G. Songer, Chairman, J. M. Williams, R. M. Nervig and L. E. Hanson, was appointed to develop a list of research priorities in animal leptospirosis, based on the input of university research personnel, producer groups, regulatory and federal research agencies and the biologics industry.

Dr. Cole, GA, presented Part III of the “Laboratory Diagnosis of Domestic Animals — Serologic Characterization”, which is attached to this report for publication.

The committee was pleased to note that the National Leptospirosis Reference Center has completed its first year of operation. Representatives of ARS and APHIS sections of the Center briefed the committee on their activities.
Dr. Dennis Murphy of APHIS, NVSL, reported that one microbiologist was recruited and received training at the Center for Disease Control in Atlanta. Two biological aides have been appointed and have received on-the-job training. One veterinary medical officer (epidemiologist) has been appointed, and will receive training under Dr. Thiermann. Dr. Murphy reported that the Center is operational. A list of services available and instructions on sample submission, which had been distributed to the veterinarian in charge of each state, also was given to the members of the committee.

Dr. Thiermann reported on the recruiting of a molecular biologist, which increased the staff of the ARS section of the National Leptospirosis Reference Center to four. He briefly reported on the following 1984 Activities: experimental infections in cattle, isolation study on swine abortions, characterization of field isolates, computerized numerical identification system, characterization of over 300 strains by restriction endonuclease analysis, quick-ready DNA extraction procedure, characterization of leptospiral proteins, development of genetic library of leptospiral DNA, and screening for expression of immunogens in these clones. Dr. W. A. Ellis from the Veterinary Research Laboratory, Belfast, N. I., spent a sabbatical during the summer of 1984 at the Center.

The committee is quite concerned about the lack of communication between the sections at the Center and the potential users. The committee recommends that the availability of services receive wide distribution through announcements in scientific and professional publications. The committee further recommends that direct communications between potential users and the Center be authorized.

The committee also recommends that the Center prepare and submit a combined annual activities report at each future leptospirosis committee meeting.
RESIDUE AVOIDANCE — MASTITIS REDUCTION

by R. D. McQueen, DVM, PhD

Current mastitis control recommendations rely heavily on the use of antibacterial drugs. That is, infusion of all quarters of all cows at drying off and intramammary/systemic treatment of clinical mastitis cases.

The first way to avoid detectable drug residues is to use a drug according to label directions. However, mistakes in identifying and/or segregating treated animals do occur. Also, in some instances veterinarians may prescribe extra-label use of drugs, when approved drugs/usage levels do not produce satisfactory response. Some producers also use unwarranted dosages and combinations without supervision. In such circumstances, on-farm/office testing of milk (urine) is needed prior to shipment of the milk (animal) for human consumption. An increasing number of economical rapid, easy-to-use tests are becoming available to veterinarians and producers for this purpose (Delvotest-P®, LAST®, CAST®, SPOT®, Penzyme®, etc.).

However, the advent of on-farm testing may detract from the fact that unrecognized, subclinical mastitis is three times more costly than losses due to acute mastitis. Also, the cost of implementing an effective mastitis control program is about $20 per cow annually, yet the program returns $3–5 from increased milk production alone for each $1 invested.

Fewer lactating cows are treated in herds with effective mastitis control programs. Herd health vaccinations, improved housing and proper feeding also decrease the incidence of respiratory and intestinal diseases, further reducing drug usage.

Reduced drug usage is possible only when the specific cause(s) of disease is determined. Increasingly, producers are confronted by advertising which emphasizes drug usage rather than diagnosis and appropriate drug usage. Current livestock profit margins and public concern for food residues emphasize the need for accurate diagnosis and more judicious drug use.

The Mastitis Control and Drug Residue Avoidance Fact Sheet was written with this emphasis, because state diagnostic laboratory services are improving and veterinarians increasingly are providing bacteriologic culture service to dairy clientele. Field experience in Illinois substantiates the value of diagnostic bacteriology in herd mastitis control programs. Herds which have lost Grade A status and are identified as Strep. ag herds have profited from blitz treatment of infected cows. Long term goals have been established and achieved in herds identified as having widespread Staph. aureus infections. Finally, herds with average bulk tank somatic cell counts have also reduced cell counts and qualified for milk price incentives.
Mastitis Control and Drug Residue Avoidance

On most dairy farms, mastitis is not only the most costly, but the most frustrating herd problem. It is influenced by a variety of factors including milking machine function, milking practices, sanitation, culling rate, environmental stresses, drug usage, and herd infection rate. A recent Minnesota study of DHIA cooperators revealed that only 5.4 percent of dairy producers had fully implemented a comprehensive control program including correct use of properly functioning milking machine, teat dipping, udder infusion at drying off, correct treatment of clinical cases, and culling cows with chronic mastitis. Producers who adopt such a program expect an immediate decline in the amount of clinical mastitis. This reduction often is not observed until the program's second year, even though the herd infection rate is declining.

Mastitis is caused by bacteria that enter the udder through the teat canal. *Streptococcus agalactiae* and *Staphylococcus aureus* are the predominant bacteria that cause subclinical (inapparent) infections as well as acute flare-ups. Subclinical infections usually are more numerous than clinical infections. In fact, the cost of decreased milk production due to subclinical mastitis (drugs, discarded milk, labor, and premature culling) is three times greater than the cost due to acute mastitis. The cost of implementing a mastitis control program is approximately $20 per cow annually with an annual return of $3 to $5 from increased milk production alone for each $1 invested. Additional profits result from: (a) fewer animals being lost to death and premature culling; (b) fewer clinical cases; (c) less milk discarded; (d) fewer drugs and veterinary expenses; and (e) less extra labor. Highly infected herds usually realize a greater increase in profits from initiating a mastitis control program. Nevertheless, implementing or continuing an effective mastitis control program is a profitable decision for all dairy farmers.

Control Program

The main objective of a herd mastitis control program is a reduction in the number of infected quarters. The number of new infections and the duration of existing infections must be decreased. New infections can be reduced by improved milking sanitation and teat dipping after each milking. Existing infections can only be reduced by treatment of clinical cases, antibiotic infusion of all quarters at drying off, and by culling. Up to 40 percent of new infections develop during the dry period. Dry cow infusions help reduce these new infections.

A secondary objective of a mastitis control program is to reduce drug residues. Drug residues in milk and meat may result from udder infusions or intramuscular injections in cows with clinical mastitis. Reasons for food residues include carelessness in animal identification, failure to follow label withdrawal times, improper drug use, and intentional sale of milk or meat from treated cows. A reduction in the number of new and existing
mastitic infections can reduce drug usage and thus the risk of food residues.

**Identification a First Priority**

The first step in improved mastitis control is to identify the specific bacteria infecting the udders and determine the infection rate of these bacteria within the herd.

Culture and antibiotic sensitivity testing of individual quarter milk samples from all lactating cows is ideal but costly and time-consuming. An effective alternative approach is to identify problem quarters of 10 to 25 percent of the cows using a California Mastitis Test (CMT) test. Milk samples of these quarters are then collected and tested to identify the bacteria present and determine their antibiotic sensitivity. Four to five consecutive daily bulk tank samples taken for bacterial culture tests and somatic cell counts (SCC) can be useful estimates of the extent of bacterial infection within the herd. The number of coliform bacteria in bulk tank milk can also help assess the sanitary condition of the udders at milking.

Evaluation of individual cow SCC records, bulk tank bacterial and cell count records, herd records of clinical mastitis, and dry cow treatments help to put a mastitis problem into perspective. With herds in danger of losing their Grade A status or milk market due to high SCC or bacterial counts, immediate drastic measures may be required to forestall severe economic consequences.

**Evaluate Equipment**

The second step in improved mastitis control is an evaluation of the milking equipment during milking to ensure a relatively stable milking vacuum of 11 to 12 inches of mercury at the claw, 45 to 60 pulsations per minute, and a pulsation ratio of 50/50 to 60/40 or equipment design. Recording equipment should be used to make these measurements. New equipment may not meet these performance standards, if improperly installed, if optional accessories adversely affect performance, or if improperly used. Deficiencies must be corrected. Regular preventive maintenance by knowledgeable service people is recommended to prevent gradual deterioration of properly functioning systems.

The milking machine can contribute to mastitis in three ways:

- **Pulsator malfunction, high milk/rest (pulsator) ratios, and pulsation rates above 60 per minute frequently cause teat end damage.**

- **Irregular milking vacuum fluctuations, liner slip/squawk, and liner flooding may inject bacteria through the teat canal into the teat and udder cisterns.**

- **The claw, and especially the liners, can assist in the mechanical transmission of bacteria from an infected quarter or the teat skin into the teat canal of another quarter of the same cow or of cows milked subsequently.**

The mechanical transmission of mastitis bacteria from cow to cow at milking occurs readily. It can be reduced by milking known infected cows
last, and by efficiently sanitizing the liners and claw between cows. In herds with bulk tank SCC or WMT above 500,000 and 10 respectively, high priority should be given to milking uninfected heifers and cows first. Upwards of 95 percent of the udder infections may be subclinical (non-visible) and will not be recognized without regular SCC/CMT testing. An SCC above 300,000 or a CMT test of 1+ or higher for two or more consecutive months often indicates infection in one or more quarters.

Dipping claws and spraying liners with udder disinfectant between cows is only partially effective in reducing bacterial spread. Disinfectant solutions that become contaminated and inactivated with milk and manure can assist in the transfer of bacteria.

Solid state back flushing units now in use in some milking parlors may reduce machine-related infections. Costs of a flush unit may reach $2,000 per milker. Units use up to 1.5 gallons of sanitizer per cow with a flush cycle from one to three minutes long. At present, such units appear to be cost effective only in very large herds, especially those with serious Staph aureus or Mycoplasma mastitis infections.

Washing Is Essential

The third step in improving mastitis control is to wash cows with a warm disinfectant udder prep solution to stimulate letdown, reduce bacterial contamination of milk, and reduce transfer of infection into the gland. The wash bucket, reused towels, wash hoses and nozzles, outside surface of milker units, and the hands of the milker may be contaminated with milk from an infected quarter and spread mastitis bacteria to other cows.

The first teat-full of milk should be taken to check for mastitis and then discarded since it has a very high bacterial count. If taken after teat washing, this milk may spread mastitis to the next cow by contaminating the operators' hands. Therefore, where practical, the first milk should be removed before teat washing.

A low pressure disinfectant spray using a hose and a single service paper towel is best, wetting and drying only the teats. Buckets and single service towels can be satisfactory, but frequently do not provide effective cleaning of heavily soiled teats. Disinfectant solutions in buckets are easily contaminated, lose potency quickly, and can actually spread infections, especially if a common cloth or sponge is used.

Hand-held teat cup washers are a recent introduction and cost about $200. A water wash to remove heavy soil followed by a disinfectant rinse will reduce disinfectant costs greatly and ensure good teat coverage with fully active disinfectant solution.

Teat Dips Give Protection

The fourth step in improved mastitis control is to dip the teats with a suitable germicidal teat dip immediately after the milker is removed. The majority of teat dips marketed today will reduce the rate of new Streptococcus agalactia and Staphylococcus aureus infections by 50 to 80 percent when used in conjunction with the other control procedures. Teat dipping
does not kill the bacteria within the gland and thus does not eliminate existing quarter infections, but does kill bacteria on the teats and teat canal ends. Because most germicides have little residual action, they are ineffective in stopping mastitis from environmental sources (coli forms, Pseudomonas, *Strep uberis*) that contaminate the teats between milkings. It takes about 30 minutes after milking for the teat canal to close. Teat sealer products, which coat and seal the teat after milking, help to reduce the exposure of the teat end to coliforms (manure contamination) between milkings. They are "peeled off" prior to milking, removing the manure contaminants in the process. Keeping udders clean by bedding well, maintaining the surface of free stalls, and scraping lots frequently can significantly reduce losses due to coliform mastitis.

**Prompt Treatment Needed**

The fifth step in improved mastitis control is correct treatment of clinical cases. Lactating cows with acute mastitis (swollen hot quarter, abnormal milk, body temperature of 103°F or higher, off feed) require prompt intravenous or intramuscular treatment because the entire body is being affected by the bacteria or their toxins. Acute cases of mastitis should be treated under veterinary supervision. Early treatment may mean the difference between saving only the cow and saving the cow and the affected quarters as well.

Mild clinical cases of mastitis (moderately swollen quarter, few to moderate amount of flakes and clots, cow still on feed) can be treated with mastitis infusion tubes. Treat for recommended period and withhold milk according to label directions. If in doubt, use the Delvotest P® before adding the milk to the bulk tank.

Each herd has its own pattern of bacterial mastitis. Treatment of *Str. agalactia*-infected quarters during lactation cures this infection in 85 to 95 percent of cows. In certain staph-infected herds, drug infusion and milk discard costs exceed the benefit of treating the repeated mild flareups. A staph infection cure rate of only 20 to 40 percent for lactating cows is common.

Drug selection should be guided by milk culture tests of previous mastitis cases and previous herd experience. Many producers use products that are only partially effective, not realizing that self-cures of certain bacterial infections are common. Mild chronic staph infections also periodically flare up and spontaneously improve during lactation. Milk culture testing allows the selection of the most effective drug. Drugs that perform well in laboratory tests may not kill bacteria that are within the gland cells or present in isolated small abscesses in the udder. Delayed treatment, inadequate dose levels, and stopping treatment too soon also cause treatment failures.

Herd somatic cell counts (SCC) identify cows that have udder inflammation. A CMT test should then be used to identify problem quarter(s). **Neither test should be used as the sole reason for treating a quarter.** Treat only when other signs indicate a clinical infection is present (abnor-
mal milk, swelling, soreness, etc.).

**Treat All Quarters**

The sixth step in improved mastitis control is to treat all quarters of all cows after the last milking at drying off.

Treatment of all quarters has the advantage of reaching all infected quarters, helps to prevent dry period infections, and does not require a laboratory culture of milk samples. Other advantages of dry cow treatment are as follows:

- The cure rate is approximately twice as high as when treatments are administered during lactation.
- Damaged tissue may be regenerated before freshening.
- Clinical mastitis at freshening is reduced.
- Marketable milk is not contaminated with drug residue.

Herd somatic cell counts can be used to identify cows with consecutive monthly high SCCs. If the infection is eliminated, the SCC will drop in the next lactation after dry treatment. Cows that continue to show high SCC in the next lactation are chronically infected and should be culled.

Dry cow treatment aids prevention of new udder infection during the four- to six-week period that the antibiotic persists in the udder. Once the next lactation is initiated, any antibiotic that persists is rapidly milked out and the gland is again susceptible to infection.

**Cull Chronics**

The seventh step in improving mastitis control is to cull cows with chronic infection that do not respond to treatment. Such cows serve as a reservoir of infection for other cows.

**Drug Residue Avoidance**

The mastitis reduction procedures described include antibiotic udder infusion during clinical mastitis and at drying off. In addition, intravenous or intramuscular drugs may be required in cows acutely ill with mastitis and other infectious diseases. Thus, steps must be taken to insure that drug residue contamination of milk or meat does not result. Drug residue concerns are based on the safety of products for human consumption. A small percentage of the population is hypersensitive to antibiotics, particularly penicillin; some to the extent that anaphylactic shock and death may occur.

Screening milk for the presence of inhibitory (antibiotic) residues has been routine for years. With the recent assay change to the more sensitive Bacillus stearothermophilus test, strict attention to drug use and withdrawal practices is required.

Most dairy farmers are conscientious about withholding milk for the prescribed time periods from cows injected or infused with mastitis drugs. Carelessness in withholding milk from treated cows is the usual cause of residue problems. The person milking does not know the milk from a
particular cow should be withheld. Antibiotic residues have also been caused by a product not known to contain antibiotics being administered to a cow.

Increased drug dosages and abnormal antibiotic elimination also cause problems. A dose of ten grams (fourfold dose increase) of tetracycline in a normal uterus will result in detectable milk levels for 48 hours or longer in most cows (twofold increase in detection period). However, in cows with inflammation of the uterus, detectable drug levels may persist in milk for several days, depending on the severity of the inflammation. Injected antibiotics may be cleared more slowly in cows with systemic infections. Milk has contained detectable antibiotic residues for as long as 15 days after systemic injections of antibiotics for acute mastitis, pneumonia, etc.

The first way to avoid detectable residues is to use a drug according to label directions, especially the dosage, route of administration, and withdrawal time. Do not underdose in an attempt to shorten withdrawal time. Reduced dosage often leads to a chronic infection that flares up periodically, requiring additional treatment. Reduced dosing may lead to the development of resistant bacteria. Relying solely on bulk tank dilution of the milk from a treated cow to avoid residue detection is not recommended. One infusion tube of penicillin (100,000 units) is enough to contaminate 45,000 pounds of milk. When in doubt, a sample of milk from the treated animal should be tested before it is added to the bulk tank. Milk haulers are very cooperative in arranging for individual cow milk tests when doubt exists regarding drug residue status. Delvotest P® kits for on-farm milk testing (about $1.00 per sample tested) can be used.

The second way to reduce the risk of drug residue is to reduce the level of mastitis and other infections in the herd. Well managed herds require fewer drug treatments. The mastitis control program outlined previously will virtually eliminate Strep. ag. infection and substantially reduce the Staph. aureus infection rate within three years. Herd health vaccinations, improved housing, and proper feeding can reduce the incidence of respiratory diseases and calf scours, further reducing drug usage.

The third way to ensure that meat does not contain drug residue is to test treated cows. In 1979, the USDA began using the S.T.O.P. test for the presence of inhibitory drugs at cattle slaughter houses. The test is easy to run and results are available overnight. At that time, dairy farmers were provided information on how to use drugs properly, including strict attention to withdrawal time. The national violation rate subsequently fell from 3.7 percent in 1978 to 0.6 percent in 1981–82. Now a similar test has been developed that enables producers to test the urine of treated cows before sending them to slaughter. Dairy farmers or their veterinarians can perform the test and obtain the results after overnight culture of the urine sample. A guidebook that provides test details is available by writing: FSIS Information, Room 1163-South, USDA, Washington, D.C. 20250.

Steps to Effective Control

Mastitis control should be continuously applied to all herds without
relaxation. Though current programs do not provide complete protection, the devastating losses from the disease can be minimized in the vast majority of herds by conscientious application of the following management and drug use practices:

- Identify the specific bacteria infecting the udders and determine the infection rate of these bacteria within the herd.
- Evaluate the milking equipment during milking.
- Wash cows with a warm disinfectant udder prep solution to stimulate letdown, reduce bacterial contamination of milk, and reduce transfer of infection into the gland.
- Dip the teats to the top with a suitable germicidal teat dip immediately after the milker is removed.
- Treat clinical cases correctly.
- Treat all quarters of all cows after the last milking at drying off.
- Cull cows with chronic infection that do not respond to treatment.

Prepared by David McQueen, extension veterinarian, University of Illinois; W. Nelson Philpot, Northern Louisiana Hillfarm Experiment Station; John McDonald, research scientist, National Animal Disease Center; Leland Allenstein, veterinary practitioner, White-water, Wisconsin.


and justice for all

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INTRODUCTION

For many years there has been considerable interest and emphasis on reducing bacterial contamination of milk, lowering somatic cell counts, and improving the overall quality of our farm milk supply. Major emphasis has been given to improving sanitary practices related to cleaning equipment, maintaining facilities and improving milking procedures. As more and more milk was upgraded to qualify under Grade “A” standards adopted and enforced by state milk regulatory agencies, the overall quality of the milk supply improved. Today, over 80 percent of the milk is Grade “A” milk produced under standards set forth in the Grade “A” Pasteurized Milk Ordinance. We continue to see a shift from manufacturing grade milk to Grade “A” milk as dairymen are forced for economic reasons to become larger and upgrade their facilities.

In the past, most of the progress which was made to improve milk quality has come about through educational efforts. Educational efforts of the Extension Service, USAHA, National Mastitis Council, farm magazines, cooperative publications have been aimed at making dairymen more aware of how much better quality milk they can produce if they would just follow recommended management practices. Very little incentive existed other than personal satisfaction gained from producing a high quality product. No real financial renumeration was used to achieve the improved quality desired at the plant or in the market.

It was not until research provided a definite link between quality of milk and yield of the manufactured product that interest began to develop to provide economic incentive payment plans for dairymen. These programs have taken many forms: quality premium payment programs, component pricing, end product pricing plans, bonus or premium payment programs. All have one common feature: that is, the basis for providing added return to dairymen comes as a direct result of more available solids in the milk. Thus, the quality of the milk and the level of somatic cells directly affects the level of solids.

Manufacture of cheese, milk powder and other dairy products such as yogurt and cottage cheese depend upon a high quality low somatic cell count milk of good flavor. Since casein is the principal milk protein in cheese manufacture, a low percentage of casein in the milk can lead to low cheese yields resulting in uneconomical cheese manufacturing operations.
According to Dr. Barbano of Cornell, a change of 0.1% in the casein level affects yield ± 0.2 to 0.24 pounds per cwt. of milk. This research is important to cheese manufacturers, since casein in cows milk has been shown to vary. In New York, for example, it has been shown to vary as much as 0.4 over the course of a year.

Therefore, to understand the economic link between low somatic cell count milk (less mastitis) and higher manufactured product yields, one must first look to the proper composition of milk and how milk of normal composition may be adversely impacted by high bacterial or high somatic cell counts.

Poor quality milk, for example, of a high somatic cell count will contain less casein as a percent of total protein than high quality milk of a low somatic cell count. Another study at Cornell indicates that a difference in somatic cell count of 100,000 can reduce casein as a percent of protein from a normal level of 77 to 78 percent to 75 percent. For example, Dr. Barbano reports one milk sample had a 500,000 somatic cell count and 77 percent casein while another had a 650,000 count and 75 percent casein. The difference in the value of the two milks (150,000) somatic cells based upon yield of cheese was 23 cents/cwt.

Another interesting phenomena occurs when milk of a high count is added to low count milk? The high count milk has been shown to rapidly attack low-count milk with the result that the higher quality milk is rapidly converted to lower quality milk very quickly. This is why most protein premium payment programs have, as a prerequisite, milk quality requirements. Most protein pricing plans take effect only when the milk has been tested to meet certain quality standards “in terms of somatic cell count and standard plate count”. Any type of a component payment program based upon product yield must, therefore, have minimum quality standards as part of the program, for it has been clearly demonstrated that somatic cells will “attack casein and convert the casein to a different type of protein which still tests out as protein, but can reduce product yield”.

It has also been clearly documented that high bacteria counts cause defects in milk and milk products. These defects often take the form of off-flavors, odors and discoloration in fluid milk. In cheese and other cultured products, defects include spoilage and inhibition of processing as a result of action of microbial enzymes on milk components. Reduced shelf-life, decreased consumer acceptability and economic loss are the ultimate result for dairymen.

Among our dairy cooperative membership today, there is a definite trend toward component pricing for protein and/or milk solids-not-fat. As mentioned earlier, it is quite prudent to integrate quality standards with component pricing plans that recognize either the protein or milk solids-not-fat portion of milk; that is, those components other than milkfat which has traditionally served as the only component of importance as far as the pricing of milk. The majority of our component pricing plans include,
therefore, evaluation for bacteria, somatic cells, added water, antibiotic residues, and sediment.

Increased attention has been given to the control of psychotrophic (cold loving) organisms that greatly reproduce at refrigerated temperatures. By employing the Preliminary Incubation (PI) Count to measure psychotrophic populations, and with follow-up to problem farms, cooperatives have greatly reduced rejection rates of tanker loads of milk by handlers.

Improved bacterial quality and end product pricing based upon components of protein or milk solids-not-fat have provided the basis for many of our cooperatives to improve profitability and return that improved income back to dairymen in the form of "bonus premiums."

A number of coops have now instituted premium bonus payment programs for dairymen who produce milk which meets specific bacterial quality parameters. In the June 10 issue, *Hoard's Dairymen* magazine reported that in Wisconsin alone, 49 plants were paying protein premiums. 26 were offering end product pricing and 112 provide for quality premiums. This trend, while originating in the Upper Mid-West, is spreading rapidly throughout the nation.

Component Pricing Slides

To provide you with some overview of what constitutes "component pricing" vs. the current classified pricing system, let me utilize some slides prepared by the Federal Milk Marketing Order Administration in St. Louis.

"A.M. PLAN"

an alternate method of component pricing

Prepared By:
MARKET ADMINISTRATOR'S OFFICE
MARYLAND HEIGHTS, MO
1. DROPLET OF MILK WITH COMPONENTS — To understand the basis for component pricing or incentive payment plans, it is imperative to understand the components of milk. Milk’s unique composition accounts for its excellent taste, as well as its nutritional and economic value. The major components of milk include water, butterfat, protein, lactose, and minerals (such as calcium and phosphorous) along with other components such as vitamins.
2. DROPLETS OF MILK WITH PERCENTAGES OF COMPONENTS — the approximate percentages of the components are:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87.75%</td>
</tr>
<tr>
<td>Protein</td>
<td>3.30%</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.70%</td>
</tr>
<tr>
<td>Minerals, etc.</td>
<td>0.60%</td>
</tr>
<tr>
<td>Butterfat</td>
<td>3.65%</td>
</tr>
</tbody>
</table>

This might be viewed as the percentage composition, by component, of an "average" quantity, or average hundredweight, of milk.
3. VERSATILITY OF MILK — This unique combination of these components accounts for milk’s high degree of versatility — milk can, and is, consumed in numerous different forms. This slide depicts a few of the many different products that can be made from milk.
4. MILK JUG — Of all these possible uses, we know that milk for fluid consumption is considered the highest or most valuable use: that is, the value of milk for fluid use is greater than the value of milk used in manufacturing. This elevated value for fluid usage is due to numerous factors — fluid milk items are more perishable (have a much shorter shelf life) than manufactured dairy products; only grade A milk, which must meet strict sanitary requirements, can be used for fluid consumption; the demand for fluid milk is more inelastic with respect to price, than the demand for most manufactured products; and fluid milk is considered an "essential food" in our diets. These factors all contribute to the higher value placed on fluid milk.
5. **CLASSIFIED PRICING** — The assignment of different values for raw milk in accordance with its final usage (i.e., what product it goes into) is called "Classified Pricing." This type of pricing system recognized that milk has different values when used in different products. Most markets provide for three distinct use categories. Milk used in Class I products has the greatest value, and includes products like whole milk, lowfat milk, skim milk, buttermilk, and whole and lowfat flavored milks. Milk used in Class II, or "soft manufactured" products such as ice cream, cottage cheese, whipping cream, yogurt, dips, etc., is assigned a value less than Class I but somewhat greater than milk used in Class III products. Class III, or "hard manufactured" products, include butter, dry milk, and hard cheeses.
6. **CLASS PRICES** — Classified pricing is used to arrive at a blend or uniform price that is paid to all producers marketing their milk within a given FMO. If we assume that the class prices in a specific FMO are:

<table>
<thead>
<tr>
<th>Class</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>$14.20 cwt.</td>
</tr>
<tr>
<td>Class II</td>
<td>$12.60 cwt.</td>
</tr>
<tr>
<td>Class III</td>
<td>$12.45 cwt.</td>
</tr>
</tbody>
</table>
and the marketwide utilization of the milk in the pool is:

<table>
<thead>
<tr>
<th>CLASS</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60%</td>
</tr>
<tr>
<td>II</td>
<td>20%</td>
</tr>
<tr>
<td>III</td>
<td>20%</td>
</tr>
</tbody>
</table>

7. **MARKET UTILIZATION** — and if we assume that the marketwide utilization of the milk in the pool is:

- Class I: 60%
- Class II: 20%
- Class III: 20%
8. BLEND PRICE COMPUTATION — Then the uniform or blend price would be: $13.53 cwt. This is determined by multiplying each class price by the utilization percentage in that class and totaling the results. The $13.53 cwt. price is adjusted upward for milk with a higher butterfat test, and is lowered for milk of lesser butterfat content through the use of a butterfat differential. Thus, any individual producer’s total milk payment is determined by the amount of milk marketed (number of hundredweights) and the butterfat test of that milk.

<table>
<thead>
<tr>
<th>CLASS</th>
<th>Price x Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$14.20 x .60 = $ 8.52</td>
</tr>
<tr>
<td>II</td>
<td>$12.60 x .20 = 2.52</td>
</tr>
<tr>
<td>III</td>
<td>$12.45 x .20 = 2.49</td>
</tr>
<tr>
<td></td>
<td><strong>$13.53</strong></td>
</tr>
</tbody>
</table>
9. **SIX VALUES IN BULK TANK** — It follows that there are six values present in the milk contained in every dairy farmer's bulk tank. These values are for Class I skim and butterfat, Class II skim and butterfat, and Class III skim and butterfat. The dollar value of each of these six items is determined by the price assigned to each class, the usage of the milk in the market, and the butterfat test of the milk in the farmer's bulk tank. Therefore, currently the value of milk is attributable to the skim and butterfat components.
10. **BULK TANK: COMPONENT PRICING** — As we shall see, the six values contained within the bulk tank would change with the implementation of component pricing. Currently, interest in establishing some form of component pricing within the Federal Order framework appears to be quite widespread. As we have stated before, “Incentive”, or premium payment, component pricing programs have been instituted by many cooperatives, so the interest in component pricing is growing. But what exactly is “component pricing”? 
Component pricing - The pricing of milk on the basis of its butterfat content along with its content of another, or several other, components such as protein, milk-solids-not-fat, fluid carrier, etc.

11. COMPONENT PRICING DEFINITION — A working definition of component pricing might read like this: The pricing of milk on the basis of its butterfat content along with its content of another, or several other components, such as protein, milk-solids-not-fat, fluid carrier, etc. This is not a formal definition, it is an explanation of what the term component pricing has come to mean. When people refer to component pricing they almost always mean some type of pricing system that would use butterfat plus one or more of the other components of milk for pricing purposes. Strictly speaking, however, any combination could be used for pricing (butterfat would not have to be one of the components). Moreover, the pricing system that we have today is technically a form of component pricing — the components being skim and butterfat. However, since the current pricing system is not considered component pricing under component pricing’s "contemporary definition", this "working definition" has come to be universally accepted.
### COMPONENTS OF MILK

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER</td>
<td>87.75%</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>3.30%</td>
</tr>
<tr>
<td>LACTOSE</td>
<td>4.70%</td>
</tr>
<tr>
<td>MINERALS, etc.</td>
<td>0.60%</td>
</tr>
<tr>
<td>BUTTERFAT</td>
<td>3.65%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

12. **DROPLETS OF MILK WITH COMPONENT PERCENTAGES** —
If we again examine the composition of milk by component percentage, we can envision many different possibilities for a component pricing system.
13. COMPONENT PRICING POSSIBILITIES — As this slide indicates, there are many possible combinations of components that could be used for pricing purposes.
14. "CALIFORNIA" COMPONENT PRICING — The method depicted here is similar to the one that has been used in California since 1962. This type of pricing scheme places a dollar value on the butterfat, the MSNF (which includes the protein, lactose, and minerals), and a value on the fluid carrier, or water component.
15. **MSNF, BUTTERFAT PRICING** — Another possibility would be to price only the MSNF and butterfat components, as this slide indicates.
16. **PROTEIN, BUTTERFAT PRICING** — Yet another possibility would be to single out one (or more) of the elements within the MSNF grouping, such as protein, and use it and butterfat for pricing purposes.
17. **TOTAL SOLIDS PRICING** — Another possibility would be to combine *all* of the solids in milk, the butterfat as well as the nonfat solids, and assign the value for milk based upon this total.

<table>
<thead>
<tr>
<th>WATER</th>
<th>PROTEIN</th>
<th>LACTOSE</th>
<th>MINERALS, etc.</th>
<th>BUTTERFAT</th>
<th>TOTAL SOLIDS</th>
</tr>
</thead>
</table>
18. **COMPONENT PRICING POSSIBILITIES** — As you can see from this slide, and the past few, the possibilities for different types of component pricing are numerous indeed, and many different components are currently used by coops in their “incentive” programs. However, in most cases, these plans still pay producers in the current manner while adding an incentive, or premium payments to those producers which market milk that has a higher percentage of the chosen component than the base percentage.

No FMO has of yet incorporated any form of component pricing. A legitimate question at this juncture might be: “Why, with all the apparent interest in component pricing, has it not been incorporated into the FMO program”? Basically, two factors have prevented this implementation in the past. The first factor relates to testing costs and procedures, and the 2nd, unrecoverable costs for components used in Class I fluid products.
19. **OLD LAB EQUIPMENT** — Until relatively recently the technology required for widespread, fast, and affordable testing of milk for components other than butterfat was not available. In the past, Babcock equipment was used by anyone buying or selling milk or cream, but it was only capable of testing for butterfat.
20. **NEW LAB EQUIPMENT** — However, in recent years we have witnessed the development of faster, more reliable, less expensive laboratory testing equipment than can be and is being used to test for various components in milk, other than just butterfat. Therefore the cost and technology has become less a prohibitive factor; however, there still exists some difficulties in the testing and verification areas that will need to be overcome before component pricing can be incorporated into the FMO program.
21. **A & B MILK JUGS** — The problem of unrecoverable raw product costs associated with component pricing remains a formidable stumbling block. Unrecoverable raw product costs would result if fluid milk handlers were forced to pay for milk used in Class I products on a component basis. The handlers that received milk with high component test (be it MSNF, protein, or some other component — other than butterfat) would be charged more per hundredweight than the handler that received raw milk with a lower component test. The problem here is that handlers receiving high component milk would have no feasible means of recovering the additional costs for the high levels of this component. Since components such as MSNF, protein, etc., cannot be separated from milk, as butterfat can, to reduce the percentage down to legal minimum (currently 8.25% MSNF) this higher cost would have to be recovered from the consumer. This too is not feasible since a handler would likely have very little success in selling his fluid milk at a higher price than his competitors simply because it contained a greater percentage of MSNF (or protein). If these two milk jugs (A & B) containing homogenized milk were on your grocery shelf, and one was priced 5 to 10 cents higher, which would you purchase?
22. **MILK JUGS WITH?** — Moreover, could you determine which of these milk jugs contained higher MSNF than the others and, if you could, would you be willing to pay the additional costs? (Assuming both meet or exceed required minimum levels). Most consumers probably would not! Thus the fluid handler which received the high solids milk would be stuck with unrecoverable costs, and this dilemma remains as the single most compelling deterrent to the implementation of component pricing in Class I markets.

How can this problem be overcome? One solution would be to raise the minimum standards for fluid milk to a level high enough to effectively eliminate the majority of these unrecoverable costs. While we have filed such a petition with FDA, recent and ongoing attempts to do this have not, as of yet, been successful. Another possible solution would be to devise a component pricing plan that would overcome this problem.
23. A.M. PLAN — This plan has been dubbed the Alternate Method Plan by USDA for purposes of illustration. A plan utilizing the same concept has been devised by the National Milk Producer's Federation (NMPF). It should be emphasized at this juncture that this is only one of many possible plans which could be implemented. Also this plan prices MSNF and butterfat, although it is readily adaptable to other components. (EMPHASIZE THIS)
REFERENCES


UDDER INFECTIONS WITH CAE VIRUS IN GOATS

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Storrs, Connecticut

The caprine arthritis encephalitis virus (CAEV) typically induces a persistent infection in the mammary glands of goats, and the virus is commonly transmitted from infected does to newborn kids through nursing. When goat farmers prevented this transmission by rearing newborns on pasteurized milk their new generations of goats were not only free of arthritis but appeared to produce more milk than their dams, and none developed the "hard udder-agalactia" problem that has plagued many goat farmers. This observation suggests that CAEV causes hard udders but does not rule out the possibility that pasteurization may have also destroyed another pathogenic agent that might be associated with this condition.

Another observation made by goat farmers was that some serologically negative (virus-free) dairy goats that were milked by milking machines, in a line with infected goats, serologically converted to positive. This suggests that CAEV transmission can occur by the intramammary route as well as the oral route.

Preliminary studies undertaken at the University of Connecticut to determine the possible role of CAEV in causing hard udders and mastitis have revealed the following:

1. CAEV has been isolated from the udders of six goats serologically positive for CAEV antibodies and free of bacterial mastitis. Two of these had no mammary gland lesions. Three had mild to severe interstitial infiltrates of mononuclear cells including macrophages, lymphocytes, and plasma cells. The more severe cases also had considerable fibrosis. It was not determined whether this fibrosis may have been caused by previous bacterial mastitis or whether it was caused by CAEV infection.

2. One of the goats had the typical hard udder described by goat owners. This goat was sacrificed three weeks postpartum. Grossly, the mammary tissue was uniformly firm and contained very little milk. Microscopic examination showed a massive infiltration of macrophages, lymphocytes and plasma cells into the gland. Normal glandular tissue was largely obliterated with only a small number of alveolar glands and tubules remaining. There was a milk fibrosis.

In addition to the CAEV isolated from this goat in tissue culture, electron microscopic examination of mammary tissue revealed numerous virus particles, smaller than CAEV, present in the cytoplasm of inflammatory cells. These particles were non-enveloped, 70-80 nm in diameter, and had prominent spikes on their surfaces. Their identity or virus type has not been determined.
As an initial pilot transmission study $2 \times 10^5$ virus infected cells from this mammary tissue, grown in tissue culture for three months, were inoculated into one udder half of a CAEV negative milking goat. Other than a transient one day elevated somatic cell count no abnormalities have been observed for two months since inoculation. This experimental goat has developed a weak positive reaction on the CAEV serum AGID test.

3. In studies of 759 goat milk samples submitted to the University of Connecticut mastitis laboratory it was determined that 11% had nonspecific mastitis, meaning that milk leukocyte counts (excluding cytoplasmic fragments) were over 1.5 million per ml. but no bacteria were present. In a similar evaluation of 150,000 bovine milk samples only 2% were found to be nonspecific. It is suspected that some of these nonspecific goat mastitis cases are due to CAEV infections, but tests have not yet been performed to assess their CAEV status.

4. To determine whether CAEV is a public health risk for humans, serum samples from four people who routinely drink CAEV infected raw milk were tested for CAEV antibodies by an AGID test. All four samples were negative, suggesting that this virus does not infect humans by the oral route. However, more samples need to be tested to establish a valid conclusion. The conclusion that may be drawn from both the goat farm observations and preliminary laboratory studies are as follows:

a. CAEV infection typically involves the mammary gland. Although these infections may cause no disease in some goats they appear to cause mild to severe interstitial mastitis resulting in hard udders in some goats and also diminished milk production. A second unidentified virus observed in these studies may also be involved in these inflammations.

b. Once established, CAEV becomes a persistent infection that probably cannot be eliminated. Therefore, control depends on prevention of infection. This can be effectively accomplished by rearing newborn goats on pasteurized or virus-free milk and segregation or culling of infected goats to prevent contact or insect transmission to uninfected animals. To prevent infection through milking practices, infected goats should be milked last.
ANTIBIOTIC RESIDUES IN COLOSTRUM, MILK, AND MEAT OF DAIRY COWS FOLLOWING ANTIBIOTIC THERAPY*

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Knoxville, TN 37901-1071

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Amherst, MA 01003

SUMMARY

Experiments were conducted to determine persistence of antibiotic residues in colostrum, milk, and meat of dairy cows and calves after antibiotic treatment. Antibiotic residues were detected qualitatively by microbiological assays utilizing Bacillus stearothermophilus or Bacillus subtilis. Four of 186 colostrum samples from cows intramammarily infused with antibiotics at drying off were positive for residues by the Delvotest. Only one was confirmed positive by the Bacillus stearothermophilus disc assay following heat treatment. All colostrum samples from 48 cows not treated at drying off were negative. The number of milk samples positive for residues after antibiotic treatment during lactation decreased with time after treatment. However, over 16% of milk samples obtained 96 hr after treatment were positive for residues by the Delvotest. Route of antibiotic administration appeared to have the greatest impact on persistence of residues in milk. Penicillin added to milk and fed to young calves for 3 days resulted in several urine samples positive for residues by the Live Animal Swab Test. However, all samples of kidney and muscle from these calves at slaughter were negative for residues by the Swab Test On Premises. No residues were detected in muscle samples from the neck or flank of cull dairy cows at slaughter by the Swab Test On Premises, but 3% of kidney samples were positive. Over 21% of urine samples from cull dairy cows were positive for residues by the Live Animal Swab Test. There was no clear association between residues in kidney and residues in urine.

INTRODUCTION

The Residue Avoidance Program (RAP), initiated in 1982, is an educational program funded by the United States Department of Agriculture, Food Safety and Inspection Service (USDA–FSIS) and administered by

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The major emphasis of RAP is to educate animal producers about the problems associated with residues, about tests that can be used to check animals and animal products for residues, and about management practices to prevent residue violations. A matrix of projects is addressing residue avoidance in several animal species. Our project was designed to evaluate the persistence of antibiotic residues in dairy products following antibiotic treatment utilizing new tests promoted for use “on the farm.”

Antibiotics are an important component in the prevention and control of diseases affecting dairy cows. Two common diseases associated with dairy cattle are mastitis and infections of the reproductive tract. These diseases are readily treated and potentially controlled by antibiotics. However, the widespread use of antibiotics by dairy producers has created potential residue problems in dairy products to be consumed by the general public. Because of the public health significance, dairy products contaminated with antibiotics are considered unfit for human consumption. While strict guidelines have been established for use of milk and meat from treated animals, little information is available on antibiotic contamination which occurs when these guidelines are followed. Furthermore, the development and use of new and more sensitive methods for the detection of antibiotic residues may amplify the residue problem. Consequently, contamination of animal products by antibiotics continues to be a major concern of state and federal regulatory agencies.

The objectives of our study were to:

1) Determine if antibiotic residues persist in bovine mammary secretion at parturition following antibiotic therapy at drying off.

2) Determine the duration of antibiotic residues in milk after intramammary, intramuscular, intrauterine, or other parenteral routes of antibiotic administration.

3) Determine residues in urine and meat of young veal calves after oral consumption of antibiotics.

4) Document the extent of antibiotic contaminated carcasses of dairy origin reaching the slaughterhouse.

MATERIALS AND METHODS

The University of Massachusetts dairy research herd and four commercial dairy farms participated in this study. All cows in these herds were on Dairy Herd Improvement Association testing programs, milked twice daily in parlors, and housed in free stall facilities.

Following the last milking of lactation, cows were dry treated in all quarters with antibiotics approved for use in dry cows. Treatments were:

1) one million units procaine penicillin G plus 1 g dihydrostreptomycin, 2) 400 mg novobiocin, 3) 500 mg benzathine cloxacillin, 4) 300 mg cephalpirin benzathine, or 5) no antibiotic. Date of infusion, calving, and length of dry period were recorded for each cow. A composite colostrum sample was
collected at the first milking after parturition and stored at 4 C until assayed.

Composite milk samples were collected from lactating cows 24, 48, 72, and 96 hr after antibiotic treatment. Type of antibiotic used, route of administration, and duration of treatment were noted.

Twenty-seven Holstein bull calves, ranging in weight from 34 to 55 kg, were purchased from a local cattle dealer. A treatment regimen was chosen to reflect management of young veal calves on dairy farms prior to slaughter. Whole milk was fed twice daily to 8% of body weight for 3 days. Calves in the control group (n = 9) received no penicillin, calves in the low group (n = 8) received 23,760 IU procaine penicillin G daily for 3 days, and calves in the high group (n = 10) received 47,520 IU procaine penicillin G daily for 3 days. These levels were chosen to approximately simulate 25 or 50% recovery of antibiotic in milk after intramammary infusion of 100,000 IU penicillin, commonly used in the treatment of mastitis.

Urine samples were collected from calves over 11 hr after morning feeding during the 3-day experimental period. Samples were stored at 4 C for 24 hr until analyzed for residues. All calves were sacrificed on day 4 of the trial. Samples of urine from the bladder and pieces of kidney and muscle were collected and transported to the laboratory on ice.

Lastly, cull dairy cows purchased by a local slaughterhouse were screened for antibiotic residues. Samples of urine, milk, kidney, and muscle were obtained at slaughter. All samples were assayed within 24 hr of collection.

Residue Determination

Milk and colostrum samples were analyzed qualitatively for antibiotic residues by the Delvotest P (GB Fermentation Industries, Inc., Des Plaines, IL). Briefly, the Delvotest utilizes Bacillus stearothermophilus var. calidolactis, a fast growing acid producing microorganism. A nutrient tablet containing bromcresol purple and 1-ml of sample were added to ampules containing agar and spores. The mixture was incubated at 65 C for 2.5 to 3 hr. In the absence of growth inhibitors, acid produced by B. stearothermophilus changed the indicator from purple to yellow. If inhibitors were present, the purple color remained.

Colostrum samples positive for antibiotic residues by the Delvotest also were screened by the B. stearothermophilus disc assay after heating samples to 82 C for 5 min. Zones of bacterial growth inhibition greater than 16 mm in diameter were interpreted as positive for residues.

Urine samples were tested for residues by the Live Animal Swab Test (LAST) advocated for use on commercial dairy farms as a screening test for the detection of antibiotic residues in urine of calves and cull dairy cows. Briefly, sterile cotton swabs were immersed in urine and placed on agar plates containing Antibiotic Medium No. 5 (Difco Laboratories, Detroit, MI) seeded with Bacillus subtilis (ATCC 66633) spores. A 5-μg neomycin disc (BBL, Cockeysville, MD) was included on each plate as a positive
control. Plates were incubated 16 to 20 hr at 29 C. Following incubation, zones of bacterial growth inhibition around the neomycin disc and each swab were measured. The presence of any zone around swabs was considered positive for residues if the zone of inhibition around the neomycin disc was > 16 mm.

Muscle and kidney samples were tested for antibiotic residues by the Swab Test On Premises (STOP) as described\textsuperscript{14}. This method is similar to the LAST, but a lower concentration of \textit{B. subtilis} spores is used. The presence of any zone around swabs was considered positive for residues if the zone of inhibition around the neomycin disc was ≥ 21 mm. The STOP is promoted for use by meat inspectors to detect antibiotic residues in animal carcasses at slaughter.

RESULTS

Four of 186 colostrum samples from cows treated with antibiotics at drying off were positive for antibiotic residues by the Delvotest (Table 1). However, only one was confirmed positive by the \textit{B. stearothermophilus} disc assay. The positive colostrum sample was from a cow calving 50 days after infusion with cephaolin at drying off. All colostrum samples from cows not treated at drying off (n=48) were negative for residues. In addition, colostrum from 53 first calf heifers was assayed and only one was positive by the Delvotest, but was not confirmed positive by the disc assay.

Most cows in this study had dry periods greater than 46 days (Table 2). Consequently, valid conclusions about residues in cows with shorter dry periods cannot be made. However, four cows in this study had dry periods less than 30 days (range 11 to 26 days) and 22 cows had dry periods less than 46 days. Colostrum samples from all these cows were negative for antibiotic residues by the Delvotest.

A variety of antibiotics and treatment schedules were used to treat cows during lactation. Thus, details regarding the use of a specific antibiotic or a specific treatment schedule are limited. However, a common denominator was the route of antibiotic administration and these data are presented in Table 3. The primary use of antibiotics in this study was for the treatment of mastitis. Data on intrauterine infusion of antibiotics were primarily derived from one herd because of a reproductive problem.

Over 50\% of milk samples obtained 24 hr after last antibiotic treatment were positive for residues by the Delvotest (Table 3). Route of antibiotic administration had a marked impact on persistence of residues in milk. Frequency of residues in milk 24 hr following intrauterine infusion was less than other routes of administration. Frequency of residues after intramuscular, intramammary or multiple routes of treatment were similar.

The number of samples positive for antibiotic residues decreased as time from last treatment increased. However, 16.5\% of milk samples obtained 4 days posttreatment were positive for residues. Only 3\% of samples taken 4 days after intrauterine treatment were positive for residues while more
than 25% were positive after intramuscular, intramammary, or multiple routes of administration.

Data on residues in urine of calves after consumption of milk containing penicillin are in Table 4. Data were grouped into 3-hr blocks of time relative to morning feeding. Number of calves urinating within a given time period ranged from a minimum of one on day 3 in the high group to a maximum of eight on day 3 in the control group. No residues were detected in urine of calves in the control group using the LAST. Residues were not consistently detected in urine of calves receiving penicillin. The number of positive urine samples within any time period ranged from zero to five for calves in low and high groups.

Residues in urine, kidney, and muscle of calves at slaughter after being fed 0, 23,760 or 47,520 IU procaine penicillin G daily for 3 days are in Table 5. Urine samples from four control calves and three calves in the low group were not collected. As observed in our daily collection periods (Table 4), urine from calves on the control diet was negative for residues. In addition, no residues were detected in kidney or muscle of control calves by the STOP. Three of five and eight of ten urine samples obtained from the bladder of calves in the low and high groups were positive for residues by the LAST. However, all calves positive for residues in urine were negative for residues in kidney and muscle.

Antibiotic residues in tissues from cull dairy cows at slaughter are presented in Table 6. Muscle tissue from the neck and flank of all cows tested were negative for residues by the STOP. However, 3.1% of kidney samples were positive for residues. Zones of *B. subtilis* growth inhibition ranged from 7 by 11 to 18 by 21 mm in size.

In addition to tissue samples, blood, urine, and milk were collected from 60 cull dairy cows at slaughter and these data are presented in Table 7. About 22% of urine samples were positive for residues by the LAST. Zones of bacterial growth inhibition ranged from 10 by 12 to 14 by 27 mm in size. Two of 60 cows (3.3%) were positive for residues in kidney tissue by the STOP. Only one cow was positive for residues in both urine and kidney. No residues were detected in flank, neck, or blood and only one of 60 milk samples was positive for residues by the Delvotest.

**DISCUSSION**

Results of this study suggest that if manufacturer's recommendations are followed, antibiotic residues in colostrum following dry cow therapy with products used in our study should not be a significant problem. Only one of 186 colostrum samples was positive for residues by both the Delvotest and *B. stearothermophilus* disc assay. Our data on antibiotic residues agree with reports on dry cow treatment with novobiocin\(^{15}\), penicillin G\(^{16}\), penicillin G plus dihydrostreptomycin\(^{17}\), and cloxacillin\(^{17,18}\). Furthermore, colostrum from cows treated at drying off and fed to young veal calves should not result in antibiotic residue problems.

The Delvotest was used in our studies because it is presently promoted
as an on-farm method for detecting antibiotic residues. The Delvotest is sensitive, fairly rapid, and simple to use. However, only one of four colostrum samples positive by the Delvotest was confirmed positive by the *B. stearothermophilus* disc assay after heat treatment. This suggests that, in some instances, natural inhibitor(s) in colostrum may produce false positive Delvotest readings. However, Delvotest false positive readings were observed in only 1.6% of colostrum samples tested.

Previous reports by Reiter19 and Reiter and Oram20 demonstrated that lactoferrin, an iron binding whey protein, inhibited *B. subtilis* and *B. stearothermophilus*. Lactoferrin is in high concentration in dry secretion and colostrum21 and also increases during acute mastitis22. Thus, lactoferrin as well as other components of mammary secretion may be associated with false positive Delvotest readings.

Antibiotic residues in milk following treatment during lactation decreased as the time after treatment increased. The frequency of antibiotic residues in milk after intrauterine infusion was markedly lower than other routes of antibiotic administration. Frequency of residues after intramuscular, intramammary, or multiple treatment routes were similar 24 hr after treatment. Over 16% of milk samples were positive for residues by the Delvotest 96 hr posttreatment. This exceeded withdrawal times noted for several of the antibiotic preparations used. The persistence of antibiotic residues in milk could be associated with several variables such as milk production at time of treatment, type of antibiotic used, type of vehicle used in antibiotic preparations, and the disease state of animals23. Furthermore, current methods of detecting antibiotic residues are significantly more sensitive than methods used in the past. Thus, milk discard times may not be long enough for some of the antibiotics currently used for the treatment of diseases affecting dairy cows. Clearly, further controlled studies using sensitive methods for the detection of antibiotic residues are needed to clarify withdrawal times to minimize the risk of antibiotic contamination of milk.

The LAST was used to detect antibiotic residues in urine of calves after consumption of milk containing penicillin. This procedure is promoted for use on commercial dairy farms to prevent residue violations from occurring 13. No residues were detected in urine from control calves suggesting that the frequency of false positives would be minimal. However, over 61% of urine samples from calves that received penicillin were also negative for residues. Thus, there was no clear relationship between presence of residues in urine and the feeding of milk containing penicillin. Furthermore, all calves positive for antibiotic residues in urine were negative for residues in kidney and muscle tissue by the STOP.

These data indicate that limited feeding of milk containing procaine penicillin G does not result in detectable residues in meat using the STOP, but does result in residues in urine using the LAST. We caution against extrapolating these results to other drugs or situations where young veal calves are consuming antibiotics in milk. Other studies 24,25,26 detected oxytetracycline, amoxicillin, and ampicillin in serum, bile, kidney, liver,
urine and muscle after oral consumption. Differences between our study and others could be attributed to lower intake of antibiotic, to type of antibiotic used, to the medium that penicillin was suspended in, or to different methods of antibiotic residue detection.

The LAST and STOP procedures also were used to detect antibiotic residues in cull dairy cows at slaughter. Over 21% of urine samples from cull cows were positive for residues by the LAST. However, only 3.3% of kidney samples and no muscle samples were positive by the STOP. Only one cow was positive for residues in both kidney and urine. Thus, these data suggest that residues in urine at slaughter are not necessarily associated with residues in meat, similar to that observed in the veal calf study. Clearly, further studies are needed to determine the reasons for the high frequency of residues in urine and lack of an apparent association between residues in urine and other tissues, especially since the LAST is promoted as an on-farm method in checking animals for antibiotic residues before they are sent to slaughter.

ACKNOWLEDGEMENTS

We are grateful to the dairymen and owner of the slaughterhouse for cooperating on this project. These studies were supported by grants from USDA–ES and the George H. Walker Milk Research Fund. We thank Mr. Dennis Kwider and Agri Mark, Inc. for providing Delvotest ampules. Technical assistance of Mary Ellen Fydenkevez, Tim McKenna, and Melinda Baker is appreciated.

<table>
<thead>
<tr>
<th>Dry Cow Product</th>
<th>No. Cows Treated</th>
<th>Colostrum Tested Positive Delvotest Confirmed$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloxacillin</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>Pen-Strep</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Cephapirin</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>4</td>
</tr>
</tbody>
</table>

$^a$Data from reference 3.

$^b$Confirmed by B. stearothermophilus disc assay.
TABLE 2. Distribution of Cows by Dry Period Length.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Days Dry</th>
<th>No. Cows</th>
<th>Positive Colostrum\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>16-30</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>31-45</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>46-60</td>
<td>105</td>
<td>1</td>
</tr>
<tr>
<td>&gt;60</td>
<td>103</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data from reference 3.

\textsuperscript{b}By \textit{B. stearothermophilus} disc assay.

TABLE 3. Residues in Milk After Antibiotic Treatment During Lactation.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Days After Treatment</th>
<th>Route of Administration</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IM\textsuperscript{b,c}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imam\textsuperscript{d}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IUT\textsuperscript{e}</td>
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</tr>
<tr>
<td></td>
<td>Other\textsuperscript{f}</td>
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</tr>
<tr>
<td>1</td>
<td>9/14</td>
<td>96/191</td>
</tr>
<tr>
<td>2</td>
<td>4/13</td>
<td>59/196</td>
</tr>
<tr>
<td>3</td>
<td>3/12</td>
<td>33/196</td>
</tr>
<tr>
<td>4</td>
<td>3/9</td>
<td>30/182</td>
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</table>

\textsuperscript{a}Detected by Delvotest.

\textsuperscript{b}No. positive/No. tested.

\textsuperscript{c}Intramuscular injection.

\textsuperscript{d}Intramammary infusion.

\textsuperscript{e}Intrauterine infusion.

\textsuperscript{f}Multiple routes of treatment.
TABLE 4. Residues in Urine of Calves After Consumption of Milk Containing Penicillin.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
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<th>Hours&lt;sup&gt;b&lt;/sup&gt;</th>
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<td></td>
<td>&lt;3</td>
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<tr>
<td></td>
<td>3</td>
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<td>0/8</td>
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<tr>
<td>Low</td>
<td>8</td>
<td>2</td>
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<td>3</td>
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<td>1/5</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>2</td>
<td>1/7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>5/7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from reference 5.

<sup>b</sup> Hours after morning feeding.

<sup>c</sup> Calves received afternoon feeding during this period.

<sup>d</sup> No. positive/no. urinating.

TABLE 5. Frequency of Residues in Urine, Kidney, and Muscle of Calves at Slaughter.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Sample</th>
<th>Urine</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>---------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Control</td>
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</tbody>
</table>

<sup>a</sup> Data from reference 5.

<sup>b</sup> Only five urine samples obtained.
TABLE 6. Residues in Kidney and Muscle of Cull Dairy Cows at Slaughter.\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kidney</th>
<th>Neck</th>
<th>Flank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Tested</td>
<td>259</td>
<td>265</td>
<td>265</td>
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<tr>
<td>No. Positive</td>
<td>8</td>
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<tr>
<td>% Positive</td>
<td>3.1</td>
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</table>

\(^a\)Detected by the STOP procedure.

TABLE 7. Antibiotic Residues in Cull Dairy Cows (n=60) at Slaughter.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kidney(^a)</th>
<th>Flank(^a)</th>
<th>Neck(^a)</th>
<th>Blood(^b)</th>
<th>Urine(^b)</th>
<th>Milk(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Positive</td>
<td>2 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>13 / 21.7</td>
<td>1 / 1.7</td>
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<tr>
<td>% Positive</td>
<td>3.3 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>21.7 / 1.7</td>
<td>1 / 1.7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Residues detected by STOP.

\(^b\)Residues detected by LAST.

\(^c\)Residues detected by Delvotest.
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5. Prange, R. W., Oliver, S. P., Duby, R. T., Tritschler, J. P.: Residues in young veal calves after consumption of milk containing penicillin. J. Dairy Sci., accepted for publication.


REPORT OF THE COMMITTEE ON MASTITIS

Chairman: C. A. Jordan, Morgan Center, VT
Vice Chairman: J. S. McDonald, Ames, IA

J. B. Adams, VA; J. M. Arnoldi, WI; R. W. Bennett, GA; A. N. Bringe, WI; R. B. Bushnell, CA; M. L. Crandall, MD; N. E. East, CA; C. F. Emerick, WA; T. J. Fuhrmann, AZ; Carl Graham, MO; J. W. Groff, PA; F. D. Gregerson, CO; M. C. Howard, CA; D. E. Jasper, CA; C. N. Jewett, AR; D. E. Johnson, WI; C. A. Kirkbride, SD; T. G. Murnane, TX; J. E. Post, CT; D. N. Rice, NE; Richard Sechrist, OH; F. E. Sterner, CO; G. H. Swenson, MI; L. A. Wager, NY; D. U. Walker, VT; R. F. Weidner, IL

The October 17, 1983, committee report was approved as read.

The five goals developed last year were reviewed and the following progress reported:

1. Strengthened our working arrangement with the three other national committees by:
   a) Continuing to play an active role in the Joint Mastitis Committee made up of representatives of USAHA, NMC, AVMA and AABP attending a meeting of that group while at the National Mastitis Council meeting in February 1984 where we emphasized the need for more basic research in mastitis, the development of more state control programs and the development of new infusion products.
   b) The Chairman is representing USAHA on the National Mastitis Council Board.
   c) We attended and participated in the February 1984 meeting of the AVMA Mastitis Committee.

2. Continued our study of Mastitis Control programs by:
   a) Dr. Wesley Bolton of the University of Vermont outlining the Vermont Milk Quality Enhancement program, the purpose of which is to improve the quality of the milk produced in Vermont by decreasing somatic cell counts, bacteria counts, increasing shelf life and improving the flavor of milk through the efforts of a State Mastitis Control program. Milk samples are collected and cultured but the main emphasis is on producer education through the use of state dairy inspectors, Extension personnel, DHIA testers, dairy fieldmen, practicing veterinarians and equipment dealers representing about 200 people making a minimum of 132,000 farm visits a year. This group will be trained by Dr. Woody Panky, recently moved from Louisiana operating under the direction of the State Mastitis Committee with all involved using the same procedure and telling the same story. There will be four technicians (2 hired at first with the dairyman paying the laboratory fees, cost of drugs and veterinary service and expenses of making any equipment changes.

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Any producer with 1,000,000 or more somatic cells will be referred to this program for help. Vermont hopes the program will be successful enough to allow them to reduce the maximum somatic cell count to 600,000 or 700,000. The emphasis is on a team effort of the above-mentioned people all working together to better solve a farmer's quality problem.

b) Dr. Leslie Wager, Director of the New York State Mastitis Control Program, outlined the makeup of a similar group in New York State, the Empire State Mastitis Council, which has been the guiding force of that program monitoring its efforts and evaluating its results, making certain that all involved speak the same language and with one voice. He reported that through the efforts of their program the number of producers with high somatic cell counts continued to drop even though the maximum allowable cell count was reduced from 1,500,000 to 1,000,000. He used the example of Region 5 which had 343 herds with cell counts over 1,000,000 in 1982 dropping down to 212 herds in 1983. Only 18% of these 212 herds had been violators at the higher level. They have installed computers which will allow them to operate out of a central laboratory instead of their present five regional labs. They are involved in a cooperative study with Cornell University to determine the significance of Stray Voltage as a cause of mastitis and poor quality milk.

3. In order to determine the progress being made in the development of milk quality programs, our clerk, John Adams, agreed to present an “Overview of Cooperative Milk Quality Programs and Monetary Incentives in the United States,” which you have just heard. In addition, he reviewed the proceedings of the recent National Mastitis Council meeting held in California, where it was emphasized that back flushing of the milking system between cows does not decrease the need for post-milk teat dipping. He noted that California's dedication to milk quality control has lowered their average somatic cell count in the State to only 300,000 or 400,000.

The Committee continued to show their dedication to Residue Avoidance by inviting Dr. Stephen P. Oliver of the University of Tennessee to present the results of his studies of “Antibiotic Residues in Colostrum, Milk and Meat in Dairy Cows following Antibiotic Therapy,” a copy of which will be printed in the proceedings of this meeting. He found that only 4 out of 186 colostrum samples collected from cows intramammarily infused with the antibiotics at drying off time were positive for residues by the Delvo-p test, with only one of those confirmed positive by the Bacillus Sterothermophilus Disc Assay following heat treatment. The number of milk samples positive for residues after the antibiotic treatment of milking cows decreased with time, but 16% of those samples obtained 96 hours after treatment were still positive. Route of administration appeared to have the greatest impact on persistence of residues in milk, penicillin added to milk and fed to young calves for three days resulted in several
urine samples positive for residues, but all samples of kidney and muscle from these calves at slaughter were negative to residues. No residues were detected in muscle samples from the neck or flank of cull dairy cows at slaughter by the Swab Test on Premises (STOP), but 3% of kidney samples were positive. Over 21% of urine samples from cull dairy cows were positive for residues by the Live Animal Swab Test (LAST). He reported that there was no clear association between residues in kidney and residues in urine.

Dr. Ralph McQueen distributed and reviewed a Residue Avoidance fact sheet which points out that Mastitis prevention is one of the prime factors in decreasing residues. He noted that current mastitis control programs rely heavily on the use of antibacterial drugs, through the infusion of all quarters of all cows on drying off and intramammary/systemic treatment of clinical cases. He listed several ways to avoid detectable drug residues emphasizing the necessity to use a drug according to directions with any deviation or the extra label use of drugs necessitating the testing of milk before adding it to the supply for shipment. He noted that an effective mastitis control program returns from $3.00 to $5.00 from increased production of milk alone for each $1.00 invested.

The committee continued its study of mastitis in dairy goats started last year by hearing a report to be printed in the proceedings from Dr. John E. Post of the University of Connecticut concerning udder infection in goats caused by the Caprine Arthritis Encephalitis Virus. This virus is the cause of the "Hard Udder Syndrome" as well as arthritis in goats, sometimes quite destructive in nature. He reported that this virus is commonly transmitted from infected does to their newborn kids in their milk. This makes it necessary to immediately segregate the kid from its mother, pasteurize the milk or feed the kids cow's milk. This new generation of CAEV free goats was not only free of arthritis but produced more milk and did not develop the Hard Udder Syndrome. He also reported that serum samples taken from four people, who regularly drink infected milk, were negative and the people should have no ill effects.

It was agreed that the committee should continue:

1. Its study of mastitis in dairy goats.
2. Continue to work closely with the Mastitis Committees of the other three national organizations.
3. Continue to encourage more states to develop mastitis control programs.
4. Continue to encourage drug companies to develop new and more effective infusion products.
5. Continue to encourage dairy cooperatives and proprietary handlers to update their present quality programs adding a monetary incentive whenever possible.
6. Seek a report on the study of bacteriological and environmental influences on somatic cell content and mastitis herd profile.
FERMENTATION – AN ALTERNATIVE TO LANDFILL AND CARCASS DISPOSAL

Charles N. Dobbins, Jr., D.V.M.
Head, Extension Veterinary Department
The University of Georgia
Athens, Georgia

The disposal of carcasses, food waste, manure or other animal products contaminated by pathogenic organisms provide major problems for regulatory agencies. In the past, burning, deep burying, and perhaps rendering offered the best means of disposal.

However, today with EPA requirements concerning burning and burial, other alternatives must be explored. Controlled fermentation offers a partial answer. Coupled with rendering, fermentation offers an excellent method of disposing of contaminated carcasses, as well as retaining some useful purpose of these carcasses.

At least two topics yesterday, discussed the field testing of a prototype unit that is capable of handling chicken, swine or calf carcasses. The process consists of an enclosed elevating conveyor belt that may also serve as a euthanizing cabinet, a grinder to reduce the particle size, a mixing chamber in which a source of carbohydrate and a Lactobacillus culture can be added and mixed.

While the prototype did have some mechanical problems, they are in the process of being modified to overcome the difficulties. Within the next four weeks, the field unit that was tested will be modified and made available in case the need arises again for massive disposal of carcasses.

I would like to discuss some of the laboratory information that has been developed over the past four years that supports the principle of fermentation as an initial step in the destruction of pathogenic organisms. While our work has dealt with representatives of various bacterial and viral groups, we have not dealt with the actual exotic organisms represented by the various groups. This is a job for Plum Island or NADL. I would simply like to introduce the virus and bacterial groups with which we have worked and give you an idea under various temperature conditions when one could expect these organisms to be destroyed.

This work was done in a tightly controlled environment in which individual organisms were introduced into food waste. Through periodic sampling, the approximate time of destruction was determined.

As you could tell from the previous slides, the Adeno Virus group represented by Infectious Canine Hepatitis was the most difficult to destroy. However, at the temperatures of 30°C and 40°C, this representa-
tive of the group was destroyed within five days. Of the Myxo Virus group, represented by Newcastle Disease Virus and the Measle Virus, the organisms were destroyed within two days at 30°C and 40°C and within three days at 20°C.

Of the bacterial groups, the gram negative bacteria were destroyed within the first day at 20°C and 30°C. Gram positive bacteria were destroyed within two days except Group E Streptococcus. Since this organism is so similar to Lactobacillus, this is not surprising.

To determine the effect of whole carcass contamination, Newcastle Disease Virus was introduced into live chickens. When the disease reached its peak, these birds were euthanized and their carcasses were included at a 20% and 40% level in relation to the carbohydrate source. In either case, the Newcastle Disease Virus was destroyed within five days at 20°C, two days at 30°C, and within 1 day at 40°C.

The same situation was developed with an infection of Salmonella typhimurium in rats. At the 20% carcass level, as well as at the 40% carcass level, Salmonella typhimurium was destroyed within the first day at 30°C and 40°C.

What we are attempting to show is that all viruses and bacteria except Group E. Streptococcus organisms were destroyed within seven days when the temperature can be maintained above 20°C.

Fermentation offers a possible answer in the safe utilization of food waste as a source of food for swine. It offers a partial answer for animal manure and other wastes that are generated at centers such as the Truman Import Center located at Fleming Key, Florida. It offers a partial answer on how to handle carcasses that must be depopulated for regulatory reasons.

Work at Ft. Benning, Georgia indicates that food waste may be collected and held for long periods of time without further decomposition when placed in an oxygen limiting container with the aid of fermentation. Potential pathogens can be destroyed which would allow food waste to be safely fed to swine without further treatment.

Fermentation offers a partial answer to the cost of operating Import Centers that generate large volumes of manure and other animal wastes. Incineration and its high cost can be greatly reduced by fermenting animal manures and wastes as they are generated. Fermentation makes it possible, through the use of oxygen limiting containers, to hold almost any organic matter without further decomposition for indefinite periods of time. After the quarantined animals are released for movement into the United States, manure and other wastes that have been stabilized and held could be released for utilization for horticultural or agricultural purposes. Should animals show infection during the quarantine period, the fermented animal waste could be incinerated along with the carcasses of the animals. Actually, fermentation could make both the carcasses and the manure suitable for rendering or other utilization.
In the Virginia outbreak of avian influenza, the Virginia poultry industry indicated that it would not utilize poultry by product meal produced from birds that were depopulated because of avian influenza. For this reason, regulatory authorities had no choice except deep burial although they did try burning. Had the Virginia poultry industry realized that the avian influenza virus could be destroyed by fermentation prior to rendering, I believe they would have accepted the resulting poultry by product meal without question. This could have saved much of the expense experienced in the outbreak and many of the problems that came with deep burial.

I hope that Plum Island or NADL will pursue the idea of fermentation where we have left off in their containment facilities. Fermentation is a viable alternative in handling carcasses, food waste and animal products contaminated with pathogenic or exotic agents.

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2. Mr. F. D. Talkington, Dr. E. B. Shotts, Dr. R. E. Wooley, Dr. W. K. Whitehead, and Dr. C. N. Dobbins, Introduction and Reisolation of Selected Gram-Negative Bacteria from Fermented Edible Wastes, American Journal of Veterinary Research, Vol. 42, No. 8, Pages 1298–1301, 1981.

3. Mr. F. D. Talkington, Dr. E. B. Shotts, Dr. R. E. Wooley, Dr. W. K. Whitehead, and Dr. C. N. Dobbins, Introduction and Reisolation of Selected Gram-Positive Bacteria from Fermented Edible Wastes, Vol. 42, No. 8, Pages 1302–1305, 1981.

4. Dr. J. A. Dickens, Dr. R. E. Vaughn, Dr. E. B. Shotts, Dr. R. E. Wooley, Dr. J. P. Gilbert, Dr. M. S. Jones, Dr. D. W. Waltman, A Process to Ferment Food Waste and Monitor the Effect on Harmful Organisms, Publication, Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens, Georgia, 1981.

5. Dr. Jeannine P. Gilbert, Dr. R. E. Wooley, Dr. E. B. Shotts, Dr. J. Andra Dickens, Viricidal Effects of Lactobacillus and Yeast Fermentation, American Society for Microbiology, Applied and Environmental Microbiology, Pages 452–458, August 1983.

### Gram Positive Bacteria

- Listeria monocytogenes
- Erysipelothrix rhusiopathiae
- Corynebacterium pseudotuberculosis
- Clostridium perfringes
- Group E Streptococcus

#### pH 4

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<th>4</th>
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### Gram Negative Bacteria

- Salmonella typhimurium
- Salmonella anatum
- Salmonella cholera – suis
- Yersinia Pseudotuberculosis
- Yersinia enterocolitica
- Pasteurella Multocida

#### pH 4

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**Myxo Virus Group**

Newcastle Disease

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**Myxo Virus Group**

Measles Virus

pH 4

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**Corona Virus Group**

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### Herpes Virus Group

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<td>Pseudorabies</td>
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<td>Infectious Bovine Rhinotracheitis (IBR)</td>
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<td>Malignant Catarrhal Fever</td>
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<td>Equine Viral Rhinopneumonitis</td>
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### Herpes Virus Group

Porcine Pseudorabies Virus

pH 4

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### Rhabdo Virus Group

**Vesicular Stomatitis**

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### Toga Virus Group

- Bovine Virus Diarrhea (BVD)
- Louping Ill
- Rift Valley Fever
- Hog Cholera
- Equine Encephalitis

**Toga Virus Group**

**Bovine Virus Diarrhea (BVD)**

**pH 4**

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Porcine Picornavirus
Foot & Mouth Disease
Epidemic Tremor
Duck Hepatitis
Teschen Disease

Picorna Virus Group
Porcine Picorna Virus
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Irido Virus Group
Frog Virus 3
African Swine Fever

Irido Virus Group
Frog Virus 3
pH 4

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**Salmonella typhimurium**

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**Salmonella typhimurium**

**RATS**

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**Salmonella tryphimurium CHICKS**
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**Salmonella tryphimurium CHICKS**
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The Environmental Residues Committee met October 22, 1984, at 1:30 p.m. Ten members and 19 guests were present. Four reports were heard.

Dr. W. B. Bixler, Center for Veterinary Medicine, FDA, reported on activities of the Center during the past year. Dr. Bixler outlined the development of a model control plan for aflatoxins in corn. The plan outlines procedures available to states for managing contamination incidents. A total of 9 states worked closely with FDA in 1983 to deal with aflatoxin contamination.

Dr. Lea Kennedy, Center for Veterinary Medicine, FDA, discussed several specific contaminants encountered. Among these were ethylene dibromide (EDB), PCB, pentachlorophenol and pesticide residues. Of 1000 grain samples surveyed for EDB, only 2 exceeded the tolerance and none was found in 537 milk samples. PCB tolerances were lowered in fish and shellfish products. Studies involving pentachlorophenol residues in animals are currently being initiated.

Dr. Jane Robens, National Research Program Leader, ARS, USDA, reported on the mycotoxin research activities of ARS. In house research has been centered in seven (7) ARS laboratories throughout the United States. Programs include mycotoxin interactions, immunomodulation, fungal ecology, aflatoxin kinetics, ammoniation of cotton products and fescue toxicosis. Dr. Robens explained the organizational and functional distinctions of various services within USDA.

Dr. W. B. Buck, Director, Animal Poison Control Center, University of Illinois, explained a plan for Development of a Center for Emergency Preparedness on Chemical Disasters in Food Animals. This program could serve to encourage a regional network of expertise to respond to toxic or contaminant emergencies, as well as a centralized database for toxicologic information.

After committee discussion, two resolutions were adopted to be forwarded to the Committee on Resolutions. The first involved support for the concept of a national and regional network of chemical emergency preparedness in livestock and a toxicology database with central coordination. A second resolution encouraged several federal agencies to continue and expand research and surveillance activities in mycotoxins.

The meeting adjourned at 4:15 p.m.
BRIEF ACCOUNT OF CAPRINE MYCOPLASMOSIS IN THE UNITED STATES WITH SPECIAL REFERENCE TO MYCOPLASMA MYCOIDES SUBSP. MYCOIDES

A. J. DaMassa, D. L. Brooks, and N. E. East, School of Veterinary Medicine, University of California, Davis, California 95616
and
A. I. Moe, State of California, Bureau of Animal Health, Modesto, California 95350

Summary

A brief account of caprine mycoplasmosis in the United States with particular emphasis on disease caused by Mycoplasma mycoides subsp. mycoides is presented together with a review of the literature on the experimental effect of caprine strains of this organism on bovines.

The recognized mycoplasma pathogens of goats in the United States are Acholeplasma oculi, Mycoplasma agalactiae, M. conjunctivae, M. capricolum, M. mycoides subsp. mycoides and M. putrefaciens. Of these, Ach. oculi and M. conjunctivae are known causes of caprine keratoconjunctivitis, but their distribution and importance are largely unknown. Mycoplasma agalactiae is rare and M. capricolum has not been found associated with the natural disease since 1955. M. putrefaciens was encountered only recently as a cause of caprine mastitis.

Caprine strains of Mycoplasma mycoides subsp. mycoides are now known to be prevalent pathogens, particularly in northern California, the only area in the United States actively sampled. The primary clinical and pathological manifestations in adult does are mastitis, polyarthritis, and pneumonia. Young kids suffer primarily from a mycoplasmemia leading to pyrexia, pneumonia, and polyarthritis. Since the winter of 1979–1980, M. mycoides subsp. mycoides infection has been confirmed in over 700 goats. Of these, about 500 either died or were euthanized while the remainder were sold for slaughter. Because the first reports of caprine M. mycoides subsp. mycoides in the United States were from eastern states, disease due to this organism is very probably more prevalent than previously suspected.

Introduction

In the 1976 edition of "Classification of Etiologic Agents on the Basis of Hazard," issued by the United States Department of Agriculture (USDA) and the United States Public Health Service (USPHS), Mycoplasma mycoides, which includes the subspecies capri and mycoides, together with
*M. agalactiae* are classified as “exotic” agents of disease, that is “Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.” A recent issue of a similar document published in 1984 as “Biosafety in Microbiological and Biomedical Laboratories,” prepared by the previously named U.S. agencies, classifies *M. agalactiae* and *M. mycoides* (including subspecies *capri* and *mycoides*) as “Nonindigenous pathogens . . .” with the following restrictions: “The importation, possession, or use . . . is prohibited or restricted by law or by USDA regulations or administrative policies.”

However, despite the restrictive “exotic” classification of *M. agalactiae* and *M. mycoides* subsp. *mycoides*, both do occur in the United States. While *M. agalactiae* is considered rare, caprine strains of *M. mycoides* subsp. *mycoides* have been known in the United States since 1969 and are now a prevalent species of mycoplasma occurring in epizootic proportions in northern California. Published scientific reports show these strains to occur also in 9 additional states.

The purpose of this report is to 1) provide a brief overview of caprine mycoplasmosis in the United States to alert goat owners and veterinarians to the presence of debilitating and lethal mycoplasmas, 2) review selected studies undertaken to assess the pathogenicity of caprine strains of *M. mycoides* subsp. *mycoides* for bovines, and 3) recommend that United States regulatory agencies remove these mycoplasmas from the “exotic” classification.

### Overview of Caprine Mycoplasmosis

#### Pathogenic Caprine Mycoplasmas Occurring in the United States

In the United States, the known pathogenic caprine mycoplasmas are as follows:

1. *Acholeplasma oculi* initially designated *Ach. oculusi,* was recovered from an outbreak of keratoconjunctivitis in Minnesota in a goat herd, which had originated in California. Conjunctivitis and pneumonic signs were demonstrable when the organism was introduced intravenously. The distribution and importance of *Ach. oculi* infection in the United States is unknown.

2. *Mycoplasma agalactiae* has been isolated twice in northern California, once from the arthritic joint of an emaciated goat doe and once from a case of caprine mastitis. The overall status of this organism is largely unknown, and for the present, *M. agalactiae* must be considered rare in the United States.

3. *Mycoplasma conjunctivae* has been isolated from goats with conjunctivitis in the United States, and studies have confirmed that experimentally cloned cultures of this organism cause an infectious form of keratoconjunctivitis. The importance and distribution of *M. conjunctivae* in the United States is largely unknown.

4. *Mycoplasma capricolum* was first recognized in northern California as
a virulent mycoplasma responsible for an acute septicemic syndrome in kids leading to pyrexia, septicemia, and high mortality.\textsuperscript{19} The cardinal lesion was a fibrinopurulent polyarthritis involving nearly every diarthrodial joint. Later studies disclosed also that \textit{M. capricolum} killed sheep\textsuperscript{17,19} and pigs\textsuperscript{18} upon experimental inoculation and that in young kids it was responsible for a acute, diffuse interstitial pneumonia.\textsuperscript{16,25,26,27} Young kids were found to be highly susceptible to infection when exposed by the oral route.\textsuperscript{26} Kids housed in close contact with infected kids quickly succumbed to a fatal mycoplasemia.\textsuperscript{26} Adult does could be easily infected via the teat canal, resulting in pyrexia, arthritis, hardened udders, interstitial pneumonia, mastitis, agalactia, and death.\textsuperscript{27} \textit{M. capricolum} has not been recovered from diseased goats in the United States since the 1955\textsuperscript{19} studies although it was recovered from the external ear of a clinically normal goat in northern California in 1983.\textsuperscript{22}

5. \textit{Mycoplasma mycoides} subsp. \textit{mycoides} as a cause of disease in goats is the subject of later sections of this report.

6. \textit{Mycoplasma putrefaciens} was in 1980 shown to produce acute mastitis in lactating goat does.\textsuperscript{3} Large numbers of the organism may be shed from the mammary secretions for approximately one month. An unusual feature of \textit{M. putrefaciens} infection is the complete absence of clinical signs other than agalactia and mastitis. Pyrexia and hardened udders do not develop, and the infection does not spread from one side of the udder to the other.\textsuperscript{13,27} The prevalence of this mycoplasma is unknown, although one study suggested that it may be widespread.\textsuperscript{1}

**Brief Background Data on Mycoplasma mycoides. subsp. mycoides**

\textit{Mycoplasma mycoides} subsp. \textit{mycoides} is the etiological agent of contagious bovine pleuropneumonia (CBPP), an important cattle disease eradicated from the United States in 1892.\textsuperscript{7} Since 1968, however, it has been known that \textit{M. mycoides} subsp. \textit{mycoides} was also responsible for a condition described as contagious caprine pleuropneumonia (CCPP).\textsuperscript{34,38,41,44,54} According to some investigators,\textsuperscript{21} bovine and caprine isolates of \textit{M. mycoides} subsp. \textit{mycoides} are divisible into “large colony” (LC) types, which are recovered from and cause disease in caprines and into “small colony” (SC) types, which cause disease in bovines. On three occasions, SC types, designated strains O, P, and Vom, have been recovered from goats in New Guinea,\textsuperscript{20,21} Sudan,\textsuperscript{20,21} and Nigeria,\textsuperscript{20,21} respectively. On two occasions, LC types have been recovered from cattle in Australia\textsuperscript{21} and France.\textsuperscript{45} Irrespective of host origin, SC types are considered to be potential bovine pathogens.

Caprine (LC) and bovine (SC) types of \textit{M. mycoides} subsp. \textit{mycoides} cannot be distinguished even by sensitive immunological and serological procedures such as immunofluorescence and growth and metabolism inhibition tests. However, they do appear to be separable by specific biochemical tests such as digestion of inspissated serum and casein,\textsuperscript{21} colony size on
Mycoplasma agar, growth and hemolysis production on ovine blood agar, survival at 45°C, and, at least with some strains, the amount of galactan produced.

Misidentification of Mycoplasma mycoides subsp. mycoides in the United States.

Since 1969 numerous reports have appeared in the scientific literature about the isolation of *M. mycoides* subsp. *capri* from goats in the United States. Those identifications were subsequently shown to be erroneous primarily because of their unfortunate comparison to the Vom mycoplasma strain, then supposed to be of subspecies *capri* but now known to be subspecies *mycoides*. Thus, the reported isolates of subspecies *capri* were, rather, *M. mycoides* subsp. *mycoides*, including also isolates reported as subsp. *capri* from Mexico. Insofar as we know, *M. mycoides* subsp. *capri* does not occur in the United States.

Causes of caprine mycoplasmal pneumonias

Caprine mycoplasmal pneumonias usually have been described under the broad, but unfortunate term “contagious caprine pleuropneumonia.” Several mycoplasmas can cause some form of caprine pneumonia. These include *M. capricolum*, *M. mycoides* subsp. *capri*, *M. mycoides* subsp. *mycoides*, and an unnamed mycoplasma designated *Mycoplasma* sp. F38. Of these mycoplasmas, only strain F38 (and other F38-like mycoplasmas) has been shown to be contagious among mature goats housed in close contact. Thus, the term “contagious” should be restricted to strain F38 unless further studies indicate otherwise. For *M. capricolum*, *M. mycoides* subsp. *capri*, and *M. mycoides* subsp. *mycoides*, reports attesting to a highly infectious nature among mature goats are lacking, although one report showed *M. capricolum* strain GM13 to be highly infectious among young kids housed in close contact.

Published Scientific Data Relating to the Occurrence of *M. mycoides* subsp. *mycoides* in Goats in the United States.

The first reported instance of *M. mycoides* subsp. *mycoides* in goats in the United States occurred in 1969 in Connecticut. Since then, its widespread distribution has been confirmed by other reports of *M. mycoides* subsp. *mycoides* in goats from Arizona, California, Connecticut, Florida, Idaho, Maryland, New York, North Carolina, Pennsylvania, and Texas. The organism has been recovered from a variety of clinical manifestations. Isolations have been made from 1) multiple joints (arthritis), 2) milk (mastitis), 3) lungs (fibrinous pleuritis, pneumonia), 4) pericardial fluid (fibrinous pericarditis), 5) brain, 6) conjunctiva, 7) peritoneal fluid (peritonitis), 8) bone marrow (osteomyelitis), 9) subcutaneous edema, 10) cervical abscess, and 11) amniotic fluid. Also, numerous isolations of *M. my-
coides subsp. mycoides have been made from external ear canals of clinically normal goats in northern California.\textsuperscript{22}

Beginning in 1979-1980, large numbers of \textit{M. mycoides} subsp. \textit{mycoides} isolates have been recovered from diseased goats in the Central Valley of California.\textsuperscript{24,28,29} In a 1200-doe commercial goat herd experiencing mastitis, polyarthritis, and pneumonia, 157 goats from a total of 605 goats cultured (26\%) were positive in their colostrum or milk for \textit{M. mycoides} subsp. \textit{mycoides}.\textsuperscript{24} As many as 1 $\times 10^7$ to 1 $\times 10^8$ colony-forming units (CFU) of the organism were shed per milliliter of colostrum or milk. The feeding to the new kid crop of pooled colostrum containing \textit{M. mycoides} subsp. \textit{mycoides} resulted in the death of about 200 kids. At one point, morbidity and mortality in kids fed that \textit{M. mycoides} subsp. \textit{mycoides} positive colostrum was about 90\%. The elimination of the organism from the colostrum, achieved by culturing and discarding the positive colostrum, reduced morbidity/mortality to less than 5\% and, of those, most were kids that had nursed their dams prior to being removed from them. The introduction of 27 lactating does from this herd into another herd (300 lactating does) without prior history of mycoplasma infection resulted in widespread mastitis in does and polyarthritis in kids, leading to death or euthanization of about 170 kids and 70 does over a 12-month period.\textsuperscript{29} In this latter outbreak, the infection of young kids was curtailed by removing the newborn from their dams before nursing, then feeding cow colostrum, pasteurized goat milk, or calf replacer.

In addition to isolations from the infected herds just mentioned, a significant number of other isolations of \textit{M. mycoides} subsp. \textit{mycoides} have been made in our laboratory either from diseased goats or from caprine tissues and/or fluids taken at necropsy. We have isolated \textit{M. mycoides} subsp. \textit{mycoides} from an estimated 700 or more goats since the winter of 1979-1980. Most either died or were euthanized while the remainder (about 200), which were at the beginning of the mycoplasmal disease process, were sold for slaughter.

\textbf{Review of Selected Studies}

\textbf{Review of the Literature on the Experimental Infectivity of Caprine Isolates of \textit{M. mycoides} subsp. \textit{mycoides} for Bovines}

\textbf{1967 Study:}\textsuperscript{31} The Y-goat caprine strain of \textit{M. mycoides} subsp. \textit{mycoides}, a well characterized LC isolate which originated in Queensland,\textsuperscript{35} Australia, was used in this study. Calves inoculated with an active culture, either behind the shoulder or by the intravenous route, showed no detectable clinical effect. No lesions were observed at necropsy 38 and 42 days postinoculation (PI). Older cattle inoculated behind the shoulder subcutaneously did not display a local reaction. Two of the steers were necropsied 1 month PI, and from one of them, Y-goat mycoplasma was recovered from a lymph node on the inoculated side.

\textbf{1969 Study:}\textsuperscript{33} In the United States two calves were inoculated intramuscularly with a caprine mycoplasma strain designated "Connecticut."
Positive blood cultures developed in one calf. Additional data were not disclosed. It is not certain whether this strain was an LC or SC type of *M. mycoides* subsp. *mycoides*, but it is strongly suspected that it was an LC type.

**1970 Study:** In the United States two calves were inoculated intramuscularly with several different mycoplasmas of caprine origin. These mycoplasmas included the Connecticut strain described previously, the Vom SC strain from Nigeria, a Mexican strain, and strain 2912 from Turkey. Also included was *M. agalactiae* (PG2 strain from Sudan), a "Texas" goat mycoplasma, and mycoplasma strains from California designated 1958 and 1967. Of the latter two, strain 1958 was probably *M. capricolum*, and strain 1967 is now known to have been *M. putrefaciens*. Thus, for purposes of this report, only the Connecticut and Mexican strains are of particular consequence because they probably were LC types of subsp. *mycoides*. Strain Vom is an SC type known to be pathogenic for cattle. The Connecticut, Mexican, and Vom strains each killed one of the two calves between 13 and 17 days PI. Calves that survived the Mexican and Vom strains developed a mycoplasmaemia while the one that survived the Connecticut strain did not. The organisms were recovered at necropsy from a variety of tissues and/or fluids.

**1976 Study:** In Kenya, the intratracheal inoculation of calves with the F30 strain of *M. mycoides* subsp. *mycoides*, a known LC type, resulted in no adverse clinical signs and no observable lesions at postmortem examination. Mycoplasmas were not isolated. When inoculated subcutaneously, four calves were also unaffected, but two animals sacrificed 28 days PI showed mild areas of hemorrhage at the inoculation site. In three animals the axillary and prescapular lymph nodes yielded Mycoplasma F30. Complement fixing antibodies were not detected in any of the calves.

**1980 Study:** In Nigeria, cattle were inoculated endobronchially with a caprine strain (Ib9) of "*M. mycoides," presumably *M. mycoides* subsp. *mycoides*, concurrently with *Trypanosoma vivax*. *Trypanosoma vivax* was used to induce a state of immunosuppression. Typical lesions of CBPP were reproduced in the imported cattle breeds studied when the dual infection was established. Note, however, that this study does not specifically state 1) whether the organism was subsp. *mycoides*, and 2) if it was subsp. *mycoides*, whether an LC organism was involved.

**1981 Study:** An LC strain (Y3343) of *M. mycoides* subsp. *mycoides* isolated in the United States (Connecticut) was used in this study. When this strain was given intravenously, one of two calves developed a slight fever and a sore and swollen left hock joint 9 to 15 days after inoculation. The second inoculated calf and a contact calf remained clinically normal. Mycoplasmaemia did not develop and mycoplasmas were not recovered at necropsy. Histologically one calf had scattered cellular necrosis with increased numbers of neutrophils in the adrenal cortex. One hock joint had
excess fibrin and hemorrhagic fluid with a synovial necrosis and was lined with a zone of granulation tissue.

1983 Study: This study used an LC strain (D44) of M. mycoides subsp. mycoides isolated from a case of caprine polyarthritis in Ontario, Canada. Six calves were placed in close contact with six goats inoculated endobronchially with $10^8$, $10^9$, or $10^{10}$ CFU of the organism. An additional two goats served as contact controls. The animals were housed in a single 18 x 18 m room with a concrete floor. Inoculated goats died within 6 days, but neither the contact calves nor the contact goats displayed adverse clinical signs. Twenty-six days after the initial goat inoculation, two calves were given $10^9$ CFU of the organism endobronchially. No errant clinical signs were noted, and no significant lesions were present at necropsy. Mycoplasmas were not isolated.

DISCUSSION

The foregoing account gives abundant and incontrovertible evidence indicating that M. mycoides subsp. mycoides is a highly prevalent mycoplasma in the goat population of the United States. Governmental regulations and policies clearly reflect concern for this agent as a cause of CBPP, but do not distinguish between caprine (LC) and bovine (SC) types of this mycoplasma. It is reassuring, however, that while caprine strains of M. mycoides subsp. mycoides are prevalent in the goat population of the United States, cattle have remained free of this important disease since its eradication in 1892. Analogous situations exist also in Australia, Canada, and West Germany. Note also that the two large goat dairies reported previously as experiencing devastating losses due to M. mycoides subsp. mycoides, are both next door neighbors to large, 500-1000-cow dairies. Additionally, we know of instances in which M. mycoides subsp. mycoides positive, caprine milk, rendered mastitic and therefore unsalable, was used to feed calves as well as (and primarily) pigs. No harmful effect was seen as a consequence.

With the exception of a single study whose data are clearly in conflict with the reports of other studies, scientific data strongly indicate that caprine LC strains of M. mycoides subsp. mycoides most probably have little effect on cattle under natural conditions. This view is also shared by at least one United States Department of Agriculture document, which states that "... the goat (strain of) M. mycoides subsp. mycoides (LC) does not appear to produce contagious pleuropneumonia in goats nor is it naturally pathogenic for cattle ..." (Foreign Animal Disease Report, March, 1984, p. 6). Published studies thus far have indicated that when given singly, caprine strains of M. mycoides subsp. mycoides can only be recovered from experimental bovines at necropsy when inoculated in large numbers by the subcutaneous, intramuscular, or intravenous routes. No isolations have been made from experimental bovines in studies in which LC organ-
isms were administered singly by the respiratory route, that is, endobronchially or intratracheally.

In one study, *M. mycoides* subsp. *mycoides* was recovered from a bovine given the organism endobronchially, but in that case the infection was purposefully compromised by a second exposure with *Trypanosoma vivax* given as a stress factor. Similarly, in France a "large colony" strain of *M. mycoides* subsp. *mycoides* was recovered from the pneumonic lesions of a calf. However, the lungs also yielded *Pasteurella multocida* and *P. hemolytica*, agents known to produce pneumonia in cattle. Such pneumonic lesions in calves yielding *P. multocida*, *P. hemolytica*, and a caprine strain of *M. mycoides* subsp. *mycoides* in one study and, *M. mycoides* subsp. *mycoides* and *T. vivax* in the other should not be construed to indicate that the caprine mycoplasma was the cause of the pneumonia.

In view of the body of scientific reports establishing the presence of *M. agalactiae* and *M. mycoides* subsp. *mycoides* in the United States, the USPHS/CDC/NIH regulations defining them as "nonindigenous pathogens..." whose "importation, possession, or use... is prohibited or restricted by law or by USDA regulations or administrative policies" is not in accordance with the facts and undermines the credibility of these agencies. The fears of the reappearance of CBPP that seem to underlie this denial of the presence of these pathogens would best be allayed by further study of caprine mycoplasmosis. The welfare of goats and cattle and the economic interests they represent would likewise best be served by such study with eventual control or eradication as the goal.

**CONCLUSIONS**

1. Caprine strains of *M. mycoides* subsp. *mycoides* are highly prevalent in the goat population of the United States.
2. The continuing designation of these caprine strains of *M. mycoides* subsp. *mycoides* and *M. agalactiae* as "nonindigenous" agents by U.S. regulatory agencies is inconsistent with the reality of the presence of these mycoplasmas in the United States.
3. The welfare of goats and cattle would best be served through orderly scientific study with *M. agalactiae* and with caprine strains of *M. mycoides* subsp. *mycoides*. Studies with subsp. *mycoides* should be allowed in a) caprines without restriction and in b) bovines with a requirement that such studies be conducted under appropriate veterinary security.
4. The majority of currently available scientific information indicates that caprine strains of *M. mycoides* subsp. *mycoides* have little effect on bovines even when introduced parenterally in large numbers. Only one study reported death of bovines when a caprine strain of this organism was introduced intramuscularly in large numbers and later, and more meaningful studies did not duplicate those results. In some studies the organism was recoverable at necropsy but only when the organism was introduced in large numbers by the intramuscular,
intravenous, or subcutaneous route. Neither adverse clinical signs developed nor were the organisms recovered at necropsy when the agent was introduced intratracheally or endobronchially in calves, or when calves were housed in close contact with diseased caprines. Thus, fears of natural infection of the bovine population by caprine (LC) strains of \( M. \) mycoides subsp. \( mycoides \) appear to be highly questionable.

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INTERCEPTION OF A VECTOR OF HEARTWATER,
AMBLYOMMA HEbraEum KOCH (ACARI:IXODIDAE) ON
BLACK RHINOCEROSSES IMPORTED INTO THE UNITED
STATES

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INTRODUCTION

Heartwater, an acute noncontagious disease of ruminants caused by the rickettsia Cowdria ruminantium, is only transmitted by ticks of the genus Amblyomma. Thus far, nine African species of Amblyomma, A. astrion Donitz, A. cohaerens Donitz, A. gemma Donitz, A. hebraeum Koch, A. lepidum Donitz, A. pomposum Donitz, A. sparsum Neumann, A. tholloni Neumann, and A. variegatum (Fabricius), have been shown to be vectors of heartwater (Uilenberg 1983). In addition, two American species, A. cajennense (Fabricius) and A. maculatum Koch, have been shown to be experimental vectors of C. ruminantium (Uilenberg 1983).

Since 1962, six of the African species (A. gemma, A. hebraeum, A. lepidum, A. sparsum, A. tholloni, and A. variegatum) have been intercepted on zoological animals or animal products imported into the United States (Table 1). Of the 30 interceptions listed, 12 have been from hides and trophies, while 18 have been from zoological animals. Rhinoceroses have accounted for 50% of the zoological animals from which vectors of heartwater have been recovered (9 of 18). Of the nine interceptions, the bont tick, A. hebraeum, has been collected from seven (78%).

Only three of the A. hebraeum interceptions have been documented to any extent — those of 1962, 1963, and 1966 (Diamant 1965; USDA 1976). Reported here are results of investigations into the most recent interception of A. hebraeum in the United States.

BACKGROUND

On March 23, 1984, two male and three female black rhinoceroses, Diceros bicorns, arrived at J. F. Kennedy Airport, New York, from South
The rhinoceroses were offloaded and housed overnight at the airport. The next day, the rhinoceroses were flown to Houston, Texas. From there, three of the rhinoceroses, two females and one male, were transported by truck to the La Coma Ranch, located near Linn, Texas, while the other two, a male and a female, were taken to the Fossil Rim Wildlife Ranch, located near Glen Rose, Texas (Figure 1). The black rhinoceroses, an endangered species (Nilsson 1983), were brought to the United States under the auspices of the African Fund for Endangered Wildlife and Game Conservation International as part of an experimental breeding program.

On May 23, 1984, one of the female rhinoceroses at the La Coma Ranch died. Postmortem conclusions indicated the rhinoceros had died from complications arising from severe biliary retention and toxic hepatosis (Farst 1984). During the course of the postmortem examination, 20 live, "very unusual" ticks were collected from the mammary folds (Huntress 1984). Four of the ticks were submitted for identification to the State-Federal Cooperative Laboratory (SFCL), Austin, Texas. The SFCL classified them as male *Amblyomma* sp., then forwarded them for specific identification to the National Veterinary Services Laboratories (NVSL), Ames, Iowa. On June 5, 1984, NVSL identified the ticks as male *A. hebraeum* and notified Emergency Programs, VS, APHIS.

Due to possible exposure of cattle on the La Coma Ranch to the tick-infested rhinoceros, a State quarantine was placed on the ranch on June 7, 1984 (Cox 1984). In addition, entomologists from Veterinary Services were sent to Texas to: 1) examine the four remaining rhinoceroses for additional ticks; 2) conduct a qualitative tick survey on both ranches; 3) examine the head of the dead rhinoceros, which had been frozen after the postmortem examination; and 4) identify the remaining 16 ticks collected during the postmortem. Two investigations were conducted: the first from June 7–20, 1984; the second from July 16–20, 1984.

**METHODS AND MATERIALS**

**Rhinoceros examination** — The two rhinoceroses at the La Coma Ranch were sedated with Etorphine (M99) before examination; those at the Fossil Rim Wildlife Ranch were examined while they were standing in a feeding chute. In addition to a careful visual examination, each rhino was "scratch" inspected (passing of hands over body areas and feeling for ticks). Particular attention was given to the axillary, inguinal, and perineal areas, the interdigital clefts, and the inside of the ears. Immediately after the inspection, each rhinoceros was sprayed with .125% coumaphos. The frozen head of the dead female rhinoceros was visually examined, and the inside of the ears were examined with the aid of an otoscope.

**Pasture survey** — Rhinoceros' pastures were surveyed by visually examining low lying vegetation in each pasture and by using a tick drag (USDA 1978). In addition to using a tick drag, 20 CO₂ traps (Ahrens 1984; USDA 1978) were placed in the 1-acre pasture that had contained the
tick-infested female rhinoceros. After the traps were removed, two sentinel animals (Jersey cow and calf) were placed in the pasture.

**Examination of cattle and wildlife** — Two hundred sixty-one (261) cattle on the La Coma Ranch were “scratch” inspected for ticks by personnel with the Fever Tick Eradication Program and then spray-dipped with .25% coumaphos. Twenty-eight (28) cattle on the premises adjacent to the La Coma Ranch were inspected and spray-dipped with .25% coumaphos after inspection. Also, three wild rabbits and two feral pigs found near the rhinoceros’ pasture were collected and examined for ticks.

There were no cattle present on the Fossil Rim Wildlife Ranch, only exotic wildlife. Rhinoceroses at this ranch were kept in a compound about a mile away from other animals; therefore, other animals on the ranch were not inspected.

**RESULTS**

Three live male *A. hebraeum* were collected from the male rhinoceros at the La Coma Ranch, while none were found on either rhinoceros at the Fossil Rim Wildlife Ranch (Table 2). No female *A. hebraeum* were found on any of the rhinoceroses. In addition to the 3 male *A. hebraeum*, 1 female and 17 male Cayenne ticks, *A. cajennense*, were collected from the rhinoceroses at the La Coma Ranch. One male and five female Lone Star ticks, *A. americanum* (Linnaeus), were found on the rhinoceroses at the Fossil Rim Wildlife Ranch. No ticks were found on the frozen head of the female rhinoceros. The 16 ticks that were collected during the postmortem examination were identified as male *A. hebraeum*.

With the exception of one *A. cajennense* nymph found immediately outside one of the pastures at the La Coma Ranch, no ticks were found in the rhinoceros’ pastures of pen areas at either ranch. No ticks were found on the sentinel animals placed in the pasture that had contained the tick-infested female rhinoceros.

One female *A. cajennense* was found on the cattle inspected at the La Coma Ranch, while one female American dog tick, *Dermacentor variabilis* (Say), one male *A. cajennense*, and one male and one female Gulf Coast tick, *A. maculatum*, were collected from cattle on the adjacent premises. Four female and two male rabbit ticks, *Haemaphysalis leporispalustris* (Packard), were found on the wild rabbits collected at the La Coma Ranch. Two *A. cajennense*, one male and one female, were found on the two feral pigs examined.

**DISCUSSION**

The impact of the most recent interception of an African tick that transmits heartwater was minimized by three factors: 1) the results of the investigation indicated that only male *A. hebraeum* were transported from Africa, thereby precluding the tick from becoming established; 2) rhinoceroses are nonruminants and are therefore not susceptible to heart-
water; and 3) the rhinoceroses came from an area in South Africa free of heartwater (Mickeljohn 1984).

That female *A. hebraeum* were not found was undoubtedly due to the fact that, compared to males, they remain on hosts for a relatively short period of time—6 to 10 days (Lounsbury 1899, 1904; Norval 1974); thus, it is assumed that any females attached to the rhinoceroses dropped off before the rhinoceroses entered the United States. On the other hand, male *A. hebraeum* may remain attached to hosts for up to 9 months (Jordaan and Baker 1981). Therefore, it was not remarkable that males were found on the rhinoceroses several months after the importation.

This is the first known record of *A. cajennense* and *A. americanum* from black rhinoceroses. Both species are endemic to the United States; *A. cajennense* is limited to four counties in southern Texas, while *A. americanum* is found in central and eastern Texas, north into Missouri, and east to the Atlantic coast (USDA 1976). The three parasitic stages of both species are known to attack a wide variety of mammals and birds (Doss et al. 1974). Thus, it was not particularly surprising that they were collected from the rhinoceroses, especially since the rhinoceroses were placed in an ecological setting favorable to attack by endemic ticks. Further, over 40 species of ticks, including 15 species of *Amblyomma*, have been recorded from black rhinoceroses (Doss et al. 1974).

There are two principal avenues by which a foreign animal disease could enter the United States: 1) importation of diseased animals or 2) importation of foreign animal disease vectors on nonregulated animals. The first avenue is unlikely because of quarantine restrictions applied to domestic livestock and wild ruminants upon entry into the United States. However, the second avenue appears more likely, especially with zoological animals not subject to quarantine restrictions. The increasing volume and rapidity of commerce via air transport have intensified the danger of introduction and establishment of an exotic vector, particularly with the recent trend towards placing zoological animals in situations which directly expose them to susceptible domestic livestock and native wildlife. The most recent interception of an efficient African vector of heartwater, *A. hebraeum*, emphasizes this danger.

**SUMMARY**

A total of 23 male *A. hebraeum*, a vector of heartwater, was collected from black rhinoceroses recently imported into the United States. No *A. hebraeum* were detected when the rhinoceros' pastures and pen areas were surveyed. Native ticks, *A. cajennense* and *A. americanum*, were also found on the rhinoceroses.

**ACKNOWLEDGEMENTS**

The authors give special thanks to Dr. Victor H. Driscoll, Mssrs. Reynaldo Hernandez, Billy Moses, Mario Alaniz, Horatio Ozuna, Raymundo Morales, and Mario Morales, Fever Tick Eradication Program, VS, APHIS, USDA, Pharr, Texas, for their valuable assistance throughout
both investigations. We also wish to thank Messrs. Calvin Bentsen and Andy McLellan, owner and foreman respectively of the La Coma Ranch and Messrs. Tom Mantzel and Casey Clark, owner and foreman respectively of the Fossil Rim Wildlife Ranch, for their cooperation and hospitality.

Thanks also are extended to Drs. Don Farst and Sherri Huntress, Gladys Porter Zoo, Brownsville, Texas, for their expert advice and assistance, and to Dr. Bob Strickland and Mr. Bob Gerrish, National Veterinary Services Laboratories, VS, APHIS, USDA, for their prompt confirmation of the identity of tick specimens collected during the investigations.

Lastly, we wish to thank Dr. Ron Davey and Mr. Elmer Ahrens, Cattle Fever Tick Research Laboratory, ARS, USDA, Mission, Texas, for the technical advice and assistance they provided during the investigation at the La Coma Ranch.

REFERENCES


Table 1. Vectors of heartwater found on animals or animal products imported into the United States, 1962-1984<sup>a</sup>

<table>
<thead>
<tr>
<th>Year Collected</th>
<th>Species</th>
<th>Animal or Product</th>
<th>Locality Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>A. hebraeum</td>
<td>Rhinoceros</td>
<td>New York</td>
</tr>
<tr>
<td>1963</td>
<td>A. hebraeum</td>
<td>Rhinoceros</td>
<td>Oklahoma</td>
</tr>
<tr>
<td>1964</td>
<td>A. gemma</td>
<td>Trophy hides</td>
<td>Maryland</td>
</tr>
<tr>
<td>1965</td>
<td>A. tholloni</td>
<td>Elephant</td>
<td>Florida</td>
</tr>
<tr>
<td></td>
<td>A. lepidum</td>
<td>Zebra hides</td>
<td>Texas, Maryland</td>
</tr>
<tr>
<td>1966</td>
<td>A. hebraeum</td>
<td>Rhinoceros</td>
<td>California, Texas</td>
</tr>
<tr>
<td>1967</td>
<td>A. gemma</td>
<td>Zebra hide</td>
<td>Florida</td>
</tr>
<tr>
<td>1968</td>
<td>A. hebraeum</td>
<td>Antelope hides</td>
<td>Texas</td>
</tr>
<tr>
<td>1969</td>
<td>A. sparsum</td>
<td>Boa constrictor</td>
<td>Washington</td>
</tr>
<tr>
<td></td>
<td>A. gemma</td>
<td>Zebra</td>
<td>New York</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Giraffe hide</td>
<td>Texas</td>
</tr>
<tr>
<td>1970</td>
<td>A. gemma</td>
<td>Zebra</td>
<td>New York</td>
</tr>
<tr>
<td></td>
<td>A. hebraeum</td>
<td>Rhinoceros</td>
<td>Texas</td>
</tr>
<tr>
<td>1971</td>
<td>A. sparsum</td>
<td>Tortoise</td>
<td>Oregon</td>
</tr>
<tr>
<td></td>
<td>A. variegatum</td>
<td>Cape buffalo hide</td>
<td>California</td>
</tr>
<tr>
<td>1973</td>
<td>A. hebraeum</td>
<td>Rhinoceros</td>
<td>Virginia</td>
</tr>
<tr>
<td></td>
<td>A. variegatum</td>
<td>Trophy hides</td>
<td>Colorado</td>
</tr>
<tr>
<td></td>
<td>A. tholloni</td>
<td>Trophy hides</td>
<td>Colorado</td>
</tr>
<tr>
<td>1974</td>
<td>A. gemma</td>
<td>Rhinoceros</td>
<td>North Carolina</td>
</tr>
<tr>
<td></td>
<td>A. tholloni</td>
<td>Animal trophies</td>
<td>Louisiana, Tennessee, California, North Carolina</td>
</tr>
<tr>
<td></td>
<td>A. variegatum</td>
<td>Rhinoceros</td>
<td>North Carolina</td>
</tr>
<tr>
<td>1977</td>
<td>A. hebraeum</td>
<td>Animal hide</td>
<td>California</td>
</tr>
<tr>
<td>1979</td>
<td>A. hebraeum</td>
<td>Cape buffalo</td>
<td>Colorado</td>
</tr>
<tr>
<td></td>
<td>A. variegatum</td>
<td>Kudu</td>
<td>Colorado</td>
</tr>
<tr>
<td>1981</td>
<td>A. hebraeum</td>
<td>Zebra hide</td>
<td>North Carolina</td>
</tr>
<tr>
<td>1984</td>
<td>A. hebraeum</td>
<td>Rhinoceroses</td>
<td>Texas</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data compiled from Becklund (1968) and USDA (1963-1983)
Table 2. Species of ticks collected from black rhinoceroses in Texas, June 9 - July 19, 1984

<table>
<thead>
<tr>
<th>Location of Rhinoceroses</th>
<th>Ticks Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>La Coma Redgate Ranch</td>
<td>A. hebraeum Male 23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>A. cajennense Male 17</td>
</tr>
<tr>
<td></td>
<td>A. cajennense Female 2</td>
</tr>
<tr>
<td>Fossil Rim Wildlife Ranch</td>
<td>A. americanum Male 6</td>
</tr>
<tr>
<td></td>
<td>A. americanum Female 5</td>
</tr>
</tbody>
</table>

<sup>a</sup> A. cajennense and A. americanum are native to the United States.

<sup>b</sup> Includes the 20 specimens collected on May 23, 1984.
Figure 1. Locations of black rhinos imported into the U.S. on March 23, 1984.
Chairman: Joe Finley, Jr., Encinal, TX
Vice Chairman: H. A. McDaniel, Silver Spring, MD

John Adams, VA; J. B. Anderson, TN; R. A. Bankowski, CA; Neal Black, MN; Joe L. Blair, VA; W. W. Buisch, MD; Ramsay Burdett, OR; M. J. Burridge, FL; S. J. Cougar, TX; R. O. Drummond, TX; W. C. H. Glaze, TX; Frank A. Hayes, GA; P. R. Henry, CO; J. L. Hyde, MD; E. T. Mallinson, MD; N. L. Meyer, VA; M. A. Mixson, AL; T. G. Murnane, TX; J. E. Novy, TX; J. S. Orsborn, CA; B. I. Osburn, CA; H. G. Purchase, MD; T. B. Ryan, NC; E. C. Sharman, MD; W. L. Sippel, FL; M. J. Tillery, MD; M. A. Van Buskirk, PA.

Chairman Finley opened the meeting at 1:30 p.m. and 48 members and guests were present.

In response to a resolution from the 1983 USAHA meeting Veterinary Services, APHIS USDA appointed two groups to review import problems. One group reviewed problems of livestock and poultry disease importation in any type of animal. The other reviewed the potential ways ectoparasites could enter the U.S. The issues and concepts raised were compiled into an "Interim Report of the Veterinary Services Zoological Animal Committee," September 14, 1984. This report was presented and discussed. Copies were provided to all members and guests. Many points from this report are reflected in subsequent presentations, discussions and resolutions.

Dr. Dan Suter, California Department of Food and Agriculture presented an enlightening paper on Emergency Preparedness in California. This paper stresses the necessity for state animal health organizations to be ready to detect and react to any exotic disease of livestock or poultry. A motion was passed recommending Dr. Suter's paper be published in the proceedings.

Dr. H. A. McDaniel presented an illustrated talk based on a paper, Interception of a vector of heartwater Amblyomma hebraeum kock (acari: ixodidae) on black rhinoceros imported into the United States by D. D. Wilson and R. D. Richards. A motion recommending publication of this manuscript in the proceedings was passed.

Dr. J. K. Atwell described carcass disposal problems in Avian Influenza eradication. The committee expressed concern that if carcass disposal problems were not resolved in advance, the next disease eradication program requiring depopulation might be in jeopardy. A resolution was passed addressing this issue.

Dr. Don Suter, speaking for Dr. Pat Smith, State Veterinarian for California, expressed concern for exotic diseases and pests. Californians are very much aware of the threat of exotic diseases and pests. All state animal health officials regard exotic diseases as part of their daily work.
They disseminate information, conduct investigations or conduct inspections on incoming animals or products almost every day.

Dr. J. K. Atwell presented some concepts on Biological Risk Management in APHIS for the Administrator, Bert Hawkins and conveyed Mr. Hawkins' request that consideration be given to problems associated with importation of zoological animals, semen, embryos, and laboratory related issues. These were addressed during subsequent discussions.

Dr. Robert Reichart briefly summarized progress toward eradication of screwworm in Mexico. Program success is truly spectacular. The attached chart depicts annual progress in eradication of screwworms from US and Mexico.

A subcommittee was appointed and instructed to develop separate resolutions asking USDA for action on unresolved problems associated with indemnity or relocation cost, Importation of Embryos and semen, and importation of domestic and zoological animals. Six resolutions on these and other issues were passed and forwarded to the committee on resolutions.
REPORT OF THE COMMITTEE
AVANCES EN LA ERRADICACION DEL GUSANO BARRENADOR DEL GANADO
PROGRESS IN SCREWWMERADICATION
1957 - 84
INTRODUCTION

In the last ten years, great strides have been made nationally in formulating plans to respond to outbreaks of exotic diseases in the United States. Regional Emergency Animal Disease Eradication Organizations (READEOs) have been set-up for five regions of the United States.

A state emergency response program may seem redundant when such an active federal program is in place. There are, however, emergency disease situations not covered by the current United States Department of Agriculture (USDA) program. Certain phases of an effective program, mainly early detection, initial response, and disease follow-up can not be adequately addressed by a federal program and are necessarily responsibilities of the states. To meet these responsibilities California has initiated an Animal Disease Emergency Preparedness and Training (AD-EPT) program.

The purpose of this paper is to demonstrate how state preparedness augments and enhances the effectiveness of a national emergency program.

EMERGENCY DISEASE DEFINITION AND CLASSIFICATION

Definition:

“Emergency diseases” are ones that have the potential for causing catastrophic economic losses (for example, foot-and-mouth disease — FMD) or a severe threat to the public health or both (e.g., Rift Valley Fever — RVF).

Classification:

In the following discussion of the classes of emergency diseases, mention of federal or state responsibility for responding to emergency disease occurrences refer to the eradication or sustained response phase. For comparisons of federal and state involvement in all aspects of emergency preparedness see later discussion of emergency disease response phases.

1. Foreign animal diseases (FADs).
   a. Those for which there are response plans or experience.

   These are diseases with which we are familiar as a threat. Examples are FMD and African swine fever (ASF). Some are diseases that are periodically introduced, e.g., exotic Newcastle disease (VVND). These diseases could enter insidiously and then explode into pandemic proportions. These are diseases for which Emergency Programs, Animal and Plant Health Inspection Services (APHIS), USDA, has developed national eradication responses or with which
there has been considerable past eradication experience (e.g., VVND, hog cholera). To date, detailed plans have been developed for six diseases. Once one of these diseases has been detected, Emergency Programs, through its Regional Emergency Animal Disease Eradication Organization (READEO), would respond with an eradication effort.

b. Those for which there are no response plans or experience.

These are diseases on the USDA, APHIS Emergency Programs list, but for which the federal response likely will be delayed due to the lack of specific response plans or experience. Containment and substantial eradication response would be left to the state. Two examples are malignant catarrhal fever and contagious bovine pleuropneumonia.

c. A changed foreign animal disease.

This would be a disease that, in the area where it is endemic, has altered its form enough to go unrecognized in California or other sections of the United States until it develops into epidemic proportions.

2. A new disease suddenly appearing in animal populations.

These diseases may be ones accidentally introduced from other life forms. In the past such examples have been vesicular exanthema (VE) from fish and ASF, presumably, from insects. It could be an emerging disease, i.e., one that starts out with little economic impact, but becomes very costly as it spreads to new areas and/or hosts. Some possibilities are Mycoplasmas or Caliciviruses from unexpected sources. VE was solely a California problem for two decades. It did not become an emergency disease, by national definition, until it spread rapidly to other states. Such an experience could be easily repeated.

3. Endemic disease mutations or altered manifestation.

These are endemic diseases that change character and therefore have an increased and sometimes disastrous economic impact. Vesicular stomatitis during 1982 occurred in epidemic proportions and appeared to have lengthened its incubation period, thereby allowing spread over long distances through shipped cattle. Avian Influenza H5N2 is also an example.

4. A point epidemic.

Point epidemics may result from toxicological disasters or from intentional introductions of traditional foreign contagious diseases for reasons of sabotage, biological warfare or terrorism. It is important to remember that such introductory routes of an otherwise traditional foreign animal disease would result in altered disease patterns. Point epidemics of foreign diseases can resemble toxicological incidents. The lack of plans for an immediate response to apparent toxicological incidents could mean a loss of critical response time in the event of misdiagnosis.
Incidents such as the Michigan PCB accident can be economically disastrous and pose a threat to human health. No federal response to this category of emergency diseases would be expected unless a national threat was demonstrated. The state has a responsibility to minimize the economic loss and public health threat in any case.

5. Other emergency disease forms as yet undiscovered.

RESPONSE PLANS FOR EMERGENCY DISEASE OCCURRENCES

The “response” to an emergency disease is the set of procedures or actions taken to prevent entry or development of an emergency disease or to limit the damage done and then eradicate any emergency disease that occurs.

Response to emergency diseases involve three phases: prior to introduction or development, upon introduction or development, and post-detection.

I. Prior to introduction/development.
   A. Exclusion and prevention phase.

   This response seeks to reduce the probability of introduction of foreign animal disease (FAD) by a variety of methods. This phase is a cooperative effort between USDA, Veterinary Services (VS) and Plant Protection and Quarantine (PPQ), and the California Department of Food and Agriculture (CDFA), Bureau of Animal Health and Division of Plant Industry. Whenever USDA diminishes its exclusionary efforts, the state must increase its activity in order to maintain the same level of protection. An example is the recent change in regulations which no longer allows the placement of seals on vessel stores which contain meat from FMD or rinderpest countries. This has caused CDFA to increase its inspection of garbage handling on ships in its ports. We also require a two hour sterilization time of aircraft and vessel garbage rather than the thirty minutes required by federal regulations.

   Some other states activities involve: a cooperative program with Mexican officials related to disease problems near the border (current emphasis — hog cholera); inspection of garbage fed swine operations; and educational activities of agricultural groups going abroad.

   B. Preparation

   This is an effort to put in place an early detection system and prepare for an immediate, effective, and efficient response to an emergency disease occurrence.

   1. To ensure minimal economic or public health loss, an emergency disease must be detected early. To increase the probability of early detection we must have: personnel trained and
REPORT OF THE COMMITTEE

experienced in the diagnosis of emergency diseases in the field and the laboratory; surveillance systems designed to detect insidious introductions of disease; surveillance of endemic diseases which mimic foreign animal diseases; a system which provides realistic incidence data; and an alert field veterinary force.

These goals are accomplished through the training of target groups and sensitizing them to the importance of animal agriculture. We have provided this training to CDFA plant quarantine officers, and USDA, PPQ inspectors. Seminars in FAD's have been presented to veterinarians in private practice. Articles regarding FAD threat have been published in both veterinary journals and livestock industry publications.

Our most important resource our field personnel, require training both by CDFA-ADEPT and USDA, Emergency Programs. California can provide certain emergency training to state employees and plans to extend it to California based VS employees if USDA approves. Foreign animal disease diagnostician training, which the state can not provide, is badly needed, particularly by state employed veterinarians.

Since FADs can mimic endemic diseases, we must develop field and laboratory surveillance systems to assist in differentiating these diseases. A functional state diagnostic laboratory system must be able to recognize etiologic agents which could be FAD and take the appropriate steps for definitive identification. In the field we urge the development of solid mutual relationships between our field personnel and private veterinary practitioners. We encourage our veterinarians to assist their colleagues in practice with difficult or unusual disease investigations. A state form with a format similar to the federal FAD investigation form (VS form 12-27) is used and training in FAD investigation procedures is being provided. Training is planned in post-mortem techniques consistent with FAD investigations, e.g., aseptic collection of tissues for etiologic studies prior to examining all organs.

A sense of immediacy and alertness on the part of the field personnel must be maintained.

This readiness is usually related to a direct involvement with the program. We hope to maintain direct involvement by: (1) using the above mentioned investigative and post-mortem procedures; (2) using all disease investigations as emergency disease surveillance; and (3) having field veterinarians become as knowledgeable as possible about one particular emergency disease and be responsible for keeping the rest of the Bureau updated.
2. The cooperation of federal, state, and local agencies and private organizations that would be impacted by such a disease occurrence must be obtained. Progress is being made in California through agreements between agencies and some enabling legislation has been enacted.

II. Upon introduction/development.

- Early detection.

Early detection is our most important job. It is not now done nor can it be done by USDA, EP or READEO, but only by trained, sensitized local field veterinarians, state and federal.

If the systems put in place in the preparation phase are effective we will greatly reduce the time between the introduction and the diagnosis of an emergency disease. It is axiomatic that time is important but let us demonstrate how important it can be in today’s modern livestock marketing system. We are currently involved in the study of marketing to be used as a model for disease transmission and movement. Our study shows one livestock auction yard in northern California that sells on the average of 3,000 cattle per sale (50 sales per year).

These cattle are sold to 120 different owners and shipped to 25 of our highest density livestock counties as well as five other states. The delay of one sale day could prove disastrous.

With the importance of early detection established, let us make a plea for increased foreign animal disease diagnostician (FADD) training for state employees. We believe they would provide: 1) a more stable geographic coverage because of fewer transfers; 2) greater availability because of fewer duties out of their area; 3) knowledge of local animal demographics; and 4) other better rapport with the private practitioners because of their more frequent involvement with local veterinary associations.

III. Post-detection.

This is the control and eradication phase and is divided into three stages: the initial response, the sustained eradication response, and the follow-up activities.

A. The initial response.

The initial response will be exclusively the responsibility of the local (i.e., state or district) emergency response organization.

The initial response is designed to halt the disease where it has been found by imposition of hold orders, quarantines, movement stoppages, etc.; define the extent of the outbreak at the time of detection by discovering the introduction source and predicting the subsequent spread; alert all impacted groups and agencies; and set in motion the initial eradication efforts.
The hold orders and initial quarantines would be effected under state laws. Movement control of people, animals, and satellite businesses will depend upon local law enforcement agencies. Initial epidemiology will necessarily be done by state trained veterinarians as they will be the first on the site following detection. Immediate control and eradication efforts would be implemented at this time.

**B. The sustained eradication response.**

Most disease introductions or developments could be expected to reach this stage. The length of the sustained response would depend upon the effectiveness of the early detection system. This response would involve the possible destruction of many animals, animal products, and any other products or articles capable of carrying the disease agent. The sustained response stage is the one in which READEO would likely be involved. Any preparations made by the state with the myriad of local, state, and federal agencies and the logistical problems that may have been solved will all assist READEO and enhance its effectiveness.

We must also be aware that under some circumstances, federal aid may not be forthcoming or may be long delayed. There are certain classes of emergency diseases, such as toxicological accidents, some endemic diseases, and new diseases to which USDA has no mandate and/or authority to respond. If a foreign emergency disease has entered the United States in other states before arriving in California, USDA assets will necessarily have been directed to the first states involved. California must be prepared to manage the sustained response alone until eradication is achieved or USDA assistance becomes available. A similar but potentially more disastrous scenario could occur in the event of two different FAD's entering separate states. Not only would resources be divided, but also expertise.

**C. Follow-up activities.**

Following an eradication effort against an emergency disease, certain activities are necessary. Surveillance must be initiated to ensure that eradication is complete and that no residual contamination or latent infection exists. The vacuum in the animal population, domestic and wild, caused by the disease and the eradication effort will mean increased importations and movements of animals. This replenishment will bring with it added danger of new introductions of disease. The disruption of normal animal populations by the emergency will also alter movement and population patterns. These will need to be re-defined during the follow-up phase.

The responsibility for ensuring eradication and rebuilding the industry will be left to the state, even if the USDA has helped out in the eradication process.
CONCLUSION

The history of animal disease control in the United States has always been one of cooperation between state and federal governments. Each state and the nation has benefited from concerted disease control efforts that recognize the national diversity. Emergency animal disease preparedness is well suited to this cooperative tradition. In fact, an effective program may well depend upon a mutually supportive approach to preparedness.

For California, and for most states, the probable magnitude of an epizootic in a concentrated livestock area, the size and quantity of logistical obstacles, and the numerous agencies, organizations, and satellite businesses that would become involved present an enormous challenge in arriving at preparedness. The states will often look to the national program for coordination, support, and encouragement. No where is that support more imperative or of farther reaching benefit to the nation than in providing state employed veterinarians with foreign animal disease diagnosticians training and field experience with animals infected with exotic emergency diseases.

The current rate of training of foreign animal disease diagnosticians is totally inadequate for the nation's needs.

We would urge that more courses be offered per year and that they be opened equally to career state and federal animal health veterinarians. This recommendation is made in the interest of increasing the readiness of the United States to deal effectively with an introduction or development of an emergency disease.
A NEW METHOD OF CARCASS DISPOSAL

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Dr. Roth and others have discussed the problem relating to depopulation during the avian influenza outbreak in Virginia. I believe all of us would agree that it would be wonderful if the carcasses that must be destroyed during the outbreak of disease could be safely rendered and utilized rather than burned, buried or otherwise destroyed with no reuse value. In the Pennsylvania and Virginia avian influenza outbreak, the poultry industry would not utilize poultry by-products coming from infected flocks. Therefore, other means of disposal were employed. On the other hand, if they could have been assured that the avian influenza virus could be destroyed prior to rendering then the story might have been different.

I would like to go into a little more detail concerning a prototype of pilot project that was tested in Virginia. While we anticipated most of the problems encountered in the field, we were not able to make all of the adjustments necessary to give the final desired performance.

For example, we knew we would have trouble trying to grind feathers and we did. Any renderer can tell you that feathers, pig skins, cow skins, and the like will cause problems periodically with the grinding equipment.

I would like to carry you through the equipment as a carcass would follow and discuss some of the features.

The unit is mounted on a 45-foot flatbed trailer and is self-contained including a diesel engine and generator as a source of electricity to operate the onboard equipment.

Starting at the rear, the first feature is an enclosed hopper and elevating belt that also can serve as a euthanasia cabinet. When the weather is good and roads are firm, this unit could back up to a chicken house and the live birds placed into the hopper. Carbon dioxide can be introduced from the top of the cabinet. A variable speed pulley allows the speed of the belt to be adjusted so that the birds are euthanized by the time they reach the top of the elevator.

In bad, wet weather, the unit can be placed on a concrete or asphalt pad and serve as a central processing unit. In this case, birds would be euthanized as was done in Pennsylvania and Virginia using a covered dumpster. The euthanized birds would then be brought to the central location, dumped on the concrete pad and placed into the hopper by the use of a front end loader.
A four inch cleanout opening is located at the lowest point of the elevator to make cleaning and disinfecting easy.

The birds come from the elevator and fall into the hopper of the grinding machine. The one utilized in the trial was a 15 inch Anco Grinder. It features teeth or anvils mounted on a central axis with other stationary anvils bolted to the frame of the grinder. This grinder is capable of grinding whole hogs, calves and with some modification, chickens.

We experienced two problems in Virginia. We first found that feathers would not pass through the grinder with any degree of regularity until we made major modifications on the front of the grinder. During the course of making adjustments, over half of the stationary anvils that are mounted onto the frame of the grinder were reversed to the outside. After the modifications necessary to allow feathers to flow through the grinder were made we did not return the stationary anvils to their original position. This allowed parts of the chicken, especially the comb, to pass through the machine without being reduced to the proper size.

The second major problem was with the shaft speed of the grinder. The speed was 50 rpm's when it arrived in Virginia. This shaft speed would not allow poundage to be processed per hour as desired. Although we were able to change some pulleys and adjust the speed somewhat, the Anco people suggested that we increase the speed to 200 rpm's. At that speed we should get adequate production (between 4,000-6,000 pounds per hour). These modifications are now being made.

There was some concern expressed that all belts and pulleys were not enclosed with proper housing. These covers were purposely left off so that field adjustment of pulleys, speeds, etc. could be made. Since the unit return to Georgia, these covers have now been installed and will fully meet EPA requirements.

The ground carcasses then are elevated by the use of a 12-inch auger to a horizontal mixer.

In the mixer, ground corn and a source of Lactobacillus culture are added. A source of water is available on board in case extra moisture is needed. It was determined that the ground corn could be added back at the grinder and assist in the movement of material through the grinder. One of the modifications being made is an extension of the auger back to the grinder to allow ground corn to be added at either location.

While the horizontal mixer was intended to work in a batch operation, it was found that the lower gate could be adjusted to allow a continuous flow. This was especially true when the ground corn is added back at the grinder. In fact, some have suggested eliminating the horizontal mixture as a part of the required equipment. This perhaps could be done under certain circumstances, however, we must be sure that the carcasses are properly blended with a source of carbohydrate and the Lactobacillus culture. In addition, moisten requirements vary, so a positive means of mixing must be available to prevent parts of carcasses from going through
the system without adequate exposure to the Lactobacillus and its growth media.

From this mixer, the material is elevated through a tube auger to a height that will accommodate trucks or any other final vessel in which the material will be left for fermentation.

Due to a problem of the material having difficulty sliding down a chute into the receiving vehicle, the chute has now been replaced with a four foot stainless steel conveyer belt that features a scraper to clean the belt. This will allow positive product removal from the processing equipment and will allow easier access of trucks and other vehicles under the discharge area.

Other features of the unit consist of the water tank to provide water to control moisture levels in the mixture and, with the aid of a high pressure pump, in cleaning.

A five ton storage bin for ground corn is located near the front of the trailer. Also located at the front of the trailer is a diesel engine and a 100 kilowatt generator to supply electrical power for the entire unit.

During the Virginia trials, the mixture of carcasses, ground corn and a source of Lactobacillus produced a product with a pH of less than 4 in 12 hours. Temperature conditions were almost ideal, being between 95° and 100° during the processing.

While we have not actually dealt with the avian influenza virus, we do know that other members of the Myxo Virus group such as Newcastle Disease and Measles Virus can be destroyed within four days.

In fact, we have yet to find a class of viruses or bacteria that cannot be destroyed under proper circumstances through the use of fermentation. Tommorow at 10:10, I will be discussing the fermentation work that we have done at the University of Georgia in exposing various classes of viruses and bacteria to the technique of fermentation.

The relatively small unit that we have been working with will grind a calf within 7-10 seconds. As soon as the final modifications are made, we hope to take the unit to Athens and determine the capability of the grinder through the processing of carcasses from the Athens Diagnostic Laboratory.

To carry this thought further, there is grinding equipment already available commercially that will grind whole cows or whole horses (minus the shoes) and will do this in a matter of less than 1/2 minute. These carcasses then can be mixed with a source of carbohydrate, a source of Lactobacillus and fermented for adequate periods of time to destroy viruses and bacteria.

What we are hoping to provide, is a method of handling carcasses on a small or large scale without the use of burning or burying.

For example, what would happen if Foot and Mouth Disease got into a 1000 cow dairy in south Florida where burying or burning is not possible.
Fermentation would allow these carcasses to be processed in orderly fashion and after the virus is destroyed, be transported to a rendering plant for utilization. The rendering temperatures would be a safety factor and if necessary the resulting material could be used in species other than those affected by the Foot and Mouth Disease Virus.

How about in the cold winter time that was experienced just before Christmas in Penn-Virginia? With relatively minor alterations, the process, working as a central unit, could be adapted to heat the mixture to an adequate level to allow fermentation to proceed. With the erection of temporary shelter over the processing unit and fermenting area, the unit could operate 24 hours per day.

Fermentation is not the final answer in carcass disposal, but it does provide a method of destroying pathogenic viruses and bacteria in preparation for rendering and other methods that would allow safe utilization of the end product.
BUFFALO POPULATION

Until the middle of the last century, the United States population of Buffalo exceeded seventy million. Today, the population is about 75,000. Fifteen thousand are in the Federal and State Parks and the remaining 60,000 exist on ranches, farms and zoos or other domestic environments. The 15,000 buffalo living in the State and Federal Parks are considered free-roaming and wild animals. This population has remained constant for many years. The population of buffalo being kept on the various ranches and farms are considered domestic and their population has quadrupled in the last 25 years.

Wyoming has a domestic buffalo population of about 3,000. Buffalo in Wyoming are amenable to the State's meat inspection laws the same as any other domestic animal. The buffalo roaming free in Yellowstone and Teton National Parks are considered wild animals and not amenable to the State's meat inspection laws.

BUFFALO ORGANIZATIONS

There are three buffalo organizations in North America. They are the National Buffalo Association, the American Buffalo Association and Canadian Buffalo Association. These associations are comprised of ranchers and farmers producing the American buffalo and other persons interested in this historic animal. Their combined memberships total about 1,400 and they represent all fifty States plus Germany, New Zealand, Japan and Norway.

BUFFALO SLAUGHTER

Ten thousand buffalo are slaughtered in the United States annually for human use. About half of these are yearlings finished in commercial feed lots for sixty to ninety days. The remainder are old bulls, non-productive cows and what the industry terms “Trophy Bulls.”

A Trophy Bull is a mature buffalo that has developed to a physique and conformation that qualifies him as an ideal taxidermy specimen. For a fee, the sportsman is delivered safely within archery or rifle range to harvest the animal. The carcass meat is usually part of the deal and is prepared under custom exemption.

The finished yearlings are usually slaughtered and processed under voluntary Federal inspection for movement into commerce. The culled bulls and cows may or may not be slaughtered under inspection.
BUFFALO MARKETING

The National Buffalo Association is actively promoting markets for buffalo products principally on the East and West coasts. They are directing their efforts toward the gourmet or American cuisine tastes. They advertise a meat product that is low in cholesterol and high in nutritive value.

JURISDICTION/AMENABILITY OF BUFFALO

There is no mandatory inspection of buffalo at the Federal level. The Federal Meat Inspection Act addresses only cattle, sheep, swine, goats, horses, mules and other equines. Therefore, buffalo are not amenable to the Food Safety Inspection Service and buffalo products come under the authority of the Food and Drug Administration.

Voluntary inspection of buffalo is provided for under the Agriculture Marketing Act of 1946.

Buffalo carcasses, meat, meat food products and meat by-products may move in interstate commerce without bearing the marks of Federal inspection.

Buffalo is amenable to the Wyoming Wholesome Meat Act and it is not allowed to enter intrastate commerce unless it is slaughtered and processed under State or Federal inspection and bears the marks of that inspection. Wyoming accepts the inspection legends representing other states with certified inspection programs.

NITRATES/NITRITE ISSUE CONCERNING BUFFALO

The Food Additives Amendment to the Food, Drug and Cosmetic Act prohibits the use of nitrates and nitrites in food products. However, meat food products derived from cattle, sheep, swine, goats, horses, mules and other equines are exempted from this amendment because of prior sanctioning of the use of nitrites and nitrates under the authority of the Federal Meat Inspection Act.

Buffalo does not qualify for this exemption because it has always been under the jurisdiction of the Food and Drug Administration and there exists no known prior sanctions for the use of nitrates and nitrites in buffalo products. The FDA will and has in the past embargoed buffalo products in the trade found to contain nitrates and/or nitrites.

There is an alternative approach to this problem. The formulation of buffalo products with the addition of meat or fat derived from domestic animals (cattle, sheep, swine or goat), will bring the finished product under the authority of the USDA. The policy is to formulate the finished weight to contain three percent of one of the meats of the above species or thirty percent of the fat content.

This procedure has been accepted for putting buffalo products within the statutory jurisdiction of FSIS. However, this approval for the use of nitrites and nitrates has come under serious review and could be rescinded.
PECULIARITIES

The buffalo is a wild, unpredictable and dangerous animal. Domestication has created some docile tendencies in the young, but the old cows and bulls remain feral. The fractious nature of this beast can cause insurmountable difficulties in rounding up, transporting, unloading and holding in pens for routine ante-mortem purposes. Few slaughter facilities are equipped properly to handle buffalo and most official slaughter plants refuse to accept them.

At one time, the Wyoming Department of Agriculture's policy allowed field slaughter of buffalo. Very little inspection was involved and often consisted only of a cursory examination of the carcass before the marks of State inspection were applied. In 1981, this policy was dropped in favor of strict adherence to the Laws and Regulations required for slaughtering livestock under State inspection.

An equally flagrant policy of allowing uninspected buffalo products in commerce was also stopped in 1981. Neighboring states that do not require buffalo to be inspected and a few boot-leg operations in Wyoming were the source of these products.

These changes resulted in a great deal of resentment and dissatisfaction among the buffalo producers. Political pressure and an attempt to change the Laws to exempt buffalo from mandatory inspection failed and the Department's position on buffalo inspection remains. The inability of the Wyoming Wholesome Meat Act to allow a variance for this species probably should be considered by the Legislature.

A proposed rule change will soon be published for comment by FSIS addressing voluntary inspection of buffalo and allowing for alternate ante-mortem methods.

VOLUNTARY INSPECTION

The following is the Background from 9 CFR 352 (Docket No. 83-038P), "Voluntary Inspection of Buffalo."

Background

The Agricultural Marketing Act of 1946, as amended provides the Secretary of Agriculture with the authority to carry out the voluntary inspection of buffalo (American bison) as well as other game animals (7 U.S.C. 1622). This authority was implemented for domesticated reindeer in 9 CFR 350.3(d). The Department uses this reindeer inspection provision to provide for inspection and certification of reindeer and other game animals such as buffalo. This inspection service enables persons to bring game animals to an official establishment for ante- and post-mortem inspection. The inspected and passed game meat is branded with the red meat USDA mark of inspection and can be sold interstate or exported (9 CFR 350.3).
The increasing consumer demand for buffalo meat and the increasing number of buffalo being raised for food have prompted buffalo associations to request regulations for buffalo that would address specific problems involved in their slaughtering and marketing. The transportation of live buffalo to an official establishment for inspection and slaughter is one of the biggest problems facing buffalo producers. The inherent unpredictable behavior of buffalo during their loading onto a transport vehicle, transporting and unloading at an official establishment has resulted in damage to transport vehicles, fences, pens and animals. The damage to ante-mortem pens at an official establishment and the increased risk of personal injury have prevented buffalo from being accepted at many slaughter establishments. There are a few establishments which have ante-mortem pens and facilities especially designed and built for buffalo. However, because of the expense, many slaughter establishments are not willing to provide reinforced pens to accommodate unruly buffalo.

Another problem facing the buffalo industry is having a brand applied to the buffalo meat that would be readily accepted by the consumer. Most consumers have become accustomed to the USDA brand on the meat they purchase. The Federal brand may only be applied to meat that has been inspected in a Federal establishment. State inspected buffalo have a State brand applied to the buffalo meat. Even though State inspected buffalo meat may be sold interstate, many consumers, as well as food businesses, will not buy buffalo meat without the Federal mark of inspection.

Recently, FSIS has been petitioned by the National Buffalo Association on behalf of the buffalo (American bison) industry for changes in the ante-mortem inspection and slaughter procedures for buffalo under the voluntary inspection program. The petitioner requested that ante-mortem inspection of buffalo be conducted on the owner's premises or outside a transport vehicle as well as at an official establishment.

Cross utilization of State and Federal veterinarians and the use of contract veterinarians were requested for the ante-mortem inspection of buffalo. The Association also requested a special Federal buffalo brand that could be used in a cooperating State or Federal establishment.

These requests were the result of the previously mentioned problems inherent to the buffalo industry. To address the voluntary inspection of buffalo specifically and remove it from the inappropriate reindeer inspection provision, a new rule is being proposed. The proposed rule would add a new part to the Voluntary Inspection and Certification Service of Subchapter B, Chapter III, Title 9, of the Federal Meat Inspection Regulations.

The proposed rule would allow three alternative locations for ante-mortem inspection. The first alternative would allow ante-mortem inspection to be performed in the field in a designated area of the owner's premises. The field ante-mortem inspection procedure would entail the ante-mortem inspection, stunning and bleeding of the animal. The carcass would then be transported to a State or Federal establishment for post-mortem inspection.
The carcass must be ear-tagged with a “U.S. Suspect” tag to identify it. This will provide the post-mortem veterinarian the means to control the post-mortem inspection and disposition of all field ante-mortem inspected buffalo. Tagging the buffalo carcass in the field with a suspect tag, along with post-mortem inspection and disposition by the veterinarian at an establishment, is consistent with the current method of handling carcasses that require delayed evisceration. Field ante-mortem inspection would reduce the risks of injury to persons handling buffalo and would eliminate the property damage to transport vehicles, fences and pens.

The second alternative would allow ante-mortem inspection to be performed outside a transport vehicle at an official establishment. The vehicle ante-mortem inspection allows a buffalo producer with a few buffalo to have ante-mortem inspection performed outside the transport vehicle on buffalo that are inside the vehicle. The veterinarian would be positioned outside the vehicle and provide such service if it can be appropriately and safely performed. The vehicle ante-mortem inspection alternative enables establishments that do not have reinforced pens to receive a few buffalo for slaughter. The ante-mortem inspected and passed buffalo would be stunned but not bled in the transport vehicle. The carcasses would be immediately taken to the slaughter floor for bleeding and post-mortem inspection.

Ante-mortem inspections performed in the field or outside a transport vehicle must ensure the safety of inspection personnel and must allow for adequate inspection.

The three alternatives are designed to resolve the unique problems involved with the ante-mortem inspection of buffalo. These ante-mortem inspection alternatives would allow more buffalo meat to enter the red meat market.

The proposed rule would allow the cross utilization of Federal and cooperative State veterinarians to perform field ante-mortem inspection. The cross utilization of Federal and State veterinarians will enable a more efficient and economical use of manpower than if Federal veterinarians were used exclusively.

The proposed rule will allow the use of a newly designed triangular brand to identify inspected and passed buffalo meat and buffalo meat food products. The Department might possibly decide to use the new triangle brand in the future to identify other species of animals that would come under the voluntary inspection program. The triangular brand may be applied at a cooperating State or Federal establishment. The triangular brand will readily identify buffalo meat which has been inspected and passed under the voluntary inspection program. The special triangular brand was designed for application to buffalo meat inspected in Federal or cooperating State establishments in lieu of the red meat brand that can only be used in Federal establishments.

Meat and meat products produced under the voluntary inspection service are certified by the Department and carry the mark of Federal inspection. To qualify for this service, the establishment, its facilities and equipment,
and its procedures would have to meet the requirements of the Federal Meat Inspection Act and the Federal Meat Inspection Regulations and would have to be, for all intents other than mandatory coverage, an official establishment. Therefore, FSIS is proposing to incorporate the relevant substantive requirements of the Meat Inspection Regulations into the Voluntary Buffalo Regulations, instead of restating those requirements.

Because the diseases in buffalo parallel diseases found in cattle, the post-mortem inspection procedures for buffalo will remain the same as are currently used for cattle. It is recommended that field-slaughtered buffalo be brought to the plant for post-mortem examination and disposition in the shortest possible time. The establishment of specific time and temperature requirement for buffalo slaughtered in the field is difficult because their carcasses would be transported different distances under varying climatic conditions. It is generally accepted, however, that the maximum time between slaughter and evisceration should not exceed 24 hours in order to minimize changes in the carcass which can affect post-mortem examination, disposition and wholesomeness. Therefore, field slaughtered buffalo must be presented for slaughter not more than 24 hours after death. Regardless of the time, if, in the judgment of the examining veterinarian, changes have occurred in the carcass which confuse the examination or affect product wholesomeness, the carcass must be condemned.
FIELD TESTING THE LAST AND SST TESTS

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Excellent progress continues to be made in the development of a practical approach for producers of meat and milk to monitor for antibiotic/sulfonamide residues in their animals prior to marketing.

Although a number of violative levels has been recorded by FSIS within the past 12 months, the magnitude of the violations is relatively low. For example, the action level of sulfonamide violation in meat is 0.1 ppm. Most of the violative levels detected fall in the range of 0.12 to 0.15 ppm. Perhaps the 0.1 ppm level, which was somewhat arbitrarily set, is too low from a cost/benefit standpoint. Maybe a slightly higher action level would be more realistic.

The Live Animal Swab Test (LAST), Swab Test on Premises (STOP) and the Sulfa Swab Test (SST) were developed for inplant detection of antibiotic/sulfonamide residues and have proved useful in the field with animals prior to marketing. The major thrust of the Georgia Residue Avoidance Program has been focused on the adaption of the LAST and SST tests under practical field conditions. These tests were proven satisfactory and approved by FSIS for use within packing plants. One of the problems in preventing antibiotic/sulfonamide residues has been the absence of a low cost method for producers monitoring their animals prior to market. The LAST, STOP and SST tests, using approved instructions were found to have certain problems when applied in the field prior to marketing of the animals. If the action level for sulfonamides is to remain at 0.1 ppm, producers must have a method of checking animals prior to market to assure that contamination does not continue even at low levels. The following are some of the problems encountered.

1. **The LAST test will not detect low level sulfonamides.** FSIS has already recognized this deficiency. The Sulfa Swab Test was already under development and will detect sulfonamides. In the early stages of the Total Residue Avoidance Program, however, the LAST test was promoted as the test to use although the violative problems were in the area of sulfonamides. Agricultural Handbook No. 601 was printed and distributed by FSIS. The STOP test utilized in packing plants also had the same deficiency in being unable to detect sulfonamides. The minimum detectable level is about 500 micrograms per milliliter for the STOP test.

   Since the Sulfa Swab Test, which is similar in nature to the LAST test will detect sulfonamides, emphasis in the Georgia Residue Avoidance Program shifted to the evaluation of this second test.

2. The LAST and Sulfa Swab Test will detect low levels of antibiotics in animal feeds at rates that are legal for use. Apparently, antibiotics such as bacitracin are approved at 50 grams per ton level of less for growth promotion purposes. These levels are not high enough to spill over into body fluids and be detected as an antibiotic residue in meat. It does interfere with the use of the LAST or SST test in evaluating the source of contamination in the feed on a farm. The solution to the feed contamination problem is partially solved by using the Sulfa Swab Test then running positive feed samples through the sulfa column test to eliminate sulfonamides as a cause of the zone of inhibition. In this manner, we can classify the cause of the positive reaction to either antibiotics or sulfonamides. Depending upon the results, appropriate action can be taken by the feed dealer or producer to eliminate sources of contamination in the feed.

3. The LAST, and SST are approved only for use with urine. From a practical standpoint, urine is difficult to collect from live market animals prior to slaughter. The alternative of using serum or saliva was investigated for possible on the farm use. FSIS has agreed that serum may be used in the conduction of the tests provided a 3 day holding period is observed following a negative test. Saliva was approved providing a holding period of seven days was observed following a negative test.

4. During the course of field application testing, it was observed that a percentage of serum and milk samples would give false positive test results. It was also observed that heat treatment in a waterbath at 55 degrees centigrade for 30 minutes will inactivate the substance causing a false positive test. While the number of false positive tests were extremely low, it was necessary to develop a method of eliminating false positives.

5. It was observed that in using body fluids, a more uniform sample could be collected, utilizing a ½ inch analytical paper disk than the recommended cotton swab. It appears that cotton swabs of different brands have different capacities of holding fluids, depending upon how tight they are wrapped. The ½ inch analytical disk collects a more uniform sample and is easier to manipulate and transport.

6. In some cases of positive animals, it was found that many of the animals had positive urine but negative kidney tests. Perhaps this is not too surprising since this is the normal way the body excretes antibiotics and sulfonamides. The positive urine test animals should be rechecked using tissue source before a violation is called.

7. Since 12 pilot counties were used in the field evaluation and over 20 different people were involved in conducting the various tests, errors in laboratory technique were noted. For
example, A. In most cases, where growth failed to occur on the test plates, an incubation temperature lower than that recommended was found to be the culprit. B. Faulty lab technique in handling the neomycin test disks and subsequently handling the cotton swab or the analytical paper disk gave false positive tests on some occasions.

8. **It was determined that freezing or other activities that might disrupt body cells will render the tissue unsuitable for testing.** Apparently PABA or other products are released when cells are ruptured, cut or otherwise altered that will result in the possible incorrect reading.

9. **The LAST test using B. subtilis spores is more satisfactory than the SST test using B. megaterium spores in testing for antibiotic residues in milk.** B. subtilis spores give results more similar although not as sensitive as B. stearothermophilus, the recognized organism used in milk regulatory work.

10. **One-half inch analytical paper disks are easily dried after dipping test body fluids for sending to a laboratory for SST testing.**

11. **The Sulfra Column Test is best used in a laboratory setting and is not a practical on the farm test.**

**SUMMARY**

The field application of approved tests for antibiotic/sulfonamide tests identified several problems and allowed the development of practical solutions. Over 30,000 tests have been conducted in all types of conditions using various species of animals, working with producers, veterinarians, county extension agents and trained laboratory personnel. It was found that with a little flexibility from the original instructions as listed in Handbook 601, the SST, LAST and Sulfra Column Test are suitable for detecting antibiotic/sulfonamides in animal body fluids such as saliva, serum, urine and milk as well as animal feeds. The cost of the tests is minimal and should not discourage their use. If the producer can collect the samples, such as body fluids or feeds and submit them to his veterinarian or to an appropriate lab for testing, a minimal cost system can easily be developed to certify animals negative to antibiotic/sulfonamide residues prior to the shipment to market.
REPORT OF THE COMMITTEE ON
FOOD ANIMAL HYGIENE

Chairman: David M. Bedell, Georgia
Vice Chairman: Joe L. Blair, Virginia

A. W. Bailey, OK; J. A. Bell, N.C.; L. G. Billingsley, CA; L. L. Beuschel, VA; A. D. Bond, D.C.; D. C. Breeden, CA; W. H. Dubbert, VA; G. B. Estes, VA; T. M. Gustafson, NE; R. E. Hall, WI; C. S. Johnson, TX; J. C. Leighby, MD; T. E. Liner, TX; C. S. McCain, OK; H. O. Miller, IL; J. K. Payne, D.C.

The Food Animal Hygiene Committee met as scheduled with eleven (11) members and seventeen (17) visitors in attendance. The following reports and papers were heard during the session.

1. The American Bison (Buffalo) in Wyoming; A Meat Inspection Challenge. Dr. Robert E. Fetzner, Director, State Meat Inspection Program, Cheyenne, Wyoming.

   The paper outlines the problems of inspection of an animal harvested from the wild—a game animal, and from domesticated herds. The total annual slaughter in the U.S. is ten thousand. The unique problems of handling this wild and semi-domesticated unpredictable dangerous animal is treated in some detail in another paper in this proceedings.

2. Field Testing—the LAST and SST Tests. Dr. Charles N. Dobbins, Jr., Head, Extension Veterinary department, University of Georgia, Athens, Georgia.

   The LAST (Live Animal Swab Test) and SST (Sulfa Swab Test) are the major thrust of the Georgia Residue Avoidance Program. The limitations and advantages of these tools as a screening procedure for residues of antibiotics and sulfonamides in body fluids, saliva, serum, urine, milk as well as animals feeds are discussed in the paper. This paper is published in its entirety in the proceedings.

3. Voluntary Inspection of Fish Processing Plants. Dr. Irving D. Sackett, Director, Field Operations, National Marine Fisheries Service, Department of Commerce, Washington, D.C.

   An overview of voluntary inspection of about one hundred (100) fish processing plants in the U.S. was presented. The plants represent 30% of the product consumed in the U.S. The remaining consumption is either not inspected or is imported.

   Another point of interest is the anti-mortem inspection. Fish are usually dead at the first point of inspection and the term anti-mortem does not apply. The exception is the commercially raised catfish.

   The total inspection budget of the agency is approximately three million dollars supporting sixty-five (65) full time employees and two hundred thirty-five (235) part-time employees through agreements with eleven (11) state and federal agencies. The cost of inspection is borne by the
processor at present. Segments of the industry have expressed a desire for mandatory inspection.

Also Dr. W. H. Dubbert gave a report on the International Salmonella Symposium and the "quick tests" being developed to enhance the quality of inspections while reducing the costs by Dr. W. H. Dubbert.
VESICULAR STOMATITIS OUTBREAKS AND SURVEILLANCE IN THE UNITED STATES
JANUARY 1980 THROUGH MAY 1984

E. W. Jenney, DVM, G. A. Erickson, DVM, PhD, and M. L. Snyder, BS

SUMMARY

Following a period of relative quiescence, New Jersey (NJ) type vesicular stomatitis (VS) was diagnosed in 673 herds in a 1982–1983 outbreak. The outbreak spread northward from Arizona and New Mexico to the Canadian border in the Rocky Mountain states and laterally due to livestock movements. The outbreak gradually disappeared during the winter of 1982 and spring of 1983. Only three cases were diagnosed by serologic tests during the summer of 1983, one in Colorado and two in Wyoming. In January 1984, NJ VS was detected in rodeo horses and Mexican roping cattle in Milam and Jim Wells counties in Texas.

In addition to testing conducted as a result of the outbreak of VS, serological surveillance of domestic animals, feral swine, deer, and other wildlife for evidence of VS infection has been done in various parts of the United States. Antibodies were detected in feral swine, horses, cattle, deer and raccoons. For the first time, VS virus was isolated from feral swine on Ossabaw Island, GA, in 1983 during a joint in-depth epizootiological study.

INTRODUCTION

Vesicular stomatitis has been a very important disease in regulatory veterinary medicine because it is clinically indistinguishable from foot-and-mouth disease (FMD) in cattle and swine, and swine vesicular disease and vesicular exanthema (caliciviruses) in swine. Accordingly, surveillance for all vesicular diseases affecting cattle and swine has been maintained in the United States since the first introduction of FMD into the U.S. in 1870.

Outbreaks of a disease with clinical signs consistent with vesicular stomatitis have occurred in the U.S. in 1904 and 1916.1 Prototype Indiana (IN) serotype virus was isolated in Indiana in 1925 and prototype New Jersey (NJ) serotype virus was isolated from affected cattle in NJ in 1926.1 Since 1925, major epizootics of the IN type have occurred in 1942, 1956, 1964, and 1965.2,3,4 Major epizootics of the NJ type have occurred in 1944, 1949, 1957, 1959, and 1963.1,3,4 Sporadic isolated NJ serotype cases have also occurred in one or more of the southern or southeastern states during the intervening years.3,4,5,6 This report presents a detailed record of VS activity from January 1980 thru May 1984.

From the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, P.O. Box 844, Ames, IA 50010.
MATERIALS AND METHODS

Investigation of Suspected Vesicular Disease — Reports of vesicular lesions in animals were routinely investigated by specially trained foreign animal disease diagnosticians. These investigations were reported to Veterinary Services headquarters and depending on the clinical signs observed and the epidemiological evidence presented, diagnostic specimens were either submitted to the Foreign Animal Disease Diagnostic Laboratory, Greenport, NY, or to the National Veterinary Services Laboratories (NVSL), Ames, Iowa. When appropriate and when vesicles were present, vesicular epithelium was submitted to NVSL for virus isolation (VI) and assay for vesicular disease antigens. In some bovine cases in which the lesions had healed, an esophageal probang sample was collected and submitted to the laboratory for VI. Serum samples were collected from affected animals and assayed by the complement fixation (CF) and neutralization (Nt) tests for type-specific VS antibody.

Surveillance — The main emphasis on surveillance activities was a study of VS in feral and domestic animals on Ossabaw Island, GA, an endemic NJ VS area. Ossabaw Island is a 25,000 acre marshy underdeveloped barrier island preserve on the Atlantic coast some 20 miles south of Savannah, Georgia. A serodiagnosis of VS was made on the island in 1965 and a surveillance program has been maintained there since that time. The Southeastern Cooperative Wildlife Disease Study (SCWDS) and the Diagnostic Virology Laboratory (DVL) of NVSL working through the Emergency Programs Staff of the Animal and Plant Health Inspection Service (APHIS) collected and assayed 632 sera from different species located on Ossabaw during 1981. An in-depth study was initiated in 1982. Feral swine were live-trapped, bled via the anterior vena cava puncture, double ear tagged, examined for vesicular lesions, and released. If vesicular lesions were observed, epithelium was harvested for VI. Swine were continuously captured in corn baited traps during the study, but recaptured pigs were rebled only if 10 days or more had elapsed since their last capture. All pigs were trapped on a modified grid system with age, tag, date, and trap site recorded. Blood samples were also collected from deer, horses, cattle, and 20 other species found on the island.

Wildlife sera were also received from feral swine and deer located in other regions of the country. Feral swine sera were submitted from Arkansas, Florida, Georgia, Hawaii, Louisiana, Mississippi, Virginia, Arizona, California, and Texas. Sera were also obtained from hunter-killed deer on military bases in New Mexico and Wisconsin.

Sera were also obtained as part of domestic animal surveillance for VS in states with a previous history of VS. In 1980, sera were received from South Carolina through the state-federal brucellosis laboratory. These sera were provided on an “as available” basis, preferably from animals.

*Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.*
located in coastal counties or counties with a history of VS. In 1983, bovine sera were collected by random sampling in Arizona sale yards and from selected herds in California and Texas.

**Serologic Tests** — Sera were tested by the CF and Nt tests for antibody against NJ and IN type VS virus (V) using microtitration techniques previously described. Swine sera and those of smaller species were tested by Nt only. Chicken-embryo-passage Atlanta strain and Chimayo strain VSV were used as the NJ and IN (subtype 1) antigens, respectively, for all CF and Nt tests. Vero-M cell cultures grown in Eagles minimum essential medium supplemented with 0.5% Eadamin S and 10% irradiated bovine fetal serum were used for all Nt tests.

Serologic diagnosis of recent infection with VSV was defined as a 4-fold or greater increase in neutralizing titer for paired serum samples, or by a positive CF antibody titer accompanied by Nt (≥1:32) on a single sample. Generally, survey sera were tested by Nt only.

**Virus Isolation and Antigen Identification by Complement-fixation Test** — Virus isolation was attempted on all vesicular epithelium samples. If sufficient tissue was received, tissue antigen was prepared for the CF test and tested for NJ and In type VS and 7 types of FMD viral antigens, as previously described. Secondary fetal bovine kidney and primary fetal swine kidney Leighton tube coverslip cell cultures were used for VI. Sometimes Vero-M cells were used. The fluorescent antibody (FA) test was used for identification of isolates.

**RESULTS**

**Investigations of Suspicious Vesicular Disease** — Clinical VS was not diagnosed in domestic animals during 1980. One horse (Guadalupe Co., Texas) and one cow (Bosque Co., Texas) were found to be serologically positive for NJ VS antibodies during August and September 1981.

The first isolation of NJ VSV of the 1982 outbreak was made on June 2 from cattle in a national forest at Camp Verde, Yavapai Co., Arizona. Clinical signs had been observed by the owner about May 23. On June 22, several positive equine sera were collected in Bernalillo Co. near Albuquerque, New Mexico. VS then appeared in northwestern New Mexico (July 6), eastern Utah (July 21), and western Colorado (July 21). By August 4, VS had crossed the Continental Divide of the Rocky Mountains into eastern Colorado (Map 1). Clinical disease was observed in Wyoming on August 11; Idaho on August 27; Montana on September 4; and South Dakota on September 15. Rapid spread of VSV occurred within infected states, with the exception of South Dakota. By September 17 VSV was isolated from a farm in Phillips Co., Montana, which adjoins the Canadian border. VS reporting was most comprehensive in Colorado. Ninety-five of Colorado’s 328 positive investigations were in Mesa Co. (Grand Junction area).

From May to mid October, disease spread was only occasionally accountable to animal movements (Map 3). However, on October 16 and 17, a large dispersal sale of a dairy herd where VS had been diagnosed a month
earlier was held at Pueblo, Colorado. Cattle from this sale spread VSV to dairies in Linn Co., Missouri; Yakima Co., Washington; and Pinal Co., Arizona (Maps 1 and 2). Fortunately, cattle moved to New York, California, and Texas from the Colorado sale were not infective. Dealer traffic in replacement dairy cattle from Idaho and adjacent Utah resulted in VSV being introduced into California and new areas of Arizona, New Mexico, and Washington. Infected Idaho cattle also introduced VSV into Nebraska and Kansas. Using epidemiological data, it was determined that VS was spread into new counties in Kansas and Nebraska through subsequent sales of infected stock until late February (Maps 2 and 4). The last positive case of the 1982–1983 outbreak was from a bull examined in Otoe Co., Nebraska, on May 25, 1983. NJ VSV was isolated and the bull died shortly after examination. Maps 3 and 4 illustrate the extent of the outbreak.

On August 10, 1983, a clinically affected horse in Garfield Co., Colorado, was reported and NJ CF and SN antibodies were demonstrated. Between August 29 and September 19, two geographically separated horses in Fremont Co., Wyoming, were also positive for NJ CF and SN antibodies. These were the only confirmed cases which appeared during the summer and fall of 1983.

In 1984, VSV was isolated from newly purchased Mexican roping cattle added to the herds of three rodeo enthusiasts in east central Texas. Lesions of NJ VSV had developed between January 16–27 in both cattle and roping horses. Two of these herds were in Milam Co. and one was in Jim Wells Co., Texas. The suspect roping cattle originated in Durango, Mexico, which had NJ VS cases diagnosed during November 1983. No further cases were reported in the US thru May 1984.

Serological Surveillance — The 1981 sample collections from Ossabaw Island are summarized in Table 1. A wide range of species was sampled, including cattle, horses, donkeys, ponies, deer, feral swine, rodents, rabbits, raccoons, selected birds, and bats. NJ VS antibodies were found in 53 percent of feral swine, 43 percent of cattle and more than a third of the equidae. Ten percent of the raccoons had VS antibodies, but none of the rodents, birds or bats were seropositive.

An in-depth wildlife surveillance on Ossabaw Island was initiated on December 15, 1981, for the 1982 season. Initially, monthly sentinel swine trappings were done. By May 1982, the trappings were increased to weekly intervals. Using the capture, tag, bleed, record, and release system on a modified grid with 22 trap sites, it was possible to detect when and where the feral swine were infected with VSV. The first seroconversions occurred on the south end of the island during the first week of June in 5 of 67 swine. These 5 juvenile feral swine, negative at 1:8 by the Nt on May 15, had seroconverted with titers of 1:2048 by June 7, 1982. During 1982, 27 of 250 juvenile and one of 57 adult feral swine seroconverted to NJ VSV.

Boletin CPA No. 16 November 1983 and CPA Quarterly Report October 1 through December 31, 1983.
A similar trapping program was used during the 1983 season. Seroconversion was again first observed in the same area on the south end of the island. One pig negative on May 25 had seroconverted by June 3. On July 6, 1983, small vesicular snout lesions were noted on a trapped pig and epithelium was harvested. This was the first time that clinically-affected animals had been found on Ossabaw Island during the 18 years of surveillance since the initial serodiagnosis of VSV on the Island. Vesicles were observed on a second feral hog on July 23 and NJ VSV was isolated at NVSL. Both swine seroconverted. A higher rate of infection, as determined by positive serology, was observed in feral swine on the island during 1983. Of 340 juvenile swine monitored, 84 seroconverted to NJ VSV. Many of the very young feral swine were presumed to have had colostral antibody titers since they were found to be seronegative on subsequent trappings. Northward movement of the virus across the Island was traced by the positive titers of the retrapped feral swine, some of which were sampled six or more times.c

Of sera collected in South Carolina in January through March 1980, 5 of 49 domestic swine and 1 of 147 horses had NJ VSV neutralizing antibodies. The positive swine were from one premise in Horry Co. and the horse was located in Berkeley Co. Ten stray horses sampled in Texas along the Mexican border during 1981 were negative for VS antibodies. An additional 231 bovine sera collected during 1983 from California, New Mexico, and Texas were negative for VSV antibodies except for one Texas cow purchased from the Pueblo, Colorado, dispersal sale. Arizona personnel collected 1134 bovine sera from market cattle during 1983 and 41 had NJ VSV neutralizing antibodies.

A summary of results for wildlife serum collections made in locations other than Ossabaw Island during 1980 through 1983 is shown in Table 2. Twenty-two of 889 feral swine were positive for NJ VS antibodies over the 4 year period. Ten of 10 one-year-old swine were positive at the Felsenthal National Wildlife Refuge in Union Co., Arkansas. Low antibody titers were detected in 12 swine of greater than 1 year of age in Louisiana, Florida, and on Rhetts Island, Georgia.

DISCUSSION

In the 1982–1983 outbreak, the disease spread within large subdivided herds for a month or more after clinical signs were first observed. VS continued to spread even after the insect population was reduced by cold weather. In a Colorado dairy herd of 800 cows, clinical disease was first observed on September 11. When a dispersal sale was held on October 16 and 17, cows from the herd introduced VS virus into herds in 3 other states. Apparently virus was being transmitted within the herd during the five intervening weeks.

In an Idaho packing company feedlot, lesions were repeatedly detected

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1Personal communication, Mr. D. E. Stallknecht, SCWDS, Athens, GA.
in cattle at slaughter and a series of VSV isolates were made over a seven week period in early 1983. Because VS virus was still cycling in the feedlot in mid-February, it was depopulated, disinfected, and repopulated without further recurrence of disease.

The incidence of new cases of VS usually decreases and gradually disappears in the fall with the decrease in the insect population, particularly after killing frosts. In a report on the 1942 outbreak of IN type VSV in Colorado, Heiny described cases that occurred from September 7 to November 1, 1942, and on November 23, 1943, with snow on the ground. In the 1982–1983 outbreak, the virus continued to spread due to cattle movement long after the first killing frost. It appeared at one time that the outbreak would not be self-limiting and would continue to spread with the return of weather favorable to insect propagation.

Field studies of vesicular stomatitis have not been as productive as workers have anticipated in resolving the mechanism of VSV survival and transmission. Detailed studies are complicated by the unpredictability of its occurrence and its relatively low economic importance to most groups of animals. Greatest monetary losses occur in dairy cattle, particularly in herds that develop teat lesions during early lactation. Mastitis is a frequent and devastating sequela to VS teat lesions.

Possible support for the biological role of buffalo gnats (Simuliidae) in the transmission of VSV was provided by a case that occurred in an Idaho dairy herd following a period of warm weather in March 1983. The foreign animal disease diagnostician associated the severity of this relatively isolated outbreak with the emergence of a large population of buffalo gnats. Approximately 60 percent of the cows developed udder and tongue vesicles in the 150 cow herd. Irrigation ditches on the farm could have contributed to the high population of Simuliidae. This case is worthy of mention in light of two isolations of NJ VSV that were obtained from Simuliidae collected in Colorado during 1982 and a previous isolation of NJ VSV from simuluids in Colombia, South America. The well aerated streams and irrigation canals of the Rocky Mountain states are ideal habitats for Simuliidae. Further information will be needed to determine whether Simuliidae are biological vectors of NJ VSV. Although VS is endemic on Ossabaw Island, a different type of epizootiology appears to be involved in western US outbreaks. The 1982 outbreak and a 1949 outbreak of NJ VS appeared to have moved northward across international borders from Mexico to Canada. The method by which VSV appears to spread or jump many miles unrelated to livestock movements remains a challenge which precludes effective control or eradication.

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*dPersonal communication, Dr. H. Myers, Oyhee Plaza, P.O. Box 9426, Boise, ID 83707.*

*Dr. Thomas P. Monath, Division of Vector-Borne Viral Diseases, Centers for Disease Control (CDC), Fort Collins, CO.*
ACKNOWLEDGEMENTS

We wish to acknowledge the assistance of the foreign animal disease diagnosticians and wildlife biologists who made the investigations and the laboratory technicians who performed the tests. Appreciation is expressed to Mrs. Eleanor West of the Ossabaw Island Foundation whose permission has made the in-depth epizootiological studies possible on Ossabaw Island.

Table 1. VS Serology Ossabaw Island: Sera Collected During 1981.

<table>
<thead>
<tr>
<th>Species Sampled</th>
<th>No. Pos/No. Tested</th>
<th>Species Samples</th>
<th>No. Pos/No. Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey squirrel</td>
<td>0/30</td>
<td>Turkey vulture</td>
<td>0/9</td>
</tr>
<tr>
<td>Fox squirrel</td>
<td>0/15</td>
<td>Florida yellow bat</td>
<td>0/11</td>
</tr>
<tr>
<td>Cotton mouse</td>
<td>0/60</td>
<td>Evening bat</td>
<td>0/7</td>
</tr>
<tr>
<td>Rice rat</td>
<td>0/1</td>
<td>Eastern pipistrela bat</td>
<td>0/6</td>
</tr>
<tr>
<td>House mouse</td>
<td>0/1</td>
<td>Semiole bat</td>
<td>0/4</td>
</tr>
<tr>
<td>Marsh rabbit</td>
<td>0/5</td>
<td>White tailed deer</td>
<td>21/101</td>
</tr>
<tr>
<td>Raccoon</td>
<td>6/60</td>
<td>Feral swine</td>
<td>54/101</td>
</tr>
<tr>
<td>Blackbird</td>
<td>0/27</td>
<td>Cattle</td>
<td>32/75</td>
</tr>
<tr>
<td>Mourning dove</td>
<td>0/30</td>
<td>Horses</td>
<td>4/13</td>
</tr>
<tr>
<td>Vulture</td>
<td>0/20</td>
<td>Donkey</td>
<td>4/10</td>
</tr>
<tr>
<td>Cattle egret</td>
<td>0/9</td>
<td>Pony</td>
<td>2/4</td>
</tr>
<tr>
<td>Wild turkey</td>
<td>0/29</td>
<td>Dog</td>
<td>0/4</td>
</tr>
<tr>
<td><strong>TOTAL TESTED</strong></td>
<td><strong>123/632</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. VS Serology: Wildlife Surveillance from January 1980 to May 1984 excluding Ossabaw Island.

<table>
<thead>
<tr>
<th>1980 State—Counties</th>
<th>Location</th>
<th>Feral Swine</th>
<th>Deer</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR—Union</td>
<td>Felsenthal NWR*</td>
<td>10/10**</td>
<td>...</td>
</tr>
<tr>
<td>FL—Glades</td>
<td></td>
<td>0/205</td>
<td>...</td>
</tr>
<tr>
<td>GA—Camden, Liberty,</td>
<td></td>
<td>0/66</td>
<td>...</td>
</tr>
<tr>
<td>Telfair, misc.</td>
<td></td>
<td></td>
<td>...</td>
</tr>
<tr>
<td>HI—Maui</td>
<td></td>
<td>0/48</td>
<td>...</td>
</tr>
<tr>
<td>LA—Grant</td>
<td></td>
<td>7/10</td>
<td>...</td>
</tr>
<tr>
<td>MS—Pearl River</td>
<td></td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td>NM—Otero</td>
<td></td>
<td></td>
<td>0/86</td>
</tr>
<tr>
<td>VA—Princess Ann</td>
<td></td>
<td>0/5</td>
<td>...</td>
</tr>
<tr>
<td><strong>TOTAL 1980</strong></td>
<td><strong>17/354</strong></td>
<td><strong>0/86</strong></td>
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### 1981 State—Counties

<table>
<thead>
<tr>
<th>Location</th>
<th>Feral Swine</th>
<th>Deer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wakulla, FL</td>
<td>4/12</td>
<td></td>
</tr>
<tr>
<td>Franklin, Brevard, Henry, Orange, FL</td>
<td>0/36</td>
<td></td>
</tr>
<tr>
<td>Liberty, McIntosh, GA</td>
<td>1/24</td>
<td></td>
</tr>
<tr>
<td>La Salle, LA</td>
<td></td>
<td>1/5</td>
</tr>
<tr>
<td>Iberia, LA</td>
<td></td>
<td>0/5</td>
</tr>
<tr>
<td>W, VA—Boone/Logan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhetts Island, FL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline, GMA***</td>
<td></td>
<td>1/5</td>
</tr>
<tr>
<td>Avery Island, LA</td>
<td></td>
<td>0/5</td>
</tr>
<tr>
<td>Bald Knob, WV, VA</td>
<td>5/80</td>
<td>1/10</td>
</tr>
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</table>

### 1982 and 1983 State—Counties

<table>
<thead>
<tr>
<th>Location</th>
<th>Feral Swine</th>
<th>Deer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otero, NM</td>
<td></td>
<td>1/99</td>
</tr>
<tr>
<td>Hardy, FL</td>
<td>0/229</td>
<td></td>
</tr>
<tr>
<td>Unknown, TX</td>
<td>0/112</td>
<td></td>
</tr>
<tr>
<td>Mojave, AZ</td>
<td>0/13</td>
<td></td>
</tr>
<tr>
<td>Merced, San Louis Obispo, Santa Clara, CA</td>
<td>0/101</td>
<td></td>
</tr>
<tr>
<td>Tehama, Ventura</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL 1982 and 1983</td>
<td>0/455</td>
<td>1/99</td>
</tr>
</tbody>
</table>

### CUMULATIVE TOTAL

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>22/889</td>
<td></td>
<td>2/195</td>
</tr>
</tbody>
</table>

*National Wildlife Refuge
**Number positive/number tested
***Game Management Area

**BIBLIOGRAPHY**

8. Jenney, E. W., Mott, L. O.: Serological studies with the virus of vesicular...
VESICULAR STOMATITIS OUTBREAKS


MAP I - VESICULAR STomatITIS OUTBREAK 1982-1983; BY COUNTIES ROCKY MOUNTAIN STATES
MAP 2 - VESICULAR STOMATITIS OUTBREAK 1982-1983, BY COUNTIES PACIFIC COAST AND CENTRAL PLAINS STATES
MAP 4 - VESICULAR STOMATITIS OUTBREAK 1982-1983; No. POSITIVE HERDS
PACIFIC COAST AND CENTRAL PLAINS STATES
RESEARCH ON VESICULAR DISEASES

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INTRODUCTION

Research on vesicular viral diseases reviewed in this report consists of studies on foot-and-mouth disease (FMD), swine vesicular disease (SVD), vesicular exanthema of swine (VES) and vesicular stomatitis (VS). This is a review of selected reports considered pertinent to the specific diseases.

Foot-and-Mouth Disease Virus (FMDV)

Interest continues in genetic engineering technology which offers newer and better methods of disease control. Monoclonal antibodies derived by the fusion of antibody producing cells with myeloma cells are improving the diagnosis and epitope delineation in infectious diseases and are being used in FMD research.

Interferon has been produced by genetic engineering in E. coli in amounts sufficient for experimental testing. Baxt et al.1 examined the epitopes on FMDV outer capsid protein VP1 and by use of monoclonal antibodies demonstrated three antigenic areas on the surface of FMDV which are involved in neutralization. One of the antigenic sites appears to be responsible for interaction with the cellular receptor sites on the surface of susceptible cells.

British workers2 have produced monoclonal antibodies against the trypsin-sensitive site on the 140S particle of FMDV and have demonstrated at least three distinct epitopes within the site. Reaction with two of these resulted in neutralization of virus infectivity.

A review of the current status of foot-and-mouth disease vaccines, finds a continuing need for properly inactivated and safety tested whole virus vaccines. This author, while acknowledging the progress made in genetically-engineered subunit vaccine, suggests that such a product is not yet ready to be produced and used commercially.3

Dutch workers have published interesting data on the inactivation of FMDV with formaldehyde. Formaldehyde is still widely used as an inactivant for FMDV vaccines. These studies indicate that the inactivation kinetics for both the adsorbed and non-adsorbed virus were identical and that no "tailing-off" was observed. Increased lactalbumen hydrolysate and quaternary amines like Tris affected inactivation and might explain published data on observed "tailing-off" when formaldehyde is used as an inactivant.4

Studies conducted in South America show that calves with maternally derived antibody fail to respond to vaccination. Apparently such antibodies may persist for 4 to 5 months.5 There is some indication in the
literature that calf response to vaccination may be improved when oil-adjuvanted vaccines are used.

Innocuity testing of formaldehyde inactivated vaccines adsorbed on aluminum hydroxide gel showed that inactivated virus could interfere with small amounts of live virus added to the vaccine and tested intradermalingually in cattle; this interference was not found when monolayers of fetal calf thyroid cells were used for detecting virus.6

British workers7 examined the antibody response in pig nasal fluid and serum following FMD infection or vaccination. They found that after virus exposure the response profile of serum and nasal mucous were similar, neutralizing activity rose to a peak at one or two weeks after exposure and subsided slowly.

After vaccination with either a single or double oil emulsion vaccine, a neutralizing response was demonstrable in the serum 3 to 7 days after the first injection, which was increased by revaccination 56 to 117 days later. The neutralizing activity was detectable in nasal fluid 7 days after the first vaccination but response after additional vaccination did not increase. The authors suggest this may relate to poor correlations observed between the serum antibody titers and protection from infection sometimes observed by contact exposure in this species. Aerosol exposure of cattle to FMDV produced pharyngeal virus growth curves and viremia patterns correlating with the dose of virus to which they were exposed and similar to intranasal inoculation.8 Electrofocusing is a sensitive method for studying genetic change and as an epidemiological technique for viruses like FMDV. Large numbers of isolates can be compared quickly and easily.9

Swine Vesicular Disease Virus (SVDV)

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Caliciviruses are becoming associated with disease manifestations that are occurring in an increasing number of animal species. The groups of interest in this report are those classified as either vesicular exanthema of swine virus (VESV) or indistinguishable from VESV. Caliciviruses have the ability to infect a wide range of host species. Two virus types infect fish, seals, domestic swine and primates, while another type from fish infects two seal species and domestic swine. Because of this ability to infect widely divergent species, the author suggest that in all probability these agents are zoonotic.12
Vesicular Stomatitis Virus (VSV):

An extensive outbreak of vesicular stomatitis (VS) occurred in the western half of the United States in 1982–83. It soon became apparent, because of the extent of the outbreak, that little was known about this virus relative to animal health. Despite the constant use of this virus as a basic model for research, there is an apparent need for additional research relating to the pathogenesis of this disease. The similarity of this disease to FMD makes rapid diagnosis of prime importance. Thus, specimens are given high priority. Initial testing is done by complement-fixation further confirmed by the fluorescent antibody virus isolation technique and the neutralization test.

The severity of the 1982–83 outbreak has renewed an interest in vaccines to aid in the control of this disease. Experiments have involved both live and inactivated vaccines. The live virus vaccine administered intramuscularly (an abnormal route) is effective in reducing the incidence of disease in cattle when administered during an epidemic. An inactivated vaccine requires a sufficient mass of inactivated virus and the use of an effective adjuvant.

A subunit vaccine has also been developed from a purified vesicular stomatitis virus preparation by selectively removing the immunogenic G glycoprotein of the virus. Experimentally, the vaccine protected cattle, and it was also possible to distinguish, serologically, animals vaccinated with the subunit preparation from those that have had the clinical disease or that were vaccinated with whole virus.

The G glycoprotein has also been sequenced and several immunogenic sites have been predicted and polypeptides organically synthesized. Several have been evaluated in mice and cattle and one afforded immunity to cattle. The G glycoprotein gene has also been placed in vaccinia virus. This combination resulted in protection of 4 of 6 cattle when their immunity was challenged.

REFERENCES


REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

Chairman: G. S. Treviño, Laredo, Texas
Vice Chairman: J. L. Hyde, College Park, Maryland

Dr. W. W. Buisch, MD; Dr. J. J. Callis, NY; Mr. Robert Combs, NV; Dr. A. H. Dardiri, NY; Dr. W. A. Deen, MD; Mr. J. B. Finley, Jr., TX; Dr. J. H. Graves, NY; Dr. C. M. Groocock, NY; Dr. F. Hamdy, DC; Dr. W. P. Heuschele, CA; Dr. J. A. House, NY; Dr. E. W. Jenney, IA; Dr. F. M. Jones, FL; Dr. D. D. King, MD; Dr. K. L. Kuttler, ID; Dr. L. L. Logan, NY; Dr. S. McConnell, TX; Dr. H. A. McDaniel, MD; Dr. P. D. McKercher, NY; Dr. N. L. Meyer, VA; Dr. James I. Moulthrop, MD; B Gen. T. G. Murnane, TX; Dr. E. I. Pilchard, MD; Dr. G. Poppensiek, NY; Dr. R. Reichard, TX; Dr. I. Ross Reid, Ottawa, ONT.; Dr. S. L. Reynolds, TX; Dr. E. C. Sharman, MD; Dr. A. W. Smith, OR; Dr. Paul Sutmoller, Brazil; Dr. Peter H. Timm, CA; Dr. S. T. Wilson, Jr., DC; Dr. T. M. Wilson, PA; Dr. John H. Wyss, FL; and Dr. R. J. Yedloutschnig, NY.

The Foreign Animal Diseases Committee met in the Texas C Room of the Hyatt Regency Hotel on Tuesday and Wednesday, afternoons, October 23 and 24, 1984, from 1:30 to 5:00 p.m. These meetings were attended by 115 persons, including members and guests. A total of 15 papers were presented at these meetings and abstracts of two of the research reports were submitted for inclusion in the Proceedings of this 88th Annual Meetings of the U. S. Animal Health Association.

During 1984 a new issue of the Foreign Animal Diseases "gray book" was published by the committee. We take this opportunity to express our infinite gratitude to the authors and reviewers who contributed unstintingly to produce this book. We have dedicated the book to the Bureau of Animal Industry and its succeeding organizations, whose devotion, fortitude, and foresight in protecting the health of America's livestock for the past century stand as brilliant monuments and have established standards in animal disease eradication unequalled by any other country in the world.

GLOBAL STATUS OF ANIMAL DISEASES EXOTIC TO THE UNITED STATES

INTRODUCTION

The status of animal diseases throughout the world was featured by the resurgence of rinderpest in several African countries. Foot and Mouth Disease outbreaks were reported in Holland and West Germany. The disease was reintroduced into Chile. Contagious bovine pleuropneumonia occurred in several countries of Africa and in France, Portugal, and Spain. There has been an apparent decrease in the incidence of African horse-sickness, Rift Valley Fever, lumpy skin disease, sheep and goat pox, dourine, glanders, African swine fever, and swine vesicular disease.

FOOT AND MOUTH DISEASE (FMD)

Holland reported three different but related outbreaks of FMD caused
by virus type O. Two outbreaks in West Germany, types A and O, were confirmed. Vaccination remains the control method of choice in Europe. In June, FMD virus type Asia-1 spread from Asia to Greece. The outbreak was apparently controlled by vaccination. Turkey confirmed the presence of types O and A-22 but denied the occurrence of Asia-1. Asia-1 virus was also reported from Israel and Lebanon, and it may be present in Syria. FMD was also reported from Malaysia, Hong Kong, Laos (Types O and Asia-1), Thailand, North Yemen (SAT-1) and Saudi Arabia (Type O). Despite the fact that Zimbabwe was the only country in Southern Africa that reported FMD outbreaks, the disease must be assumed to be present in other countries from which reports have not been transmitted. In the Western Hemisphere, the incidence of FMD was higher than that experienced the previous year in Argentina (types O and C), Colombia and Ecuador (type O). The disease was reintroduced into Chile in March of 1984 (type 0-1). A total of 7,399 animals were slaughtered (2,208 cattle, 1,146 sheep, 3,876 goats, and 169 pigs) and the carcasses were disposed of by burying and burning. Brazil, Peru, Venezuela, and Uruguay reported lower incidences of FMD.

RINDERPEST
A new campaign to control rinderpest through vaccination is scheduled to begin in Africa on December 1, 1984. The effort, financed mostly by European donors, hopefully will eliminate the immense economic losses caused by this disease. A large epidemic occurred in Nigeria, and reports of its occurrence have been documented in the Ivory Coast, Mali, Benin, and Mauritania. Occasional outbreaks occur in Israel. Recent evidence suggests that epizootics are occurring across the northern part of the African continent and may be spread by cattle destined for slaughter to Saudi Arabia and Kuwait. Veterinary Services has stationed a veterinary virologist at the Central Veterinary Laboratory in Mali who is currently working with this disease. Rinderpest is a notable example of a disease for which there exists an effective vaccine but funds and organized efforts to utilize it must be provided to eliminate it.

CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)
CBPP has been confirmed in Nigeria, Angola, Mali, and Namibia, but probably occurs in other countries which have failed to report it. Portugal had large outbreaks in the summer of 1983. France is combatting CBPP along its border with Spain, which reported a case in June of 1984.

LUMPY SKIN DISEASE
This disease, reported only from South Africa and Madagascar, apparently has decreased in incidence.

SHEEP AND GOAT POX
Israel reported this entity in April of 1984, but information of its occurrence in other countries was not documented.
DOURINE

This equine disease was reported only from South Africa, Namibia, and Italy.

GLANDERS

Turkey was the only country reporting glanders. Namibia, erroneously reported last year as having dourine within its borders, has not experienced this entity since 1926. Veterinary Services has recently dispatched a veterinarian to Turkey to obtain fresh material from field cases for the preparation of diagnostic reagents.

AFRICAN SWINE FEVER (ASF)

It is gratifying to report that ASF has apparently been eradicated from the Western Hemisphere, and no new cases have been recorded from Haiti. However, ASF is still present in Italy, Spain, and Portugal, and in Africa, still persists in Cameroon, Angola, and Zambia.

SWINE VESICULAR DISEASE (SVD)

Decreased incidence of SVD has been noted. Singular outbreaks were reported from France (November, 1983) and Italy (August, 1984). Disquieting serologic activity was detected in sentinel swine at a West German quarantine station processing zoological animals destined for export to the United States.

HOG CHOLERA (HC)

Hog cholera still prevails in most of the swine producing areas of the world, with West Germany reporting about 100 outbreaks per month. The United States remains free of this disease.

HEARTWATER

This disease, caused by the rickettsial organism Cowdria ruminantium, transmitted by ticks of the Genus Amblyoma, is widespread in Africa. In the Caribbean it has apparently been confined to Guadeloupe and Reunion, where the vector ticks are plentiful. Efforts to develop effective serologic diagnostic tests are ongoing at Plum Island.

VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE, VESICULAR STOMATITIS, AND AVIAN INFLUENZA

These entities are covered in detail in the following attached Insert #1 — Emergency Programs Progress Report.

A report dealing with vesicular disease research, prepared by Dr. P. D. McKercher of Plum Island Animal Disease Center is included for the Proceedings. (See attached Report — Insert #2).

Abstracts of two other scientific papers presented before the Foreign Animal Disease Committee are included for the Proceedings to be published later this year.

A discussion of "Research Objectives to improve U. S. Diagnostic Capabilities for Heartwater Disease," prepared by L. L. Logan, et al., was presented by Dr. Logan. (See attached Abstract — Insert #3).
Another interesting paper, authored by E. W. Jenney et al., was presented by Dr. Jenney. An abstract of the paper, entitled "Vesicular Stomatitis Outbreaks and Surveillance in the U.S.," is attached—Insert #4.

The Foreign Animal Disease Committee submitted three resolutions to the Executive Committee of USAHA.

The meetings adjourned at 5:15 p.m. on Wednesday, October 24, 1984.

EMERGENCY PROGRAMS PROGRESS REPORT

During fiscal year 1984 (October 1, 1983 through September 30, 1984), there were 167 investigations in the United States for foreign animal diseases besides those conducted because of the avian influenza in Pennsylvania, New Jersey, Maryland, and Virginia.

Vesicular stomatitis was diagnosed at many premises in the United States during 1982 and 1983, but for fiscal year 1984, only one case was diagnosed. It occurred on a Texas premises in roping horses used to work Mexican-source roping steers that had been imported on January 9 and moved to the ranch on January 12, 1984. Symptoms were confined to the horses, and positive serology to the calves.

Newcastle disease in pet birds as a result of smuggling, especially from Mexico, is a perennial spring and summer occurrence. Total costs related to the fiscal year 1984 outbreaks amounted to approximately $265,000. This year differed from past years in that young parrots moving through Texas caused problems. In past years, only those through California were incriminated in velogenic viscerotropic Newcastle disease (VVND) outbreaks.

Baby Amazon parrots illegally imported into California were found to be infected with VVND during the spring of 1984. This is the sixth consecutive year for this to occur. A mini-task force was activated in southern California to traceout 47 shipments made by a bird wholesaler. This wholesale operation was positive for VVND and had a common source of "yellow napes" with another California positive VVND case. Birds purchased from the former and moved to Utah were also positive.

Another California supplier shipped juvenile yellow napes to a wholesaler in Kansas City, Missouri. Exotic Newcastle virus was isolated from some of these yellow napes. Traceouts of sales from the wholesaler involved the following States: Iowa, Kansas, Oklahoma, Texas, Louisiana, and Nebraska. One additional positive case was confirmed in Enid, Oklahoma.

A wholesale dealer in Alabama that acquired a number of extremely young spectacled Amazon parrots had VVND diagnosed in his birds on May 30, 1984. Sales and contacts resulted in approximately 100 premises investigated in Alabama, 13 in Florida, and 30 in Georgia. Six additional VVND cases resulted from movements related to him and involved four cases in Alabama, one case in Georgia, and two cases in Florida.

One VVND case unrelated to the others was also diagnosed in Alabama.
on May 31, 1984, and six traceouts involving Alabama, Georgia, and North Carolina failed to detect additional infection.

Two individuals in Florida split a shipment of 10 baby yellow napes from California. One hundred birds on the two Florida premises were depopulated when VVND was diagnosed from these yellow napes. No additional spread occurred.

Another outbreak in Florida resulted from purchases of baby Amazon parrots in the Brownsville, Texas, area and moved to Florida by the owner. No sales were made from these premises and no additional cases occurred. The exact source for the birds could not be detected.

In early May through June 1984, Newcastle was found in pigeons from several lofts in and around the New York City area. Mortality was reported to be as high as 90 percent in some lofts. Eight investigations in New York, three in New Jersey, and one in Vermont (son of an owner in New York) were made in this period. It is believed that possibly 65–75 lofts were affected. Also, Belgium pigeon Newcastle vaccine labeled bottles were found. Newcastle virus isolated from the bottles and the investigations was characterized as lentogenic. In the first study no inoculated chickens sickened or were killed by the virus. To study the isolated virus, four pigeons units were inoculated at the National Veterinary Services Laboratories (NVSL), Ames, Iowa for each of eight infected pigeon lofts and the vaccine bottle isolations. No clinical symptoms were demonstrated by the test pigeons for the vaccine isolate. For the eight loft isolates, there were five units that had dead birds, many with central nervous system (CNS) signs. Study continues on the virus.

On November 9, 1983, a Federal Extraordinary Emergency was declared for portions of Pennsylvania because a highly pathogenic form of avian influenza virus (H5N2) had developed. The Northern Regional Emergency Animal Disease Eradication Organization (READEO) was activated on November 4, 1983, and was located at Lancaster, Pennsylvania. The virus continued to spread and three other States, including New Jersey, Maryland, and Virginia, became involved. New Jersey had their only positive flock depopulated on November 24, 1983, and Maryland had their last of three positive flocks depopulated on January 25, 1984. The Shenandoah Valley portion of Virginia was quarantined on January 27, 1984, and was released on September 14, 1984. The original Pennsylvania area was expanded after the original November 9, 1983 quarantine because of disease spread. However, by June 8, 1984, the quarantine area west of the Susquehanna River could be lifted. The rest (eastern portion) qualified for release on October 4, 1984, except for seven premises that although depopulated had not been cleaned and disinfected (C&D) and/or had not concluded their required 30-day down time following C&D.

The program at first was directed at the highly pathogenic form of the virus. Soon it was found necessary, especially because of the mixture of the high and low pathogenic forms found, to depopulate any flocks infected with H5N2 virus. Later flocks with antibodies were depopulated because
of the concern that even though virus could not be isolated the potential for harboring dormant virus existed. Of interest is that Virginia poultry were found infected with only the low pathogenic form of the virus.

STATISTICAL INFORMATION FOR AVIAN INFLUENZA

Pennsylvania (includes New Jersey and Maryland): A total of 652 premises had sick call investigations. Two hundred and eighty-nine positive flocks, thirty-nine previously infected low-path flocks, and fifty-one seropositive flocks were declared and depopulated. Thirty-eight premises were self-depopulated without indemnity. The last virus isolation was from a flock depopulated March 31, 1984.

Classification of birds depopulated:

<table>
<thead>
<tr>
<th></th>
<th>Layers</th>
<th>Broilers</th>
<th>Breeders</th>
<th>Turkeys</th>
<th>Other</th>
<th>Total</th>
<th>Flocks</th>
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<tr>
<td>Low Path</td>
<td>7,388,287</td>
<td>3,745,661</td>
<td>246,892</td>
<td>83,602</td>
<td>36,330</td>
<td>11,500,772</td>
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<td>Seropositive</td>
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<td>839</td>
<td>12,370</td>
<td>156</td>
<td>2,460</td>
<td>2,031,909</td>
<td>51</td>
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</tbody>
</table>

Indemnity: $35,856,874

Virginia: One hundred and eighty-nine premises had sick call investigations, with 65 premises declared positive and depopulated. Four seropositive flocks were declared and depopulated. In addition, the State and industry depopulated four premises prior to activation of the task force on January 26, 1984. The last virus was isolated from a flock depopulated on July 1, 1984.

Classification of birds depopulated:

<table>
<thead>
<tr>
<th></th>
<th>Layers</th>
<th>Broilers</th>
<th>Breeders</th>
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<th>Other</th>
<th>Total</th>
<th>Flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Path</td>
<td>36,244</td>
<td>214,863</td>
<td>141,409</td>
<td>856,748</td>
<td>0</td>
<td>1,249,264</td>
<td>65</td>
</tr>
<tr>
<td>Seropositive</td>
<td>2,850</td>
<td>0</td>
<td>10,601</td>
<td>5,937</td>
<td>4</td>
<td>19,392</td>
<td>4</td>
</tr>
</tbody>
</table>

Indemnity: $5,784,418

Surveillance activity will continue for 6 months following quarantine release for each of Virginia and the two areas of Pennsylvania.

OTHER INVESTIGATIONS AND ACTIVITIES

On March 23, 1984, five black rhinos from South Africa arrived at New York. On May 23, 1984, 20 male Amblyomma hebraeum Koch, the bont tick, were found on postmortem for one of the animals at a Texas ranch. The other two rhinos on the ranch were scratched disclosing three more male ticks. No ticks were found on the other two rhinos at another ranch. Tick drags, sentinel cattle, and additional scratchings have not disclosed additional ticks. Surveillance is continuing.

One foreign animal disease diagnostician course was conducted during the year. With the addition of 14 newly trained diagnosticians, we now have available in the United States a total of 266 veterinarians trained to investigate foreign animal diseases.
REPORT OF THE COMMITTEE

The separation of the diagnostic section from the Agricultural Research Service at the Plum Island Animal Disease Center is now complete. The diagnostic laboratory is now administratively under Veterinary Services. Dr. D. W. Luchsinger heads the laboratory which is referred to as the Foreign Animal Disease Diagnostic Laboratory (FADDL).

The five Regional Emergency Animal Disease Eradication Organizations (READEO) are fully staffed and remain ready to respond rapidly to outbreaks of emergency diseases. Two of the teams have been on active duty for most of this year. The Southeastern READEO was in Virginia and the Northern READEO was in Pennsylvania for avian influenza. The Western READEO team acted on a mini-task force in the spring on the southern California traceout for Newcastle in pet birds. All READEO teams contributed heavily to the two more active ones during the year. As a result, a test exercise was not held this year nor will one be needed this coming year. Over 42 percent of Veterinary Services personnel served at least one tour of duty with either the Pennsylvania or Virginia task force. Two, three, or even four 30-day tours were not unusual.

The Technical Support Staff of Emergency Programs now has approximately 53,000 articles on foreign animal diseases, covering 38 diseases and entomological items in the data bank. The data bank is fast becoming recognized worldwide for its coverage. Information is routinely exchanged with several countries.

RESEARCH ON VESICULAR DISEASES

Presented by Dr. P. D. McKercher

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combination resulted in protection of 4 of 6 cattle when their immunity was challenged (16).

REFERENCES


The objectives of heartwater research at the Plum Island Animal Disease Center (PIADC) are to study the clinical aspects of *Cowdria ruminantium* in goats and mice, to develop methods to produce *Cowdria* antigen and to evaluate diagnostic techniques for *Cowdria*. Two mouse adapted strains of heartwater were received from South Africa in infected *Amblyomma hebraeum* ticks during 1983–84. A Caribbean strain from Guadeloupe was received in frozen goat blood in December 1983. Recently a *Cowdria* isolate was made at the PIADC from adult *A. variegatum* ticks received from the Central Veterinary Laboratory in Bamako, Mali. Techniques were developed to allow ticks to infect goats with heartwater. The clinical course of the disease produced by each of these four strains is being evaluated in both goats and mice. Preliminary trials indicate that the first three strains are antigenically different. An animal immune to one strain is not protected against a challenge with either of the other two strains. This antigenic variation could have significant importance when attempting to develop a diagnostic test or a vaccine.

A bank of sera from goats and mice recovered from first and second challenges and cross challenge studies has been established for future serological studies. Evaluation of the efficacy of fluorescent antibody or indirect fluorescent antibody tests for heartwater using mouse peritoneal macrophages as antigen is in the preliminary stages.

Several bovine and murine cell lines have been selected for attempted *in vitro* propagation of *Cowdria*. Pilot trials to grow *Cowdria in vitro* are in progress.

Colonies of *A. hebraeum*, *A. variegatum* and *A. maculatum* are being maintained for transmission studies and for developing means of detecting *Cowdria* infections in ticks.

Abstract from the Paper Entitled

"Vesicular Stomatitis Outbreaks and Surveillance in the United States"

Presented by E. W. Jenney et al.

**SUMMARY**

Following a period of relative quiescence, New Jersey (NJ) type vesicular stomatitis (VS) was diagnosed in 673 herds in a 1982–1983 outbreak. The outbreak spread northward from Arizona and New Mexico to the Canadian border in the Rocky Mountain states and laterally due to livestock movements. The outbreak gradually disappeared during the winter of 1982 and spring of 1983. Only three cases were diagnosed by serologic tests during the summer of 1983, one in Colorado and two in Wyoming. In January 1984, NJ VS was detected in rodeo horses and Mexican roping cattle in Milam and Jim Wells counties in Texas.

In addition to testing conducted as a result of the outbreak of VS,
serological surveillance of domestic animals, feral swine, deer, and other wildlife for evidence of VS infection has been done in various parts of the United States. Antibodies were detected in feral swine, horses, cattle, deer and raccoons. For the first time, VS virus was isolated from feral swine on Ossabaw Island, GA, in 1983 during a joint in-depth epizootiological study.
ACUTE EQUINE DIARRHEA SYNDROME
(A.E.D.S.) – A SITUATION REPORT


INTRODUCTION

Acute Equine Diarrhea Syndrome (AEDS) is also known as Potomac Fever, Potomac Horse Fever and Equine Monocytic Erlichiosis. This is a preliminary report on a newly reported disease entity seen in Maryland, Virginia and Pennsylvania. The summer of 1984 was the sixth year that AEDS has been recognized in Maryland with the majority of cases located in Montgomery County. Since the veterinary profession is still "pioneering" with AEDS many important questions remain unanswered.

Definition

An acute disease entity of horses characterized by loss of appetite, depression, fever, leukopenia, ileus or explosive diarrhea, dehydration, laminitis (20–30% among cases) and terminal shock. The authors have circumstantiated evidence this is a clinically discernable “specific” disease of horses and ponies with a spectrum of clinical signs. The severity of clinical signs varies to transient fever, depression without diarrhea to hypovolemic shock, diarrhea and death.

Causative Agent

As of this writing the causative agent has not been identified; but preliminary work (electron photomicrographs of erlichia within macrophages in the wall of the large colon of a pony experimentally infected with blood from field cases of A.E.D.S. [Perry–Virginia Tech–VMRCVM] and blood from an A.E.D.S. affected horse [Jenny–USDA–N.V.S.L.] was placed in a pony at the University of Illinois and erlichia was present in tissue culture inoculated with the harvested blood from this pony. Then this tissue culture material was injected into a second pony which showed signs of A.E.D.S. and its blood revealed an erlichial agent, [C. Holland & M. Ristic]), indicates that a priorly unidentified erlichial parasite may be a possible causative agent.1,2 Several viruses have been isolated from AEDS cases; however, none have been shown to be the causative agent of this

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**Presenter
disease entity. Early an adeno-virus was photographed, but this virus grew poorly in tissue culture (Dutta). A corona-like virus was isolated but when placed in ponies did not cause illness. Although not isolated, the agent is likely present in whole blood. The disease can be transmitted from natural cases to normal horses and ponies using whole blood. The incubation period between time of inoculation and development of fever and leukopenia varies from 8 to 15 days based on limited studies.

Clinical Signs

A typical case of the disease would be as follows: History reported by the owner, “This horse was a little slow to clean up his grain,” at the next feeding the horse would be “completely off feed” with fever in the range of 103–104.5°F being usual. Auscultation over the paralumbar fossa reveals few borborygmal sounds, suggesting ileus early in the disease process, and after 24–48 hours, just prior to the onset of diarrhea, higher pitched tinkling sounds, suggestive of excessive fluid and gas accumulation. These typical fluid sounds and occasional high pitched “pings” are especially prominent over the cecum. Fecal consistency may be normal at the initial veterinary examination, but the patient often develops diarrhea within the following 24–48 hours. Diarrhea is a common occurrence but does not occur in all cases. The diarrhea can be profuse, “pipe stream” in character with a duration of 24 hours to 10 days. In some horses the diarrhea may be less profuse and transient, in others no diarrhea occurs. Colic of variable severity (mild abdominal pain or crampiness to severe intense pain resembling a “surgical” colic) occurs in a small proportion of horses. Most horses that have colic also develop moderately severe diarrhea and seem to have a higher incidence of laminitis than other affected horses.

Laminitis occurs in 20–30% of the cases, the onset usually occurs within 3 days of the initial diarrhea. Laminitis has been observed simultaneously with the initial diarrhea and in certain cases (in July 1983) laminitis was evident before diarrhea. Rotation of the 3rd phalanx occurs, and in rare instances sloughing of the hoof may result. In one extreme case the hooves of both rear feet were “shed” after the onset of severe colic, followed by a profuse diarrhea. Often the horse has to be euthanized due to the consequences of laminitis. Usually the diarrhea has abated by the time the horse is euthanized due to laminitis. Anderson noted only three cases with laminitis that survived (avoiding death or euthanasia); they involved months of difficulty for the horse and owner. In the 60–70% of the horses that survive Potomac Fever, recovery is usually complete, with the horse returning to its former performance level.

Clinical Pathology

Notable laboratory findings nearly always include a very depressed white blood count (2–5,000 WBC/ul), and PCV’s from 40% to 65% or higher, depending on the severity of the diarrhea. The plasma protein concentration is often elevated early in the disease process due to dehydration and hemoconcentration and in severely affected horses decrease
to lower than normal concentrations as the diarrhea resolves. Fecal analysis has been negative for flagellates, and, more notably, for *Salmonella* species.

**Epidemiological Observations**

Anecdotal history in Maryland revealed in 1979, 1980 and 1981 that most cases were seen within a mile or two of the Potomac River in a geographical strip approximately six miles long. In the summer of 1982 closer observations disclosed that early cases (May and June) were “along the river” but as the summer progressed cases were seen in the uplands of Montgomery County, Maryland. Several cases were also recorded in adjacent Maryland counties, namely Frederick and Howard, up to a distance approximately 20 miles from the Potomac River. Cases were reported as follows: June 11, July 23, August 53, September 21, October 5. Among these 113 cases, 28 died or were euthanized. They occurred on 51 premises. In the summer of 1983 116 cases of AEDS were reported, among these 42 died or were euthanized for humane reasons. These cases occurred on 75 farms in Maryland. With exception of one case each in Carroll, Frederick and Howard counties, all cases have been in Montgomery County. Cases reported in 1983 were: May 1, June 8, July 67, August 25, September 11, October 2 and November 2. Again, geographically in Montgomery County many cases were located along the Potomac River and as the summer progressed, cases were reported in areas away from the river. In the summer of 1984 in Maryland 97 cases (as of October 10) were reported (principally in Montgomery County) with 4 deaths; also 12 horses were euthanized due to an unfavorable prognosis. In the 1984 summer season Virginia reported 6 cases in Fairfax, Loudoun and Frederick Counties with one death. Pastures on several Maryland affected premises were inspected for poisonous plants. Several kinds of poisonous plants were present; however, it was evident that such plants were not being eaten by horses. All ages and breeds of horses appear to be susceptible to this disease. One herd of 8 horses in Montgomery County, Maryland having one newly introduced horse clinically ill in 1984 was tested by the experimental indirect fluorescent antibody test. It revealed all herd members as positive except one yearling. One week later this yearling was clinically ill with Potomac Horse Fever and 3 weeks later reacted positively against *erlichia sennetsu* antigen. Generally the attack rate for AEDS in a herd is lower (5–7%). During the summer of 1984 work has been done to characterize blood feeding, anthropods on A.E.D.S. affected and non affected farms in Montgomery County. The objective of this work is to define the vector aspects of this seasonal disease.

**An Outline of Pathologic Findings**

**Gross:** Heart: Severe petechial and ecchymotic hemorrhages: In fat at heart base and along the coronary vessels; on the endocardium and adventitial surfaces of ascending and thoracic aorta.

**Lungs:** Congested, occasionally consolidated.
Liver: Mottled, slightly swollen.

Gastrointestinal Tract: Stomach Ulceration of mucosal surface in the glandular and pyloric regions.

   Duodenum: Regional patchy areas of congestion.

   Small Intestine: Fluid contents with slight excess mucus.

Cecum, anterior and mid-colon: Focal areas of congestion – watery contents.

Other lesions: Vesicles on oral mucosa – most often on the upper lip (recorded in 3 cases).

Microscopic

   Necrotizing entero-colitis: Most severe in the cecal and colonic segments.

   Breakdown of the vascular system: Vasculitis of smaller blood vessels, both veins and arteries.

   Disseminated intravascular coagulation: Microscopic thrombi in blood vessels, especially in cecal and colonic regions.

Fluid accumulation in the lungs: Occasional pneumonia.

Hemorrhage and disruption of the normal architectural arrangements in the liver.

Lymphoidal depletion within the spleen and lymph nodes.

Differential Diagnosis

A. Since salmonella species reside in the gastrointestinal tract of many horses, salmonellosis must be ruled out. Indeed despite diligent and repetitive culturing the great majority were negative for salmonella. The leukopenia and the hypoproteinemia as a sequela occurs in both diseases.6

B. Certain colic conditions need to be differentiated from AEDS, the history or presence of fever (102–105°F), response to medication to relieve pain (most colics respond better than AEDS to medication); rectal palpation may help differentiate. The Packed Cell Volume will be the same in the two conditions while the white blood cell count will be depressed in AEDS and usually not in colic.7

C. Both Colitis X and AEDS have clinical signs similar to endotoxemia and it is difficult to differentiate the two entities. Colitis X appears to be more fulminating and there is usually a history of stress to the horse, whereas this is not the case for AEDS horses.
Treatment

Many combinations of therapy have been tried since no one therapeutic modality has been demonstrated the best. Correction of the dehydration by fluid replacement is always indicated and the amount needed is based on laboratory and clinical evaluations. Non-steroidal anti-inflammatory drugs such as flunixin meglumine and phenylbutazone are often used at various stages of the disease. A variety of antibiotics designed to control gram-negative bacteremia have been used, but no consistent results seem to be noted even with the use of massive amounts of gentocin. In some cases, large amounts of Pepto Bismol® or Kaopectate® have been given by stomach tube, but their true value in the control of the diarrhea has not been established. The judicious use of steroids, and even atropine, has been tried; again clinical reports are conflicting. Some practitioners, including Anderson, feel that low dose heparin therapy (30-40,000 units I.M. QID) helps combat Disseminated Intravascular Coagulation (DIC). Free choice water with NaCl, KCl and NaHCO₃ added is indicated. A number of horses will consume large quantities of the electrolyte water when they will not drink fresh water.

Tetracycline therapy against erlichiosis in dogs is specific therapy. In horses the side effects of therapeutic levels of tetracyclines are so severe that many clinicians are reticent to use this pharmaceutical.

The treatment can be very costly, with veterinary bills quickly running into thousands of dollars of those patients that do not respond quickly.

One of the many frustrating aspects of the disease is the inability to accurately predict the outcome. Some cases that seem mild never recover, and others with more alarming initial signs recover nicely. As mentioned earlier, and signs of laminitis are reason for grave concern.

Summary

1. This is a preliminary report on AEDS, a newly recognized specific disease syndrome of horses.
2. The causative agent and methods of transmission are not completely established as of this report (even though a new erlichial parasite has been provisionally implicated).
3. So far this disease has only been reported in the summer and fall in the states of Maryland, New Jersey, Pennsylvania and Virginia, but is likely to occur throughout the country.
4. Field investigation and research work need to be pursued to better define AEDS.

Acknowledgements

The principal scientific investigations have been a cooperative effort by: Equine Practitioners (EP) in Montgomery County, Maryland; The Maryland Department of Agriculture (MDA), Animal Health Section; The University of Pennsylvania (New Bolton Center); the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM); and the U.S. De-
department of Agriculture (USDA) National Veterinary Services Laboratory. The authors thank the following scientists for their efforts in these investigations: E. Gard, E. Gaughan, H. Holbrook, R. Scullin, P. Radue, J. Magurn, H. Larson, J. Sessions (EP); J. Huang, E. Sacchi, S. Joseph (MDA); J. Palmer (U of PA); S. Dutta, F. Troutt (VMRCVM); E. Schmidtmann and A. Jenny (USDA).

BIBLIOGRAPHY


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. C. L. Campbell, Florida
Vice Chairman: Dr. Ralph C. Knowles, Delaware

Dr. J. B. Anderson, TN; Dr. Jesus Casteneda Garcia, Venezuela; Dr. George C. Cilley, NH; Dr. W. W. Clark, TX; Dr. Leroy Coggins, NC; Dr. P. M. Epple, MD; Dr. G. B. Estes, VA; Dr. C. A. Gipson, MD; Dr. R. C. Goulding, CA; Dr. R. I. Hail, KY; Dr. John B. Healy, CA; Dr. Floyd Jones, FL; Dr. M. J. Keman, NY; Gen. Wayne O. Kester, CO; Mr. Michael Nolan, D.C.; Dr. S. R. Nusbaum, NJ; Ms. M. A. Owen, MA; Dr. William E. Pace, FL; Dr. Linda Schlater, IA, Mr. John Smiley, ME; Dr. M. B. Teigland, FL; Dr. C. D. Vail, CO; Dr. Thomas E. Walton, CO.

The Committee on Infectious Diseases of Horses convened in Fort Worth, Texas, on October 22, 1984, with some 40 members and visitors in attendance. A quorum present. Numerous items of interest to the equine industry were discussed.


In 1983, there 931 cases submitted to NVSL of which 19 were positive for EEE. Eastern equine encephalitis was diagnosed in Arkansas, Indiana, Louisiana, Mississippi, Michigan, Rhode Island and Tennessee. There were 77 positive WEE cases from Arkansas, Idaho, Illinois, Iowa, Kansas, Minnesota, Montana, Nebraska, North Dakota, Oklahoma, South Dakota, Wisconsin and Wyoming.

In 1984, there were only 5 positive WEE cases of which 1 was confirmed and the others were presumptive. There were 5 positive EEE cases of which 2 were confirmed. To obtain additional information on incidence, Dr. Theodore Tsai of the Centers for Disease Control, Ft. Collins, Colorado; Dr. Sidney Nusbaum, New Jersey; and Dr. Harvey Rubin, Kissimee, Florida, were contacted. There were outbreaks of EEE reported in New Jersey and Florida, but very few WEE cases. Most of these cases were presumptive; however, 15 of the 20 New Jersey cases were confirmed.

In 1983, 67 horses in 24 states had antibody against VEE, and 26 horses in 16 states were positive in 1984. Many of the horses had stable antibody titers or low CF and HI antibody titers which would indicate that the VEE exposure was not recent. Some of the horses also had histories of VEE vaccination; however, in some cases there was no apparent explanation for the VEE antibody titers.
In conclusion, there was an epizootic of EEE in 1983 and scattered WEE cases. In 1984, there were the fewest cases of WEE since we started testing. There were scattered EEE cases with a severe outbreak in New Jersey and Florida. Antibody was detected against VEE in a few horses, but there was no evidence of recent infection with the virulent subtype.

Dr. Chester Gipson reported on the incidence of equine infectious anemia in the United States during FY 1984, the individual state breakdown being reflected in attached Exhibit 1. It can be noted that of the 709,923 equidae tested throughout the nation in this period 2,711 were positive — an incidence rate of .38 of 1%.

Dr. Gipson also reported that Veterinary Services was in the final stages of revising Form 10-11, the single horse EIA test chart which has been previously recommended by this committee and approved by the Association. It should be noted that when these test charts become available to the several states, the multiple horse test charts now in use should be recalled.

Discussion ensued relative to withdrawal of approval of laboratories currently certified to conduct AGID tests throughout the country. It was reported that some laboratories may be manufacturing their own antigen, rather than procuring approved antigen from licensed manufacturers. It was agreed that Drs. Pearson and Gipson would coordinate their efforts with VS Biological Licensing on a priority basis to resolve this problem.

Last year’s report endorsed a proposed protocol designed to determine if equidae with complement fixation (CF) titers of 4+ in 1:10 or less dilution for Babesia equi after treatment with Imizole® are capable of transmitting piroplasmosis. The project was implemented and, although, it is not yet complete, Dr. Gipson reported that several conclusions had been reached. One of the significant observations is that the organism does not appear to be as highly pathogenic as it had been thought to be. Another involved demonstrating the degree of organism circulation in the blood stream, heretofore thought to be minimal. The project is being continued and we expect to bring you up-to-date on its progress next year.

On the subject of contagious equine metritis, Mr. Mike Nolan reported that he has been visiting numerous veterinarians, horse owners and veterinary colleges throughout the United States relative to the feasibility of amending CEM import rules, particularly as they relate to mares, hoping that the practice of performing clitoral sinusectomies might be fully accomplished within this country — thus negating the inconvenience to owners of having the operation performed prior to debarkation. His observations and discussions have led to the conclusion that changing those procedures is not a viable option; therefore, it is recommended that no amendments to the CEM import rules be made at this time.

Drs. Jack Pitcher, Jim Smith and Robert Hail provided the Committee a detailed account of this year’s episode of equine viral arteritis experienced in some thirty-seven Kentucky thoroughbred horse farms. The majority of the spread of EVA was demonstrated to be through breeding sheds with exposed mares returning to home farms and, in turn, exposing their
cohorts. Equine viral arteritis appears currently to be confined to the State of Kentucky and now seems to be relegated to a quiescent stage; however, in considering the future of EVA, the following points should be remembered:

1– EVA is and has been for at least 30 years, endemic in the United States.
2– In most states, the disease is not reportable;
3– Much more knowledge about the disease is needed;
4– A commercial modified live virus vaccine may be available in 1985;
5– The import bans applied by the Tri-Partite European countries are applicable to all breeds of horses in the United States.

A resolution with reference to further research on equine viral arteritis will be proposed to the general assembly on Friday.

A request was presented to the Committee that it recommend to APHIS a change in the Code of Federal Regulations to allow equine infectious anemia reactors to move in interstate commerce through one horse assembly point for sale to immediate slaughter, providing that the animals have been properly identified and permitted prior to said movement. Following extended discussion, the Committee concurred in the request, referring the matter to Veterinary Services for refinement, if approved by the Executive Committee.

We were privileged to hear a presentation on the subject of Potomac Horse Fever by Dr. Robert Whitlock of the University of Pennsylvania. The observations presented parallel those findings disclosed in the paper which has just been presented by Dr. Knowles.
EQUINE INFECTIOUS ANEMIA
AGID TESTS REPORTED FY 1984

Exhibit 1

Total positive 2,711
Total negative 709,923

*States with pending EIA reports
EXPANDING AND MAINTAINING LIVESTOCK EXPORTS

by John K. Atwell, D.V.M.
Deputy Administrator, Veterinary Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Presented before the U.S. Animal Health Association
Fort Worth, Texas; October 23, 1984

INTRODUCTION

If I were to ask, I am sure this audience would give an overwhelmingly favorable response to the question of the need for "Maintaining and Expanding Our Livestock Export Markets." Like prosperity, it's something everyone is in favor of. And just like prosperity, it's something that is not all that easy to achieve.

But, the record of U.S. livestock exports shows pretty clearly that this can be a "sometime thing." Cattle exports from fiscal year 1980 through fiscal year 1983 show increases of roughly 100 percent of two successive years followed by a drop of more than two-thirds. Similar wide year-to-year variations occur for exports of horses, sheep and goats, and swine.

### EXPORTED ANIMALS

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<td>66,900</td>
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(Source: USAHA Annual Reports)

However much these figures may vary from year to year, livestock exports must still be reckoned as a significant item in the import-export picture. Agriculture exports have stood for a number of years as one of the bright spots in our international balance of payments. Exports of agricultural commodities currently more than double the value of agricultural commodity imports — $39 billion compared to $15.3 billion. Exports of animals and animal products exceeded imports by 14.3 percent. While imports of live animals, excluding poultry, currently exceed exports, we have an outstanding potential that awaits development. Our exports of poultry, if we include poultry products, are one of agriculture's great success stories.
VALUE OF EXPORTS AND IMPORTS BY PRINCIPAL GROUPS,
FISCAL YEAR 1982
(Selected Entries)

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<th>Exports (1,000 dollars)</th>
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<td>Animals and Animal Products $ 4,072,542</td>
<td>$ 3,552,193</td>
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<tr>
<td>Live Animals, Excluding Poultry $ 246,665</td>
<td>$ 404,461</td>
</tr>
<tr>
<td>and Cattle $ 60,270</td>
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<tr>
<td>Poultry and Poultry Products $ 578,967</td>
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International economic conditions certainly affect our imports and exports, but we can't do a great deal about the economic situation. The fact remains, however, that we are not doing as much as we can in areas that we can control. These are areas, which, if fully exploited, would present a considerably better export picture.

The purpose of these remarks is to discuss why we have not yet made the most of our export potential, and what we can do (and are doing) to improve it.

THE POSITIVE SIDE

Regardless of our international balance-of-payments, the first thing we need to recognize is that the United States has a good thing going in its livestock exports. There are at least five good reasons for promoting our exports.

1. The first reason is to make money. That's the basic incentive. We can and do make money when we export quality livestock.

2. The second reason is that we have a surplus available for marketing abroad, especially in the dairy industry. We have an adequate supply of cattle to sell to the world livestock market.

3. Third, U.S. livestock have become an important genetic pool for many parts of the world. Our poultry and dairy industries in particular have gained this recognition.

4. Fourth, the United States has superior breeding, the "super-genetics," desired by much of the world. This is true of swine, beef cattle, and horses.

5. Through our animal health programs and the health management of most herds and flocks, our livestock and poultry enjoy a health status that is superior to most of the world.

In short, when importers look to the United States, they are looking for superior genetics and freedom from diseases.

ADDRESSING THE PROBLEMS

With all these advantages, why aren't we doing better?
There are some problems, some negative aspects, that keep us from realizing our full potential. They come in several categories.

1. First, there are worldwide economic conditions. They affect the ability of foreign importers to buy our livestock. In most cases, there is not very much we can do about those influences.

2. Second, we are affected by international competition. If we are to compete effectively in the international marketplace, we must address the way we do business, the way we negotiate. And we must examine our dealings with our competitors.

3. Third, in the past we have been hindered by problems concerning the health and quality of the livestock we export. Some foreign health restrictions have adversely affected our livestock exports while we, ourselves, have experienced problems in our own export health certification procedures. These are areas where we can work for improvements.

4. And finally, we have suffered in the world marketplace because of weakness in the way we do business.

Having highlighted these problems, I would like to review them in greater detail and perhaps suggest possibilities for improvement.

WORLDWIDE ECONOMIC CONDITIONS

Worldwide economic conditions include such variables as the strength of the dollar on the world market, the international debt of different countries, inflation, and endemic poverty in various regions and nations.

Most of us rejoice in a strong dollar. It’s great for travelers to other nations. It gets us a better price on many foreign goods. And it is usually regarded as a good sign for our national economy.

But turn the situation around. Put yourself in the position of the person trying to sell American exports abroad; it’s a different ball game. When foreign currencies buy less at U.S. prices, we sell less, and that affects our livestock sales, as well as industrial products and other commodities. The demand may still be there. The ability to pay is not.

The same thing happens when a foreign nation is overburdened by a potentially disastrous international debt. Remedies are needed, and they include austere economic restrictions, which limit all but essential foreign purchases. Galloping inflation is usually a companion to debt-ridden economies, and without hard currencies foreign buyers can buy very little.

And finally, there are large areas of oppressive poverty in the world. Overpopulation, inadequate resource development, or the abuse and exhaustion of resources too often blunt attempts to build up a nation’s livestock industry. In such countries, improved livestock and poultry industries might offer one ray of hope for the future were the potential for development available. And improved industries might well look to the United States for genetic stock.

Our response?
There is very little that we in the animal health business can do about world economics. We can hope for the stabilization of the world and regional economies. That will depend on international policies, financing, and economic aid.

To a limited extent, APHIS personnel have been privileged to work in international aid programs for the improvement of livestock industries in less developed regions. We participate in such broad government programs as the Caribbean Basin Initiative. These help. And international cooperation for the eradication of diseases and pests can strengthen livestock industries in less developed nations, which eventually may result in a demand for good U.S. breeding stock. Screwworm eradication in Mexico and the eradication of African swine fever in Haiti and the Dominican Republic must be counted as long-range benefits for those countries, as well as for the United States.

We can encourage such programs. However, the overall solution to world economic problems lies mainly beyond our immediate efforts.

So let's move on to areas in which we are more directly involved.

COMPETITION

In our efforts to expand and maintain [our] livestock exports, we must recognize the competition. In any commercial venture worthy of the effort, there is going to be competition. And that usually works out for the better. It assures top performance.

In the international livestock export market, we have some topnotch competition. It begins with some outstanding genetic stock in a number of countries, especially Canada, Denmark, The Netherlands, and West Germany.

What makes this competition a little tougher to deal with is the strong government-industry relationship that exists in a number of countries. Through government regulations, these nations control the export operation much more closely. They assure that only qualified organizations are recognized to conduct export business. And often as not, they provide government support and assistance. In countries such as Canada or The Netherlands, for example, the government is much more directly involved in export sales than in the United States.

Coupled with strong government involvement, many livestock exporting nations have adopted a consistent, sustained export policy. They have made a long-term commitment to develop and serve the export market. This means assurance of quality animals consistently; it means maintaining close liaison with both suppliers and customers; it means facilitation of export operations; and it means not being deterred by the ups and downs of the market, either at home or in the international sector.

And yet another tough nut for us to crack is artificial barriers. There are a variety of regulations imposed by other nations, or by regional economic organizations, that restrict imports. Some may be presented with bona fide reasons as to why restrictions are necessary; others may be
patently economic in nature. The end result, in any case, is a limitation on U.S. entry into those markets.

Our response?

We in the United States need not be alarmed by foreign livestock with top genetic qualities. We too have top quality, and our breeders are continually improving the bloodlines. And without apologies, we seek to obtain new genetic stock from abroad through our import programs. To quote the song from the musical "Annie Get Your Gun," "Anything you can do, I can do better."

However, if we are to meet the competition on an equal footing, we must get organized. In a political and economic climate of deregulation, it may not be practical to regulate the export industry in the same way that other nations apply so effectively. Yet the government and industry must approach exports with a united policy that assures quality and blocks out those who would peddle runts and culls. Since it is the industry that does the exporting, and industry that ultimately will be regulated, we look to industry to suggest policies and regulations to achieve this end.

On our part, with the help of the State Department and the USDA’s Foreign Agricultural Service, we will continue to negotiate against barriers and assure equitable treatment in the international marketplace.

HEALTH REGULATIONS, LIVESTOCK QUALITY

Problems relating to the import health regulations imposed by other countries are of immediate concern to APHIS, Veterinary Services. We must also overcome the negative impacts that result when our exported livestock fail to meet the quality expected by our customers. These problems can be summarized briefly.

As I indicated, we have experienced problems when export shipments failed to meet the standards of quality that the foreign importers had every right to expect. This is at variance from our usual performance, so it is a matter of special concern.

Closely related to this is the performance of accredited veterinarians. They fulfill their responsibilities by examining, testing, and certifying the health of exported animals. Their certification is assurance that exported animals meet both USDA export health requirements and the import requirements of the receiving nation. APHIS veterinary officials endorse those certifications to complete the export procedure. This procedure has worked well over the years, but on some unfortunate occasions it broke down; the veterinarians did not meet their responsibilities. Our export markets suffered serious damage.

We need to ensure that all U.S. Origin Export Health Certificates are completed accurately and legibly. Basically the certificate is a good, concise document; but when it is not completed legibly and clearly, foreign veterinary officials have had difficulty understanding it.

APHIS, Veterinary Services officials have negotiated with foreign governments the tests and certifications required for different categories
of livestock, poultry, and semen. Usually, individual country requirements are reasonable and do not present extreme demands on the U.S. exporter, the accredited veterinarian, or the diagnostic laboratory. However, on some occasions, we have found ourselves with conditions that were all but impossible to live with.

Foreign country import requirements, when they present stringent demands, can sometimes be traced back to the stringent demands that we have required of other nations. We have no intention of relaxing what we regard as necessary import health requirements; however, they can be mirrored in the requirements that we must meet for other countries.

We also must pay special attention to health requirements that specify that a state, an area, or the herd of origin be certified free of a particular disease. This is no problem with respect to many diseases, but such requirements have not been addressed in the case of others. For example, bovine leukosis is a matter of concern to some nations, and they have established bovine-leukosis-free areas. This gives them a talking point, a competitive edge, and possibly a negative impact on us at some later date.

Beyond these particular concerns, we should recognize that APHIS, Veterinary Services, and the industry have not worked as closely as we should to monitor trends in export markets and in the health requirements that are imposed.

Our response?

The starting point for addressing these problems is in the procedure for selecting and certifying exported livestock. If we work together to assure quality exports, and if we make sure that export health certifications are performed consistently in good faith and with attention to every detail, we may find fewer roadblocks put in our way by veterinary officials in other countries.

From our part in APHIS, Veterinary Services, we are closely monitoring all export health certifications, and we are checking to be sure that the general health condition of the animals is evaluated in light of the stress of shipment. We want to certify livestock “healthy” in the fullest sense of the word.

To reinforce our supervision of export health certification, we are strengthening compliance activity. This means investigating all suspected violations of animal health regulations. Where we find wrongdoing or neglect, we initiate legal or disciplinary action.

A more positive approach to livestock certification is the re-evaluation, now underway, of the Veterinary Accreditation Program. In cooperation with the schools of veterinary medicine, we have analyzed the tasks required of accredited veterinarians, [we have] conducted a workshop to develop training requirements, and [have] organized a task force to recommend new standards whereby veterinary practitioners establish and maintain accreditation. We have not yet determined what changes may be made in the accreditation program; however, the result will be better and more professional adherence to health certification requirements.
Beyond these direct actions on our part, we will continue to negotiate for more equitable import health requirements from foreign governments. The People's Republic of China is just one example of where we have made considerable progress in developing workable requirements. Successful negotiations depend, of course, on equitable relationships between trading partners.

Unilateral actions on the part of APHIS are not the final answer. APHIS and the livestock export industry must develop better communications to unite their efforts vis-a-vis foreign governments. We will be looking to industry for their commitment to a long-term, united export program.

THE WAY WE DO BUSINESS

We can deplore international economic conditions that hinder our export market development. We can analyze our competition and try to deal with it. We can take a variety of steps to meet health requirements and provide greater quality assurance. In the end, however, we are talking about business. How business functions has an awful lot to do with its success or failure.

I am not a businessman, so perhaps I am out of my territory on this subject. But I am an observer whose agency deals with some of the factors that affect our livestock export business. I have alluded to some of those factors in my discussion about the competition. Some other factors crop up when we discuss assurance of livestock quality and negotiations with U.S. trading partners.

So it is time to talk about business.

The American free enterprize system, with all of its advantages, competes at a disadvantage against some countries that use a close government-industry relationship as a marketing tool. With our antitrust philosophy, it is highly unlikely that we will adopt such a policy. We are not likely to see quasigovernmental corporations mobilizing our export program under a centralized authority.

However, even without a centralized export operation, American exporters have often displayed another weakness. This is a tendency to treat livestock exports as a source of income from surplus animals, rather than as a long-term commitment to market a quality product and to service an established and expanding market. If we are to compete effectively, we must look at exports not as individual shipments, but as a sustained marketing effort.

Frankly, we in government must also acknowledge that perhaps we could be more involved in market development. Even without the close government-industry relationship that exists in some other countries, opportunities exist for us to work together more closely.

Our APHIS responsibilities for the health and quality of livestock put us in a natural working relationship with livestock producers and exporters. While this does not authorize us to engage in market development, we can work informally with the industry in support of a united industry effort.
Naturally we will seek continued support from other agencies such as the Foreign Agricultural Service and the Office of International Cooperation and Development.

This brings us to what industry can do. As an observer, frankly, I get the impression of an industry that is not well organized, whose efforts are diverse and fragmented. The lack of a united effort brings with it a lack of self-regulation. Free market operations do not always assure the quality control necessary for market development.

Our response?

The response to this diverse and fragmented business must begin with a closer working relationship between industry and the government. Even within the free enterprise system, there are things government can do to support market development. We can administer animal health programs that assure improved livestock quality and guarantee uniform and reliable export health certifications. We can negotiate equitable health requirements wherever our livestock are shipped.

And the industry can help by pulling together and imposing discipline and self-regulation. We can respond to foreign competition by developing specific disease-free herds, states, or regions when international market requirements make such programs advisable. Most of all we need industry-wide commitments to the concept of sustained marketing.

CONCLUSION

What are we going to do?

Many of the economic problems affecting livestock exports are beyond the capabilities of USDA, andAPHIS in particular. Industry cannot be expected to tackle worldwide economic problems. There are many problems that no government in the world can overcome.

But we can start from where we are. APHIS can address animal health programs that will improve the demand for livestock exports. We can negotiate more aggressively with foreign governments. We can assure consistent quality for our foreign customers through our export health certification. We can make sure that accredited veterinarians consistently perform with the highest professional standards.

The industry can make a commitment to sustained market development. Exporters can work toward development of a more cohesive organization. They can support animal health programs that place us in the most advantageous marketing position. Most of all, we need industry support and suggestions for shutting out the fast-buck artist and the man who pawns off culls. Industry can set standards of quality and seek ways to maintain them.

But most significantly, industry and government can strengthen the lines of communication. A united front for market development will put us on a common footing with many of our more aggressive and effective competitors.

Toward the end, APHIS has been meeting with a broad spectrum of
representatives of the livestock export industry. Three such meetings have been held. We are reviewing the Veterinary Accreditation Program to see what is needed to assure the highest possible performance. We are strengthening enforcement of animal health regulations to make sure all livestock movements — interstate and international — are in compliance.

I am happy to see that the livestock industry has taken the initiative to organize a joint USDA-industry Livestock Export Task Force that will address all of the issues I have discussed.

We have a good thing going in our superior U.S. livestock. We have superior breeding, an outstanding genetic pool to meet world needs. And we have an abundant supply. All we need now is a united and sustained commitment for the development of a worldwide market for American livestock and poultry.
MAINTAINING AND EXPANDING OUR LIVESTOCK EXPORT MARKET

Maurice E. Mix, Executive Assistant, International Affairs
Holstein Association—Brattleboro, VT

It is a distinct privilege to speak at the General Session of this convention.

Dr. Atwell and I have been asked to present papers on the topic, “Maintaining and Expanding Our Livestock Export Market.” I will endeavor to present the views of the livestock industry as seen by the Holstein Association, the world’s largest purebred dairy cattle organization.

The focus of my remarks will be attuned to the dairy export industry, my comments, I hope, will reflect the view of most breeders and exporters whether their primary interest is in dairy—beef—or swine.

My lifetime experience has been in the production, promotion, and sale of dairy cattle genetics (cattle—semen—embryos), and addressing animal health concerns that relate to the export of U.S. dairy genetics. I have been blessed with both the opportunity and privilege of traveling many times in every state of the union plus 53 foreign countries.

No one at Holstein (nor any other breed organization) would profess to have the expertise or knowledge to undertake and supervise the vast neverending task of animal disease research, testing, quarantine and disease control. These tasks are assigned to specific agencies represented in this room. APHIS, for instance, is the agency of USDA whose mission in part is to protect U.S. plant and animal resources and certify U.S. agricultural export products in accordance with foreign government requirements. Holstein, on the other hand, represents 44,000 Registered Holstein members in every state who are in the business of breeding Registered Holstein cattle. Holsteins comprise the overwhelming majority of the dairy cattle going for export, and the bulls from whom over $25 million of Holstein semen will be exported in 1984, and the majority of donors from whom fresh and frozen embryos are exported. No breed of dairy cattle in the world has reached such high standards of productivity—nor is there a breed or strain of dairy cattle in greater demand overseas, than the U.S. Holstein. Holstein is a Cooperator with the Foreign Agricultural Service. You can readily see why Holstein is so vitally concerned about expanding and maintaining the dairy cattle export market and reducing or eliminating some of the constraints in our path, whether they be political—financial—or animal health related.

There is no statement I could make which more accurately depicts the role of American agriculture in the world than to say that the world and U.S. agriculture are becoming increasingly interdependent. American agriculture can no longer live without the export market—this is inescapable and irreversible. Nor can American dairy farmers or breed as-
sociations (like Holstein) stand aside as silent partners as efforts are made to expand world markets for Holstein genetics.

The present administration has strongly indicated their commitment to do everything possible to develop-promote-support-expand exports of U.S. agricultural products, including livestock. Organizations such as APHIS, and breed association like Holstein, are in key positions to influence that commitment, either positively or negatively.

History over the past 8 years records a sharp decline in the number of U.S. dairy cattle (for breeding purposes) exported to other countries. Holstein transfer records indicate only 3,829 Registered Holsteins exported in 1983 as compared to 29,052 from West Germany; 14,932 from Canada, and Holland 9,557. It is important to understand that this is happening at a time when the U.S. Registered Holstein—the most sought after breed of dairy cattle in the world—frequently is severely limited or unable to meet health specs of even some developing countries who may have little real knowledge of which diseases exist in their country, but know very well from our veterinary journals every disease that exists within our boundaries and what tests our laboratories can perform. What are the major reasons for the decrease in U.S. exports? Here are but a few—

1. Trends in animal health export regulations which are becoming increasingly complicated, excessive, expensive, and in many instances, frequently being used as non-tariff trade barriers.
2. The high cost of the U.S. dollar in relation to other foreign currencies.
3. Severe competition from other countries using U.S. Holstein semen to upgrade native Friesian cattle, and selling 50% or 75% U.S. Holstein blood at a cut-rate price.
4. Substantial subsidies provided by E.C. governments to their dairy farmers—low interest—delayed repayment provisions or interest free loans available to customer countries.
5. Lack of a competitive credit program in the U.S. for potential foreign customers.

So as to focus on the topic to which I have been assigned, I wish to address the first constraint—the trend in animal health export regulations.

We wholeheartedly agree that it is an appropriate role and function of APHIS to certify compliance of animals in the process of being exported with health specifications set forth in a sales/purchase contract. It is equally appropriate for USDA-APHIS to enter into an understanding with foreign governments that such certification will be performed as a service to the exporter—the importer—and to the government of the country making the importation.

Beyond the above commitment between governments, Holstein seriously questions the advisability of APHIS entering into specific animal health agreements on a bilateral basis. Animal health agreements, like non-health specifications, are generally negotiable between buyer and seller, and in the final Letter of Credit, are seldom like the original tender
or the published import health requirements of a country at the time of the first sales contract.

Holstein exporters object to situations like the one which occurred on June 1, 1983 when an animal health export document was signed with the People's Republic of China, knowing very well that the health regulations specified in that document were totally unrealistic. They do not accept the explanation that it was appropriate to have some kind of agreement, rather than to have none. If the reason is a valid one, then why do we not have an agreement with the U.K. who also have issued unrealistic import demands? It is important that input into animal export agreements combine the scientific and political decision-makers and also the exporter and source of supply. Major attention must be paid to the practicality and the economics of fulfilling animal health requirements before signatures go on a document.

We feel the Livestock Division of the Foreign Agricultural Service should be the depository and primary source of import health specifications for each country which makes such information available. Attaches should keep these reference files up-to-date. From these FAS files, potential exporters to a given country could obtain the stated health specifications prior to sales efforts or known market interests. That is when negotiations on import health requirements might start and when APHIS and interested exporters could join hands in an effort to establish a realistic, workable set of health specifications with the potential importer and his government. Such a service to one exporter or organization would be service to all. Basically, this is a matter of backup and commercial support. The Canadians have been operating very satisfactorily in such a fashion for years.

Within APHIS, we believe that it would be most effective to have a central or single authoritative point for the interpretation of foreign health specifications and that interpretations should be consistent with those of other countries competing for the same markets. This would also include centralized authority and interpretation over points of embarkation where final health certificates are issued.

It follows that our domestic regulatory and disease control programs must be expanded to establish herd free status certification for diseases like BLV and Bluetongue which high potential import countries are demanding with increasing frequency. Uniform test results are absolutely essential in building confidence in a health program. APHIS must supervise and coordinate the activities of each licensed laboratory so as to assure prompt service, accurate testing procedures, and results that are highly repeatable both in the U.S. and in importing countries.

Finally, the Administration's agriculture export philosophy as well as policy must be translated more realistically into positive attitudes and even more positive action toward the promotion, facilitating and expediting of exports . . . in this case, livestock and related germ plasm. It's a matter of finding ways by which things can get done, not reasons why they
can't be done. In the case of APHIS, we need their help to remove animal health road-blocks, (often very real non-tariff barriers) rather than helping to set them in place.

Holstein has made their position very clear with respect to both Agricultural Market Development (USDA-FAS) and Animal Plant Health Services (APHIS). We have challenged our Congressmen to give full support to the efforts of the Foreign Agricultural Service/USDA in its cooperative foreign market development programs through favorable congressional support of this important and necessary joint effort of government and the private sector.

With respect to APHIS, Holstein wishes to go on record and voice their appreciation for the assistance given exporters in all phases of their work, especially that related to export marketing, while at the same time urging that consideration be continually given to the real need for expanding the capability and improving procedures for handling required animal health tests and the quarantine of animals for export. Special emphasis must be given to those diseases considered of major significance in international trade, i.e., Leukosis, Bluetongue, etc.

Further, that APHIS and responsible administrators within USDA establish high priorities on the allocation of funds and personnel to well-documented major animal health problems before initiating new rules and procedures for matters not now covered, and which do not at this time, present animal health problems of major consequence, domestically or internationally.

We ask that a special effort be put forth within APHIS to establish a positive attitude and understanding at all levels towards exports, the negotiations of export health requirements with foreign government counterparts, and the coordination of such efforts with the Foreign Agricultural Service and appropriate private sector organizations.

In closing, let me say that we are very concerned about the ability of the livestock export industry in this country to compete with far more aggressive, opportunistic, export oriented countries. We have an ample resource of the most sought after dairy cattle germ plasm in the world. We have one of the cleanest animal health records to be found anywhere on the globe. Let's unite and get this message to our friends overseas and take a more positive approach to exporting our livestock genetics.
REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: Clint Booth, Texas
Vice Chairman: Dan Childs, Florida

J. N. Armstrong, NV; W. A. Bailey, MD; C. T. Barns, Jr., VA; J. L. Blair, VA; R. B. Caffey, MD; Glenn Conatser, IN; Jack Dahl, ND; R. L. Evinger, ID; W. H. Fales, MO; D. J. Gilhooley, HI; R. C. Goulding, CA; W. B. Grene, FL; Frank Harding, IL; W. C. Hare, Canada; Rube Harrington, Jr., IA; D. E. Herrick, MD; Michele Howard, CA; R. C. Knowles, MD; M. L. Main, SD; Bob Mathis, AZ; John McVicar, NY; M. E. Mix, VT; M. J. Nolan, DC; Brent Perry, TX; D. A. Price, CO; William Prichard, OR; G. B. Rea, OR; Charles Reid, FL; T. D. Rich, MO; J. D. Roswurm, CA; R. H. Rumler, VT; S. V. Timberlake, Jr., NY; J. S. Walker, LI, NY; H. A. Waters, VA; C. R. Weston, NH; Walker Wilson, TX; G. O. Winegar, MD; R. J. Yedloutschnig, NY.

Dr. R. B. Caffey reviewed the past year's activity in Plant Protection and Quarantine and Dr. D. E. Herrick assisted by Dr. G. O. Winegar reviewed the past year's activity by the Import-Export Program of Veterinary Services. Their reports are attached as Appendix 1.

Dr. H. A. Waters presented the report of the export subcommittee which is attached as Appendix 2.

Mr. Shelby V. Timberlake, Jr., presented the report of the Embryo Movement Subcommittee which was newly formed this year. The report is included as Appendix 3.

Mr. Tom Cook presented a report of the Livestock Export Task Force which is attached as Appendix 4.

Dr. D. E. Herrick discussed the interim report of September 14, 1984, the Veterinary Services zoological animal committee. The interim report suggested possible additional measures that APHIS could establish to prevent the introduction of exotic diseases and ectoparasites with the importation of a wider range of zoological species than are now regulated.

Dr. J. W. Holcombe spoke in support of measures to control the introduction of exotic diseases and ectoparasites through the importation of zoological animals.

Dr. Joe Bearden discussed the resolution to be presented to the Committee on Infectious Disease of Cattle relative to importation of bovine semen. The resolution would request USDA to change the import requirements of semen to meet CSS and state health standards. The resolution was supported by the Import-Export Committee.

Dr. N. L. Meyer of the Foreign Animal Disease Committee presented that committee's resolution relative to importation of zoo animals which strongly supports the principles expressed in the Veterinary Services Interim Report of September 14, 1984. The Import-Export Committee voted unanimously to support that resolution.
REPORT OF THE COMMITTEE

The committee passed the following resolutions:

1. User Fees — which urges that the costs of export testing be borne by the exporter.

2. Embryo transfer — which urges support of increased research on embryo transfer and formation of a committee to set research priorities.

3. Embryo regulations — which urges USDA to request OIE to delay issuance of rigid embryo transfer standards and to sponsor an international conference to exchange information on embryo transfer.

Dr. D. E. Herrick announced that a proposed rulemaking to amend USDA regulations for the importation of animal embryos was published for public comment in the Federal Register. If there is no serious public opposition, animal embryos would be permitted importation from countries free of foot and mouth disease and other serious animal diseases exotic to the United States.

The committee adjourned at 5:30 p.m.

APHIS REPORT
TO THE
IMPORT-EXPORT COMMITTEE OF THE USAHA

Import Animals

The regulations governing the importation of stallions from countries where contagious equine metritis (CEM) exists have been modified. We now require that all procedures relating to the treatment and specimen collection from the stallion and test mares be monitored by a State-employed veterinarian. Test mares must now be permanently identified with a tattoo and can only be used once as a test animal for an imported stallion. Stallions can now be cultured for CEM prior to treatment and specimens can be sent to approved State laboratories rather than the previous requirement that all specimens be sent to the National Veterinary Services Laboratories in Ames, Iowa. The eleven States approved to receive stallions from CEM countries are California, Colorado, Kentucky, Louisiana, Maryland, New York, North Carolina, Ohio, South Carolina, Tennessee, and Virginia.

The eight States approved to receive mares from CEM countries are California, Colorado, Kentucky, Louisiana, Maryland, New York, South Carolina, Tennessee, and Virginia. Mares received without clitoral sinusectomies or with incomplete clitoral sinusectomies will now be referred directly to the New York State College of Veterinary Medicine, Cornell University, Ithaca, New York or the University of California Veterinary College, Davis, California, for corrective surgery.

A total of 291 horses were imported for the 1984 Olympic Games in Los Angeles, California. The horses were monitored by Veterinary Services while on the Olympic grounds at the Santa Anita Racetrack.

Negotiations are still underway with the People's Republic of China
(PRC) to finalize a protocol for the importation of swine semen from PRC. Alternative protocols on the importation of swine from PRC have also been developed. These protocols are designed to maintain adequate biosecurity on swine imported from PRC and will utilize the Harry S. Truman Animal Import Center (HSTAIC) to a greater extent than normal. None of these alternative protocols have been presented to the authorities in PRC.

Due to the introduction of foot-and-mouth disease into Chile in an area remote from their isolation facility and because alpacas and llamas for export to the United States were already in embarkation quarantine, special permission was given to import this consignment through HSTAIC. A total of 333 alpacas and llamas was imported into HSTAIC in August. These animals are expected to be released in November to allow sufficient time to clean and disinfect the facilities before the arrival of the European consignment.

A total of 91 head of cattle from Europe are expected to arrive from the quarantine station in Brest, France, in mid-December for a 90-day quarantine at HSTAIC. These cattle are from Italy, France, Germany, Switzerland, and the United Kingdom. The majority of the animals originated in France.

The previous European shipment of 228 animals was released from HSTAIC in March 1984. This was the fifth importation of cattle into HSTAIC. A summation of all importations through September 30, 1984, is given below (this is an estimation pending final reports).

<table>
<thead>
<tr>
<th>Animals Imported</th>
<th>FY 1982</th>
<th>FY 1983</th>
<th>FY 1984</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>795,435</td>
<td>1,224,076</td>
<td>908,128</td>
</tr>
<tr>
<td>Swine</td>
<td>224,004</td>
<td>416,224</td>
<td>964,391</td>
</tr>
<tr>
<td>Horses</td>
<td>32,398</td>
<td>36,232</td>
<td>32,007</td>
</tr>
<tr>
<td>Sheep</td>
<td>8,968</td>
<td>9,980</td>
<td>2,043</td>
</tr>
<tr>
<td>Others</td>
<td>15,061</td>
<td>3,494</td>
<td>5,945</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1,075,866</strong></td>
<td><strong>1,690,006</strong></td>
<td><strong>1,912,514</strong></td>
</tr>
</tbody>
</table>

| Cattle (Canadian border ports)         | 475,193   | 457,550    | 340,609   |
| Cattle (Air & Ocean ports)             | 171       | 150        | 178       |
| Cattle (Mexican border ports)          | 320,071   | 766,376    | 567,341   |
| **TOTAL**                              | **795,435** | **1,224,076** | **908,128** |

| Swine (Canadian border ports)          | 223,533   | 416,056    | 964,340   |
| Swine (Air & Ocean ports)              | 471       | 168        | 51        |
| **TOTAL**                              | **224,004** | **416,224**    | **964,391** |

| Horses (Canadian border ports)         | 25,840    | 27,227     | 23,644    |
| Horses (Air & Ocean border ports)       | 4,317     | 4,536      | 4,939     |
| Horses (Mexican border ports)           | 2,241     | 4,469      | 3,424     |
| **TOTAL**                              | **32,398** | **36,232**    | **32,007** |
AVIAN IMPORT ACTIVITIES

A. Commercial Bird Program

1. There were seven infected velogenic viscerotropic Newcastle disease (VVND) and velogenic Newcastle disease (VND) infected commercial bird lots from six countries. Those countries were Bolivia, El Salvador, Honduras, Indonesia, Taiwan, and Tanzania. These were put down or returned to the country of origin. There was no VND or VVND introduced into the United States with legally imported birds, poultry, or hatching eggs during FY 84.

2. A regulation was finalized which allows five existing commercial bird quarantine stations to move from Detroit, Michigan, and Seattle, Washington, to Miami Florida; (These cities will be closed to commercial bird entries after these stations relocate.) Two additional quarantine stations were approved for Miami, Florida, and Brownsville, Texas, under this same regulation. The bird station operators for these cities were drawn from 123 applicants for the Miami opening, and 68 applicants for the Brownsville opening. This indicates considerable interest by the public to own bird quarantine stations.

3. The minimum space reservation fee for quarantine of pet and commercial birds and poultry in USDA animal import centers was increased from $40 to $80 and time limitation on refunds by regulation change. A proposed regulation has been drafted to further increase space reservation fees based on amount of space reserved.

4. Approximately 300 penquins and other Antarctic birds were hatched under USDA quarantine supervision at Sea World, San Diego, California. Sea World Research Institute is establishing a self-perpetuating colony of “high” Antarctic penguins and other bird species. This is a special research project.

B. Pet Bird Program

1. There were two VVND infected pet bird lots originating in Mexico and the Philippines that were refused entry and destroyed.

2. Though a few pet bird owners had complaints about inconvenience, cost, and some deaths of their birds; generally, the program is operating smoothly.

C. Smuggled Bird Program

1. Plans have been approved to include construction of a permanent smuggled bird quarantine facility in the new Customs border inspection station at Otay Mesa, California. According to conversations with the U.S. Department of the Interior, Fish and Wildlife Service, and U.S. Customs, smuggled bird seizures are down because of budget restrictions and personnel ceilings. (Because of less surveillance, our smuggled bird facilities are not being fully utilized.)

D. Poultry and Hatching Eggs Program

1. USDA required quarantine of hatching eggs from Ireland because of
an avian influenza (AI) outbreak in their National flock of chickens and turkeys. Blood tests of brooding ducks and poults for AI will be conducted while in Veterinary Services (VS) quarantine.

2. An import regulation has been initiated this year requiring pullorum-typhoid disease testing of captive gamebird hatching egg flocks of Canadian origin. Canadian veterinary officials have requested that USDA require gamebird hatching egg importations from Canada to have pullorum-typhoid-free certification. The Canadian flock(s) of origin must be tested negative or be on an approved government program.

3. A regulation was initiated which increases import restrictions of hatching egg, breaking egg, and table egg certification from countries infected with VVND, VND, AI, adenovirus 127 (egg drop syndrome), and pullorum-typhoid disease. A VS Memorandum was written requiring pasteurization processing of breaking eggs from Ireland and all countries infected with AI, VVND, VND, and other serious economic diseases of poultry.

Export Animals

The Export Animals Staff spent substantial time this fiscal year in negotiating with those countries which placed a total or partial ban on importation of U.S. poultry or poultry products due to lethal avian influenza (AI) outbreaks in this country.

Canada was the first country to lift their remaining prohibition on U.S. poultry after the last Federal quarantine was removed. Argentina, Peru, India, and Venezuela, are the only countries which had a total ban on poultry and poultry products from the United States. Taiwan will not accept poultry or poultry products from a State where AI has occurred during the past 12 months. Some countries banned poultry and poultry products from the State of California even though it was not affected with lethal AI and was not under Federal quarantine.

England, France, Ireland, and Italy, banned importation of U.S. horses for at least 2 months due to an outbreak of equine viral arteritis (EVA) in the State of Kentucky. The ban was released on July 16, 1984, but a serologic test for EVA is required. For horses originating from Kentucky, a 30-day period outside the State must be completed prior to exportation.

In the Fiscal Year 1983–84, only one isolation of vesicular stomatitis (VS) was made in the United States. The single case of VS occurred in the State of Texas. Taiwan does not accept livestock from States where VS has occurred during the 12 months prior to exportation and Brazil does not accept cattle or swine from like areas.

A new health agreement was signed with the People's Republic of China (PRC) in May 1984 for exportation of cattle, swine, and poultry from the United States. A shipment of cattle is being prepared from the States of Wisconsin, Minnesota, and Iowa. According to Chinese Protocol, testing can begin November 1, 1984, in the 18 North Central and Northeastern
States which have a low incidence of bluetongue disease. A draft of the import health requirements of the PRC for bovine semen from the U.S. was received in September 1984. Comments on these requirements are being solicited from the semen industry prior to responding to the PRC.

No bovine semen was shipped to Great Britain in the 1983–84 collection season due to conditions in the import health requirements. Revisions in the requirements for the 1984–85 collection season have been made. Great Britain will now recognize a “designated accredited veterinarian” for supervision of an AI center. The requirements still require extensive testing and procedural monitoring. Three AI centers are planning to collect semen in the 1984–85 season for shipment to Great Britain.

A livestock export committee (task force) was formed during this fiscal year to work on export problems and to improve the export image of the United States. The committee is composed of members from the livestock industry, Foreign Agricultural Service, and Veterinary Services. The committee has met three times; in Washington, in Hyattsville, and in Chicago, and plans to meet in Washington in December 1984.

The Korean market is still open to the U.S. livestock industry. A sudden increase in livestock export to Korea during the fourth quarter of this fiscal year has been noticed. Korea has revised their import health requirements and a serological test for leukosis or a certification statement on herd history for the past 3 years is now necessary. This requirement will have a serious impact on selection procedures for the Korean market.

During this Fiscal Year 1983–84, several new memoranda of the 592 series, VS notices, and policy memorandums were sent to the field relative to the requirements of importing countries.

In Fiscal Year 1983–84, cattle, sheep, and goats were shipped to Australia directly from the United States. Previously, livestock was exported by way of Canada where a quarantine period was served.

An agreement was signed by the Government of Egypt for the import health requirements for cattle from the United States.

A final agreement was reached with Japan for exportation of bovine semen from the United States. The United States will be the first country to ship bovine semen to Japan on a commercial basis.

The exportation of bovine embryos is increasing substantially each year. The majority of the embryos exported this Fiscal Year were exported to France, Canada, Hungary, Netherlands, and Venezuela. Several other South American countries also imported U.S. embryos.

Due to the irregularities in export certifications by an accredited veterinarian that occurred in Fiscal Year 1982–83, exports of cattle to Korea were drastically reduced this fiscal year. Increases in exports of cattle to Mexico in FY 83–84 have occurred. Two large shipments of dairy cattle to Tunisia occurred in 1983–84 which is a new market for U.S. cattle.

All livestock, live poultry, and hatching eggs offered for export from the United States were inspected for evidence of communicable disease, proper
health certification to meet regulatory requirements, and compliance with Veterinary Services health agreements with the importing country. The following tables summarize the livestock and poultry health certificates endorsed for exportation from the United States for the past 2 fiscal years.

**Year Ending September 30, 1983**

<table>
<thead>
<tr>
<th></th>
<th>Canada</th>
<th>Mexico</th>
<th>Other</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>16,206</td>
<td>7,215</td>
<td>33,692</td>
<td>57,113</td>
</tr>
<tr>
<td>Horses</td>
<td>33,576</td>
<td>1,487</td>
<td>3,008</td>
<td>38,071</td>
</tr>
<tr>
<td>Sheep/Goats</td>
<td>36,810</td>
<td>146,572</td>
<td>3,485</td>
<td>186,867</td>
</tr>
<tr>
<td>Swine</td>
<td>692</td>
<td>3,315</td>
<td>34,813</td>
<td>38,820</td>
</tr>
<tr>
<td><strong>Total Livestock</strong></td>
<td>87,284</td>
<td>158,589</td>
<td>74,998</td>
<td>320,871</td>
</tr>
<tr>
<td>Poultry</td>
<td></td>
<td></td>
<td></td>
<td>32,901,304</td>
</tr>
<tr>
<td>Hatching eggs (doz.)</td>
<td></td>
<td></td>
<td></td>
<td>15,722,320</td>
</tr>
<tr>
<td>Semen</td>
<td></td>
<td></td>
<td>22.7 million units</td>
<td></td>
</tr>
<tr>
<td>Embryos</td>
<td></td>
<td></td>
<td>2,365 (estimated)</td>
<td></td>
</tr>
</tbody>
</table>

**Year Ending September 30, 1984**

<table>
<thead>
<tr>
<th></th>
<th>Canada</th>
<th>Mexico</th>
<th>Other</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>15,729</td>
<td>22,921</td>
<td>20,569</td>
<td>59,219</td>
</tr>
<tr>
<td>Horses**</td>
<td>8,495</td>
<td>614</td>
<td>2,407</td>
<td>11,516</td>
</tr>
<tr>
<td>Sheep/Goats</td>
<td>42,478</td>
<td>249,576</td>
<td>1,271</td>
<td>293,325</td>
</tr>
<tr>
<td>Swine</td>
<td>81</td>
<td>1,371</td>
<td>12,163</td>
<td>13,615</td>
</tr>
<tr>
<td><strong>Total Livestock</strong></td>
<td>66,783</td>
<td>274,482</td>
<td>36,410</td>
<td>377,675</td>
</tr>
<tr>
<td>Poultry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Baby chicks</td>
<td>11,587,180</td>
<td>810,371</td>
<td>13,370,440</td>
<td>25,767,991</td>
</tr>
<tr>
<td>(b) Turkey poults</td>
<td>760,902</td>
<td>14,424</td>
<td>282,644</td>
<td>1,057,970</td>
</tr>
<tr>
<td>(c) Other poultry</td>
<td>2,877,251</td>
<td>28,065</td>
<td>140,949</td>
<td>3,046,265</td>
</tr>
<tr>
<td><strong>Total Poultry</strong></td>
<td>15,225,333</td>
<td>852,860</td>
<td>13,794,033</td>
<td>29,872,226</td>
</tr>
<tr>
<td>Hatching eggs (doz.)</td>
<td></td>
<td></td>
<td>13,535,587</td>
<td></td>
</tr>
<tr>
<td>Bull Semen</td>
<td>1,119,457</td>
<td>929,626</td>
<td>23,478,893</td>
<td>25,527,967</td>
</tr>
<tr>
<td>(dollar value)</td>
<td>(dollar value)</td>
<td>(dollar value)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos</td>
<td></td>
<td></td>
<td>5,685 (estimated)</td>
<td></td>
</tr>
</tbody>
</table>

**ANIMAL PRODUCTS**

**Undercooked Argentine Beef** — Veterinary Services (VS) was notified on December 24, 1983, that three shipments of cooked frozen beef from Argentina (Establishments 1383 and 1930) were found not thoroughly cooked. This was discovered during port of arrival examination in New Orleans, LA. None of the products had been distributed in retail commerce. Based on these findings, it was determined that three production codes from Establishment 1383 and 7 production codes from Establishment 1930 were undercooked. Both shipments were rejected and returned to Argentina. Veterinary Services took the position that no further shipments from these plants would be considered until an official report was received on the cause of undercooking. It also would be required to
show that remedial action was taken before new shipments from that plant would be considered.

**Change in Disease Status for Denmark, France and Dominican Republic** — Denmark was removed from the list of countries designated as being affected with FMD on January 16, 1984. The Department recognized France as being free of African swine fever on June 13, 1984. On March 12, 1984, the Dominican Republic was declared free of both African swine fever and hog cholera.

**Parma Hams from Italy** — The Department has received numerous requests to allow the importation of Parma hams from Italy. Research studies using swine vesicular disease virus indicated that the process used to produce Parma hams inactivates the virus. Current studies are now underway to determine the effect of the processing on FMD, hog cholera and African swine fever viruses. If current studies prove that FMD, hog cholera and African swine fever viruses are inactivated during the processing of Parma hams, Veterinary Services will amend current regulations to provide for the entry of these products into the United States. Regulations will have to provide sufficient time period for processing the hams to ensure that all four viruses are inactivated prior to shipment to the United States.

**CURRENT STATUS OF PASSENGER BAGGAGE INSPECTION**

Animal and Plant Health Inspection Service commitment to protect American agriculture and the ever-increasing number of travelers has necessitated the testing and implementation of new methods of expeditiously detecting prohibited agricultural items in baggage and still maintain effectiveness.

**X-ray Imaging of Baggage** — The use of X-rays to detect agricultural contraband in baggage has proven highly successful at predeparture clearance sites in Puerto Rico and Hawaii. Interceptions have increased significantly with less manpower. Tests in Hawaii have shown that a flow rate of 800–1,200 pieces of baggage per hour can be maintained with less manpower than is required now. With a remote control system, it could be operated by one or two persons during off peak periods. Stationing PPQ Officers at X-rays used by airport security has also proven to be highly productive. Officers have been able to identify a large percentage of the images correctly. This will increase as officers gain experience in image recognition.

It is anticipated that in the near future all predeparture clearances will be facilitated with X-ray systems. The use of X-rays in foreign arrival areas is not presently being planned but no doubt will be considered after more experience is gained.

**Detector Dogs** — For the past 3 years, PPQ has used trained detector dogs to identify foreign mail packages containing meat products, plants, and fruit. The program has been highly successful. In addition, PPQ has begun using passively trained dogs in the airport environment at Los
Angeles and San Francisco, California, in order to detect prohibited materials carried in passenger baggage. These dogs are smaller breeds, well trained in obedience, and will not alert aggressively on luggage containing agricultural contraband.

Public reaction to having a dog in their midst has been favorable. Also, over 90 percent of the alerts have resulted in seizures! Dogs have alerted on meat, cream, a saddle, oranges, apples, bananas, a wide variety of other fruits, and even a trash can where someone had dumped fruit to avoid inspection. Each time they alert and the handler feels it will be positive, they are rewarded with a treat. They can be given about 45–50 treats per day. It is hoped that this program will expand to other international airports. Some have shown reluctance in initiating this until favorable reaction is attained and has proven successful at the two pilot airports.

**Carbon Dioxide (CO₂) Detectors** — Several gas detection devices have been tested. CO₂ was chosen because of the large amounts discharged by fruits and plants long after harvest. The prototype sensing units developed by the Agricultural Research Service were successful in detecting fresh fruits, plants, and vegetables in baggage but to a lesser degree in detecting meats. Problems have arisen in the use of this type of sensing unit. It is larger and cumbersome; a negative reading does not necessarily mean there is nothing; those fruits wrapped in plastic, etc., trapping the CO₂ will not give a reading; positive readings could be fruit but also dirty clothes; a continual fresh air supply is needed resulting in pipes through ceilings; and a lesser degree of accuracy (70 percent expected but most feel it is less).

**“Red Door/Green Door”** — The purpose of this passenger inspection system is to facilitate the processing of travelers through the Federal Inspection Service (FIS) inspection requirements without negatively impacting on enforcement needs of the Agencies. PPQ’s role in this system is as follows: Officers using profiling techniques monitor the baggage claim areas screening passengers who are likely to be bringing agricultural items into the U.S. Depending on the physical layout of the facility, this may be done before or after the passengers have gone through primary U.S. Customs Service (Customs)/U.S. Immigration and Naturalization Service (INS) inspections. PPQ Officers also provide coverage at agricultural secondary counters and are available for Customs secondary counters when items of agricultural interest are found. To date, enforcement has improved. The real challenge facing PPQ Officers is to identify more travelers who are carrying contraband. Further profile refining and acquired expertise will improve this process. PPQ has recently developed a profiling videotape addressing the four sections of the world and is using this as an aide for new officers and for Customs and INS officer training.

**Civil Penalties** — The purpose of enforcing inspection with civil penalty assessments where justified is for deterrence purposes, not punishment. Merely seizing the prohibited items to dispose of the pest risk
only irritates the traveler; and so, they are often more than willing to take the risk again. Hopefully, the civil penalty system will get travelers’ attention when they realize that one hidden piece of fruit or meat could cost them $50 or even more. The system went nationwide in March 1984; and to date, 12,078 penalties have been assessed and $341,845 collected. Civil penalties for baggage violations will continue to be a prominent strategy to gain compliance with quarantine regulations.

It appears that no one system will be the answer, but use of one or a combination of the new technologies addressing certain variable situations will enhance future detection capabilities and enable us to continue our prevention of pest entry into the United States.

FISCAL YEAR 1984 REPORT OF ANIMAL PRODUCTS
IMPORTED/EXPORTED
(DOES NOT INCLUDE MONTH OF SEPTEMBER)

Vessel and aircraft arrivals

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel and aircraft</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arrivals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40,719 vessels boarded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,356 lots consisting of 1,099,560 kilograms of garbage were removed from these vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,686 garbage handling discrepancies corrected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>272,510 aircraft arrived from foreign locations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3,757,436 kilograms of garbage were removed from these aircraft

Meat and products refused entry/confiscated

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ship passenger baggage</td>
<td>264 lots</td>
<td>1,433 kilograms</td>
</tr>
<tr>
<td>Aircraft passenger baggage</td>
<td>54,355 lots</td>
<td>72,141 kilograms</td>
</tr>
<tr>
<td>Border crossing</td>
<td>19,658 lots</td>
<td>22,603 kilograms</td>
</tr>
<tr>
<td>Post office</td>
<td>7,587 lots</td>
<td>11,004 kilograms</td>
</tr>
<tr>
<td>Commercial ruminant shipments</td>
<td>147 lots</td>
<td>226,818 kilograms</td>
</tr>
<tr>
<td>Commercial pork shipments</td>
<td>115 lots</td>
<td>337,418 kilograms</td>
</tr>
<tr>
<td>Commercial poultry shipments</td>
<td>24 lots</td>
<td>1,989 kilograms</td>
</tr>
</tbody>
</table>

Meat and meat products checked

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>42,765 lots</td>
<td></td>
<td>725,549,214 kilograms</td>
</tr>
</tbody>
</table>

Footwear cleaned and disinfected

12,645 referred and inspected 755 cleaned and disinfected

Export certification

6,632 certificates 929,393,154 kilograms

Commercial animal products imported

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Weight</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,733 lots</td>
<td></td>
<td>44,383,089 kilograms</td>
<td>restricted entry</td>
</tr>
<tr>
<td>24,391 lots</td>
<td></td>
<td>331,369,198 kilograms</td>
<td>unrestricted entry</td>
</tr>
<tr>
<td>308 lots</td>
<td></td>
<td>332,277 kilograms</td>
<td>refused entry</td>
</tr>
</tbody>
</table>

ORGANISMS AND VECTORS

In Fiscal Year 1984, 994 permits were issued for the importation and interstate transportation of animal disease organisms and vectors.
Cell Lines and Hybridomas — An increasing number of requests are being made for the importation of cell lines and hybridomas. Since animal serum is used in the media to grow the cells, the possibility exists that such materials might be contaminated by animal pathogens and inadvertently lead to the introduction of exotic animal diseases into the United States. Cell lines from countries with exotic animal diseases are usually required to be safety tested at the Foreign Animal Disease Diagnostic Laboratory at the Plum Island Animal Disease Center, Greenport, New York, before entry into the United States is permitted. The Parent Committee on Foreign Pathogens and Vectors has approved the policy of \textit{in vitro} and laboratory animal testing of cell lines and hybridomas from Japan, Australia, and the United Kingdom. For cell lines originating in foot-and-mouth disease countries, safety testing in domestic animals is required.

Representatives of Veterinary Services and the National Institutes of Health, are currently planning studies designed to establish an effective \textit{in vitro} or laboratory animal test for the detection of FMD and other exotic pathogens.

\textbf{CONSTRUCTION-USAHA}

\textbf{Otay Mesa, California} — The General Service Administration has awarded the contract at a cost of $358,000. Construction will begin around October 15, 1984, and should be completed by the end of April 1985.

\textbf{Sweetgrass, Montana} — Administrative Services Division of APHIS is reviewing plans in order to reduce costs. Construction bids for the facility came back much higher than the $495,000 which was approved by the administrator of APHIS. Customs had originally requested a weighing scale for animals in the facility but has recently withdrawn this request.

\textbf{Detroit, Michigan} — A second soil boring test at deeper levels was completed verifying that the site was not suitable for construction. Another site will have to be located before APHIS can build a new station.

\textbf{Houlton, Maine} — The facility is now under construction and is scheduled to be completed by May of 1985.

\textbf{COMMITTEE ON IMPORT-EXPORT EXPORT SUBCOMMITTEE}

The Export Subcommittee met at 1:30 p.m. on October 23, 1984. Twenty-three USAHA members were present, several of which were subcommittee
REPORT OF THE COMMITTEE

members. Twelve persons were from industry, eleven represented government agencies.

H. A. Waters, Chairman, introduced G. O. Winegar. Dr. Winegar reported that APHIS had implemented the recommendations in USAHA Resolution #10 regarding testing of animals at the point of origin to avoid rejections enroute or at destination.

Dr. Winegar presented and discussed the Export Animal Health Program Report (copy attached).

Dr. Waters reported on the meeting of livestock exporters and breed association representatives at which he represented USAHA on June 19, 1984, in Washington, D.C. From this meeting a Livestock Export Task Force was formed. This task force has invited comments from USAHA regarding quality inspection standards for livestock. The Export Subcommittee developed the recommendation to the task force that standards be developed and enforced regarding minimum weight for age of animals.

The meeting was adjourned at 3:30 p.m.

Respectfully submitted,

Harold A. Waters, Chairman

TO: MR. CLINT BOOTH, CHAIRMAN
IMPORT - EXPORT COMMITTEE USAHA

FROM: THE EMBRYO MOVEMENT SUBCOMMITTEE
IMPORT EXPORT COMMITTEE, USAHA

The meeting of our committee was called to order at 10:00 a.m. on October 23, 1984 in the VanZandt Room of the Hyatt Regency Hotel, Fort Worth, Texas. Members of the committee in attendance were:

S. V. Timberlake, Chairman
Dr. S. R. Bolin
Dr. Richard Bowen
Dr. John Cobb
Mr. Howard Hansen
Dr. W. C. D. Hare
Dr. D. C. Kraemer
Dr. George Winegar
Mr. Clint Booth, Ex Officio

Members of USAHA present to discuss the subject of embryos moving in international trade.

Drs. Acree, Winegar, Walker, Callis, Bowen, Hare, and Carmichael were asked to give their views on the current problems facing the movement of embryos; and the status of overseas health requirements, regulations, and research was thoroughly discussed. Also steps that should be taken to promote the import and export of embryos to and from the U. S. and to protect the interests of these shippers. It was emphasized by those participating in our discussion that USAHA must enlist the support of the
Secretary of Agriculture and the USDA representative to OIE to sponsor an international symposium as soon as possible to exchange information on current research data and its implications, and for the USDA representative to OIE to urge delay by his counterparts in issuing rigid regulations until additional research data becomes available.

Resolutions in regard to the foregoing are attached for delivery to your committee for consideration and approval. Drs. Callis, Walker, Waters, Winegar, Bowen, Hare, Bolin, and Mr. Hansen drafted these resolutions and are to be commended for the completion of same in short order.

The meeting was adjourned at 12:15 p.m.

Respectfully submitted,

Shelby V. Timberlake, Jr.
Chairman,

LIVESTOCK EXPORT TASK FORCE

An industry group entitled Livestock Export Task Force has recently been formed to examine export related issues such as health requirements, credit, government regulations and industry standards. This group is composed of representatives from industry and government and will examine the relationships among government agencies, livestock exporters and related organizations in the exporting process.

Initially the task force will gather information to be included in recommendations at all levels in government and industry. The primary beneficiaries of this group's activities over the next several months will be the livestock exporters themselves and their foreign clients. Ultimately, the entire U.S. livestock industry should benefit from improved quality and quantity of exports.

It has long been apparent to many livestock industry leaders that improved communication among industry groups, exporters and government agencies could be highly beneficial in enhancing U.S. livestock exports. With this in mind, several of these industry leaders agreed informally to form a group which would represent a broad spectrum of the livestock industry. From these initial efforts has evolved the Livestock Export Task Force, which utilizes the advice of government agencies, primarily the Foreign Agricultural Service (FAS) and the Animal Plant Health Inspection Service (APHIS), as well as the technical expertise of various industry groups and associations.

The organization of this task force is composed of a General Chairman and four committees, Export Procedures, Qualification and Standards, Marketing, and Government and Industry Relations. An Executive Committee, responsible for coordination between general meetings, will include the General Chairman plus the four Committee Chairmen. Whereas the entire group will meet about every three months to assimilate information and formulate recommendations, most of the data gathering and basic discussion will take place at the committee level.

Since the Livestock Export Task Force is charged with making recom-
mendations at all levels to enhance U.S. livestock exports, this group requests the support and assistance of individuals and organizations involved in this business.

Suggestions are welcome and should be directed to any one of the following:

General Chairman
Livestock Export Task Force
James R. Stafford
Holstein-Friesian Services, Inc.
1 South Main St.
Brattleboro, VT 05301
(802) 254-4551

Export Procedures Chairman
Livestock Export Task Force
C. T. Barnes
AMS Inc.
3601 Mayland Ct.
Richmond, VA 23229
(804) 747-8855

Qualification and Standards Chairman
Livestock Export Task Force
Robert Smith
HAS Trading
9604 Garden Plain Rd.
Morrison, IL 61270
(815) 772-4200

Marketing Chairman
Livestock Export Task Force
Claude Dobbins
Livestock Exporters Assn.
4801 Kenmore Ave. #718
Alexandria, VA 22304
(202) 223-3600

Government and Industry Relations Chairman
Livestock Export Task Force
Al Keating
American Farm Bureau Federation
225 Touhy Ave.
Park Ridge, IL 60068
(312) 399-5749
CARRIER ERYTHROCYTES: NATURE'S DRUG DELIVERY SYSTEM

J. R. DeLoach
USDA-ARS, Veterinary Toxicology and Entomology Research Laboratory, Veterinary Toxicology Research Unit, College Station, TX 77841

The biological origin of the red blood cell (RBC) and its function was an enigma after the discovery of the red corpuscle in 1658. After traveling many erroneous roads, investigators were able to clearly define the RBC and its function. The developing RBC has the capacity to synthesize hemoglobin; however, the adult RBC has lost this capacity and serves only to carry hemoglobin. Thus RBC's have one primary function and that is to transport or carry hemoglobin for the transport of respiratory gases. The lifespan of the adult RBC is finite which has important physiological implications. RBC's are in a constant state of turnover with daily production equal to daily destruction.

RBC's have been proposed and used as carriers of molecules other than hemoglobin [for review see ref]. The concept of RBC as cellular carriers of drugs and enzymes began to evolve in the early 1970's with the first reported encapsulation of an enzyme by a process of hypotonic dilution of the cells. However, it was not until a high efficiency entrapment procedure with minimal cell disruption was developed that preparation of a carrier erythrocyte with normal survival characteristics was possible.

Encapsulation within RBC can be achieved because of their dynamic membrane and because of their response to reduced salt concentration (reduced osmotic pressure). When RBC's are exposed to hypoosmotic conditions, they swell and rupture. However, when RBC are exposed to predetermined hypoosmotic conditions such that maximum swelling occurs, their membrane becomes permeable to molecules as large as 600,000 molecular weight. Under controlled conditions, RBC can be made permeable to exogenous molecules and will entrap these added molecules when the salt concentration is restored to isosmotic conditions. Their membrane again becomes impermeable to molecules larger than about 200 molecular weight. A final process of annealing allows the membrane to heal. The least disruptive and most efficient process of preparing the carrier RBC (C-RBC) is one of hypotonic dialysis. C-RBC thus prepared have transport functions and circulating survival characteristics equal to normal RBC. C-RBC can be prepared from almost any animal. The technological basis for preparation of C-RBC has recently been expanded to include a continuous flow hollow-fiber dialysis system. This technological achievement is more amendable to commercialization than the hypotonic dialysis system.

C-RBC possess many properties of an ideal carrier. They are biodegradable, have a large volume, are easily prepared and either circulate for long times or alternatively can be targeted to the reticuloendothelial system.
C-RBC have the same problems of those associated with storage of whole blood and the problem of antigenic determinants. Nevertheless, C-RBC are a unique carrier and have several veterinary and medical applications.

A futuristic look at C-RBC has to include their application in the expanding field of immunomodulators. Modulators such as interferon, interleukin–2, and muramyl dipeptide have been shown to be more effective in vivo when encapsulated. The target site for these molecules is the macrophage. RBC are naturally targeted for fixed macrophages of the RES and thus can easily carry modulators to these cells. Targeting of C-RBC to circulating macrophages presents a greater challenge. However, osaponization of RBC with antibodies can facilitate their phagocytosis.

A second promising area for the future is the application of C-RBC to vaccines. If an antigen requires processing by macrophages of liver or spleen before eliciting a protective immune response, then C-RBC would be appropriate carriers of such antigens. A second release mechanism for antigens could come from the slow release of antigenic molecules from circulating C-RBC.

Research with small laboratory animals and large livestock animals indicates that drug-loaded C-RBC can be used for prophylactic treatment of protozoan diseases. Tropical veterinary medicine could benefit from a slow-release carrier or perhaps from a carrier targeted to the RES. Since circulation of C-RBC has been observed for 100 days, it is theoretically possible to offer drug release for that period of time. Recent research with dogs indicate that drug-loaded C-RBC targeted to the RES results in secondary drug release from the RES.

In human medicine, several diseases are treatable with C-RBC. L-asparaginase therapy with encapsulated enzyme shows that serum asparagine levels are significantly reduced for long time periods with one administration of enzyme-loaded RBC. β-Thalassemia patients who undergo extensive blood transfusion therapy are burdened by iron overload. Successful iron chelation therapy with drug-loaded RBC has been achieved in laboratory animals. Inborn errors of metabolism, in certain instances, may be amendable to treatment with C-RBC. For example, Gaucher's disease, a lysosomal storage disease, could be amendable to therapy with adequate amounts of active enzyme entrapped in C-RBC.

Recent research with pig RBC and encapsulation of an allosteric effector has doubled the oxygen-carrying capacity of hemoglobin. Transfusion of effector-loaded RBC in pigs resulted in diminished physiological responses normally observed with strenuous exercise. Thus, C-RBC which are naturally occurring liposomes containing protein in their membrane may have a future in the field of drug and protein carriers for targeting and slow-release of drugs and proteins.

**REFERENCES**

The Commonwealth of Puerto Rico is one of the easternmost islands of the Greater Antilles group of Caribbean Islands. It is approximately 100 miles long and 35 miles wide. It is bounded on the north by the Atlantic Ocean while its southern shores are washed by the Caribbean Sea. It has an area of approximately 3,400 square miles and a population of 3,500,000 people. The livestock population is estimated at 40,000 cattle herds with 600,000 head and a horse population numbering 8,000 head.

Physically, it has a central mountain chain with northern and southern coastal plains. The northern half of the Island receives more rainfall and has larger rivers than the drier southern half. The mean temperature ranges about 75 to 80 degrees the year round.

The Tick Eradication Program in Puerto Rico was established to eradicate the Tropical Cattle Fever Tick and the Bont Tick from the Island. It is a cooperative program between the Commonwealth of Puerto Rico Department of Agriculture and the U.S.D.A., A.P.H.I.S., Veterinary Services. The eradication of Boophilus microplus ticks in Puerto Rico began in 1979 after a tick free period of 20 years (1954–1974).

The first Tick Program in Puerto Rico began in 1936 and continued until 1941 when Puerto Rico was declared free. Surveillance continued at a low level until end of World War II when surveillance was increased. Ticks were reintroduced from St. Croix in 1950. The last ticks were collected in December, 1952 and the Island was declared free in 1954. Boophilus were again recognized in January of 1978.

The Amblyomma Program

In June of 1974 Amblyomma variegatum, the Tropical Bont Tick was confirmed in the Cidra-Cayey area. Surveys were made, but eradication was not started until 1981. The Tropical Bont Tick is a three host tick native of south Sahara Africa. At each stage of its development (larva, nymph and adult) this tick takes a blood meal from its host, drops to the ground and moults. It then crawls up a blade of grass and waits for a new host. This tick can harbor the agents of the rickettsial disease, Heartwater, caused by Cowdria ruminantium and dermatophilosis, a skin condition caused by Dermatophilus congolensis. It is also associated with the causative of Q Fever, Coxiella burnetii and Tick Typhus. by R. conori.

By April 1981, 152 herds were under systematic treatment, which meant the application of Coumaphos (Coral) or Ciorid pesticide every week. This schedule was later changed to every two weeks. Trials were made with various antibiotics on livestock affected with dermatophilosis. Treatment with Coral or Ciorid and a long acting penicillin proved to be effective to control this disease. Systematic treatment was continued in the Cidra-Cayey area until March, 1983. The last Amblyomma tick was
found in that area in September 1982. The area was declared Amblyomma free in July, 1984.

Amblyomma ticks were found near the municipalities of Ponce, Cabo Rojo and on the island of Vieques after they were first seen in the Cidra-Cayey area: As ticks were found, the infested premises were quarantined and put under systematic treatment. There are two premises in Ponce on which ticks still are seen. One Amblyomma male was found in Cabo Rojo and one male on the island of Vieques in 1984. At present (September 1984) only 62 premises in Puerto Rico remain under quarantine for infestation by Amblyomma variegatum.

In October 1983, an Amblyomma cajennense (Cayenne tick) was found in the Rincon area, on the western end of the island. An extensive search of the area was conducted and no other ticks of this species have been found. From where it originated, is still unknown.

The Boophilus Program

In January of 1978 Boophilus ticks were found in the abattoir at Mayaguez on cattle from the San Sebastian Market. The cattle originated from a farm in Utuado. After nearly a year of survey to determine the extent of the infestation, the present Tick Eradication Program was started. The island of Puerto Rico was placed under federal quarantine in 1978 as a result of reinfestation by Boophilus microplus.

Boophilus microplus, the Tropical Cattle Fever Tick, is a one host tick, which lives in tropical and sub-tropical areas of North, Central and South America, and also in Africa, Australia and tropical areas of Asia. Besides its role as vector of the agents of Babesiosis (Piroplasmosis) and Anaplasmosis, it causes anemia, weakness, loss of weight and reduction of milk production.

Puerto Rico cattle ticks develop through 3 stages in their life cycle. Eggs are laid by female ticks which hatch into six-legged larva. These climb up a blade of grass and wait to contact a host animal. The larva crawl up through the hair and take a blood meal by piercing the animals skin with its specialized mouth parts. The tick then goes through a moult on the host or drops to the ground depending on the species. Amblyomma ticks fall off and moult on the ground, while Boophilus ticks moult on the host animal. This moult produces a nymph which takes a blood meal and drops off the host if it is an Amblyomma tick, or moult in place if it is a Boophilus tick. The adult ticks mate, take a blood meal and then the female drops off to find a humid place on the ground to lay eggs. If conditions of temperature and humidity are right, the eggs will hatch quickly and the lifecycle will continue. The female Amblyomma tick will lay up to 20,000 eggs. A female Boophilus will lay up to 4,500 eggs.

Our Tick Eradication Program is based on systematic treatment of herds for a minimum of ten treatments (30 weeks) in order to break the reproductive cycle of the tick. The Program personnel treat all susceptible animals in the quarantine area with a suitable pesticide every three
weeks. Between treatments, the livestock go out to pasture and collect more ticks which are then killed when the cattle are resprayed. Systematic treatment consists of treating every susceptible animal on a premises every three weeks without fail. The treatment cycle must not be broken. The herd owner must gather his entire herd every treatment day or the cycle will be considered broken and a new series of treatments will have to be started.

**Reinfestations**

Reinfestations are a major problem in this Program. Some predisposing causes in Puerto Rico are: (1) the limited area of the Island where livestock can be pastured, which does not allow for vacating of pastures and (2) the high density of the livestock population. The principle cause of re-infestation is the illegal movement of cattle across the quarantine lines, from infested areas to areas under treatment. First, there is the movement of cattle by dealers and then there is the movement due to split herds. There are many dairy herds in Puerto Rico. Some of the larger herds are split into the milking line, dry cows and heifer herds. As heifers or dry cows freshen, the dairyman will want them on the milking line as soon as possible. If the dry cows or heifers are outside of the treatment zone, they will more than likely be ticky. (95% of cattle outside of the quarantined areas are infested.) Due to the economic necessity of getting newly freshened cows onto the milking line, quarantines are violated and re-infestations occur. Since July 1984, a 24-hour patrol of the quarantine line has been initiated and this has greatly reduced illegal movements. Puerto Rico has another movement problem. That of the movement of infested grass. In general, pastures are overpopulated and overgrazed. In times of drought food for animals becomes a problem. Herd owners go out along the roadways, into vacant lots and deserted pastures, and cut grass to bring home to their livestock. This grass is often infested with ticks. It is illegal to move grass across the quarantine line. We try to stop the practice and are searching for ways to solve the problem. Livestock need the feed. Imported hay is very expensive and fumigation of this grass has proven impractical. We are investigating whether the pelletizing of hay or grass will be helpful.

There are many stray animals in Puerto Rico. Farmers with small acreage put their animals out to pasture on roadways and in open areas. These carry ticks from vacant premises, roadways and infested farms to herds under treatment and to herds already treated and released. The Program has constructed two detention areas for stray animals with corrals for the different species. These are located in the east and west quarantine zones. Stray animals are picked up with judicial authority, then are scratched, treated and held until claimed by the owner or auctioned off and sold for slaughter. The confiscation pens are under the control of the quarantine line patrol.
Quarantine Areas

The Puerto Rico Eradication Program has two *Boophilus microplus* quarantine areas, one on the eastern end of the Island with headquarters at Juncos, the other on the western end of the Island with headquarters at Arecibo.

The Juncos Quarantine Station has an area of 342 square miles bounded on the east by the Atlantic Ocean. The station is divided into 4 substations: Juncos, Las Piedras, Humacao and Yabucoa. The quarantine line is 77 miles long and is manned 24 hours a day, 365 days a year. This station has been selected as the area from which expansion should proceed. The Tick Eradication Program will expand from the present area north to the Ocean, including the El Yunque National Forest and east to the Ocean. This expansion would give a north-south quarantine line which will be much shorter and more easily protected.

By FY 1985 the quarantine area should be pushed west to a line from San Juan, and south to the Caribbean. By FY 1986 the quarantine line should have moved west to a north-south line dividing off the eastern one-third of the Island. The following year the quarantine line is projected to divide the Island in half. By FY 1989, the Program should join the quarantine line of the Arecibo station.

The Arecibo station is in the northwest corner of the Commonwealth. Its present area is about the same as that of the Juncos station. It has five substations located at Isabela, Moca, Quebradillas, San Sebastian and Aguada. This quarantine zone will expand toward the south, straightening its quarantine line to a north-south direction, in order to maintain its quarantine security without draining too much the limited funds of the Program.

At present there are 1,279 herds under treatment in the Juncos station with 42,350 animals. There is a total of 3,501 premises in the Juncos quarantine area. At the Arecibo station there are 1,582 herds under treatment with 17,500 animals. There is a total of 4,004 premises in the Arecibo quarantine area. In FY 1984, 2,595 premises were freed of ticks in both stations and 844 premises were reinfested, leaving a net-free balance of 1,751 herds freed of *Boophilus microplus*.

There were 143 premises released from quarantine for *Amblyomma variegatum* infestation with no reinfestations.

Each of the Boophilus quarantine areas are under the direction of a Station Director who reports to the Program Director. Each of the two stations are divided into substations. Each substation is the responsibility of a field supervisor. Most of the field supervisors are federal employees, some are state employees.

The field supervisors are responsible for all activity at the substations. They have up to 10 or 12 groups of 2 or 3 PRDA cattle tick inspectors. The groups are trained as tick inspectors who scratch animals for ticks and/or treat them with pesticides. Still other groups check animals for ticks and give movement permits if the animals are free of ticks. Some work on spray
dip machines, others put in ear tags to identify the animals. Each group consists of a group leader and one or two inspectors.

Around each quarantine area there exists a quarantine line which is guarded by quarantine line personnel. Personnel on the quarantine line are divided into patrols who ride the quarantine line in jeeps and cars, and treatment station personnel who operate treatment stations along the quarantine line. The treatment stations have a corral and a treatment chute where animals entering the quarantine area can be off-loaded from trucks, scratched to assure that they are tick free, sprayed preventively with an appropriate pesticide, and then reloaded. The driver then receives a permit to enter the quarantine area. If one or more of the animals is found to have ticks, the load is rejected for entry. The animals will be sprayed but denied entrance into the zone.

Puerto Rico has many hills and small dirt roads which must be traveled in order to get to the cattle farms. For this reason 4 wheel drive vehicles are used by the Program to haul the heavy equipment necessary to spray the cattle.

The cattle are treated with pesticide sprayed at 100 psi from 200 gallon high pressure spraying machines. When there are more than 50 animals in a herd of cattle, a spray dip machine is used. This is a mobile unit with a metal chamber supplied with spray jets placed in a way that pesticide can be sprayed to thoroughly wet an animal inside the chamber. An animal to be treated enters one end of the machine, the jets are turned on for 10 second blasts, then the other end opens and the animal goes out. It is economical because pesticide is recycled.

When there are many animals to be treated a hydrovat may be used. This is a portable cage which can be lowered into a pesticide solution in a stationary tank. Livestock are driven into the cage and lowered into the pesticide solution. It is then raised hydraulically to ground level and the livestock are let out. The hydrovat is much faster than is the spray dip machine.

There are now only 2 dipping vats in operation in Puerto Rico. A dip vat facility is under construction at the San Sebastian Livestock Market within the Arecibo quarantine zone on the western end of the Island, and others are planned. The earlier Tick Program used 400 dip vats for treating livestock. Most of these have been filled in or are beyond repair.

Security and Safety

Security is an important item in the Tick Program. The Program has hundreds of thousands of dollars in pesticides, equipment and supplies. These must be kept secure from theft, vandalism and deterioration. When possible we use guard dogs for protection from theft and vandalism. They are dependable, alert and inexpensive.

The Program is very concerned for the safety of its employees. TEP employees wear protective clothing when spraying livestock. They receive more protection than required by EPA or suggested by the manufacturer's
Because of their continual contact with a pesticide drenched atmosphere, employees are required to wear raingear, boots, respirator masks, goggles and gloves. The P.R. Tick Program uses the most effective and safe pesticides permitted by the EPA. In the 1936–54 program, arsenicals were used and the animals sometimes showed toxic symptoms. The EPA later prohibited use of these products. The present program started in 1979 using CoRal (Coumaphos) for beef cattle and horses. Ciorid (Crototoxophos) was used for treatment of dairy herds. Both of these organophosphate products cause toxic reactions: reduction of the cholinesterase level in man and animals with toxic symptoms of weakness, headache, dizziness, incoordination, nausea, slow heart rate or an influenza-like illness. Before adequate protective measures were instituted 40% of our employees had to be shifted away from exposure to pesticide due to low cholinesterase levels. Today the Program uses TAKTIC (Amitraz) on all animals susceptible to ticks except horses and sheep. It uses Atroban (Permethrin) for horses, sheep and in dip vats for all species. This product is very stable in dip vats.

Atroban is used as a 0.050% solution in water and is available to the Puerto Rico Tick Project under a 24C Registration. It can also be used as 0.5% wipe-on for application in the false nostrils of horses in the control of Dermacentor nitens, the tropical horse tick. It is of unusually low toxicity to mammalian species, has a broad pesticidal spectrum and is rapidly metabolized and eliminated so as to present little concern with potential residues in meat or milk.

Taktic is an extremely active acaricide. It has an extended residual action, being effective against ticks at all stages of development. It acts on the metabolic processes of the tick by enzyme inhibition. This results in the stimulation of tick activity, causing it to remove its mouth parts from the animal, move itself rapidly in a disorganized manner, drop off and die. Gravid female ticks tend not to lay eggs but even eggs that are laid are generally not viable. Taktic is used in Puerto Rico under an EPA Section 18 Emergency Exemption.

Taktic has proved very valuable to the Program. This product has allowed the Program to prolong the interval between treatment cycles against Boophilus ticks from 2 to 3 weeks. It is equally as effective against Amblyomma ticks. When the Program was using organophosphate products: Coral, Ciorid and Deltox, there were incidents of toxicity in both employees and livestock. Those employees working in the field were tested every two months to control their blood cholinesterase level. Any lowering of this vital blood constituent below the employee's base level was reason for his immediate removal from any contact with organophosphates.

The Authority for the Cattle Fever Tick Program

The regulation that governs the control and eradication of Boophilus ticks is based on Puerto Rico law #106 promulgated in 1936 and revised in 1980. It describes the structure and implementation of the Program, the duties of herd owners to the Program and establishes a quarantine for
areas under treatment. Stray animals are regulated under this law, also the movement of animals, materials, feed and equipment. The law provides for inspection, treatment, surveillance and termination of quarantine of susceptible animals. It also provides penalties for violations of the provisions of the law.

Law #60 regulates in comparable manner the Program to eradicate the Tropical Bont tick, *Amblyomma variegatum*. Under this law, farms where Amblyomma ticks were found were placed under individual quarantine and treated.

Compliance is very good because with an average of 2,918 herds treated monthly, there were only 253 violations of these laws, prosecuted during FY 1984. This is less than 10% over the course of a year.

The Tick Eradication Program Budget

The Tick Program budget is made up of funds from APHIS, VS, Legislated funds from Puerto Rico (RC) and from special project funds from the Food and Nutrition Service of USDA (PAN). This last is special project money from the Block Grant which replaced food stamps in Puerto Rico.

1984 funding was as follows: There were 1.7 million dollars from APHIS, Veterinary Services, 1.25 million dollars from legislated funds for the Puerto Rican Department of Agriculture and 8.6 million dollars from PAN funds. The total budget was 11.5 million dollars. Below is a chart showing the amount of funds budgeted for the program since 1978.

The full PAN funds for the 1984 budget were not approved until May 25, 1984. As a result of this late funding, vehicles, equipment and supplies, although ordered in 1984 will not arrive until FY 1985. The trucks which are badly needed will not be delivered until January 1985. In the meantime, we are using federal vehicles and some jeeps bought with PAN money. Some federal vehicles and equipment have come in from other states having excess trucks and excess cattle spraying machines. The 1985 budget will be essentially the same as 1984.

RESUME OF FINANCIAL INPUTS FROM THE BEGINNING OF THE TICK PROGRAM

<table>
<thead>
<tr>
<th>USDA APHIS VS</th>
<th>Other Federal</th>
<th>Commonwealth</th>
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<td>8,694,144**</td>
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*Comprehensive Employment Act Funds
**Nutritional Assistance Plan Funds
REPORT OF THE COMMITTEE ON PARASITIC DISEASES AND PARASITICIDES

Chairman: R. L. Pyles, New Mexico
Vice Chairman: John H. Grey, Colorado

L. G. Biehl, IL; A. R. Burgess, WY; A. A. Chadwick, DE; J. E. Christy, IL; R. O. Drummond, TX; Robert Gadd, SD; Bill Gallagher, SD; S. C. Gartman, TX; J. F. Hudelson, CO; Ralph Jones, SD; N. W. Kruse, NE; N. H. Lang, IA; R. P. McDonald, TX; C. H. Miranda, SC; J. H. Niemi, SD; J. R. Pemberton, IA; Glenn O. Schubert, MD; M. G. Scroggs, TX; P. L. Smith, CA; R. K. Strickland, IA; William Utterback, CA.

This committee met on Monday, October 22, 1984, at the Hyatt Regency Hotel, Fort Worth, Texas.

The meeting was called to order by the chairman at 1:35 p.m. Approximately 30 persons were in attendance, twelve of which were committee members.

Dr. Glen O. Schubert, USDA, APHIS, VS, presented summaries for the past fiscal year on the Puerto Rico Tick Eradication Program, the Texas Tick Eradication Program and the cases of psoroptic cattle scabies reported from the various states. These summaries are as follows:

**Puerto Rico Tick Eradication in FY 1984**

A net total of 1,750 herds were freed of ticks during the year October 1, 1983, through September 30, 1984. This brings a total of 5,059 new free herds in Puerto Rico. The numbers are:

- FY 1980 — 133
- FY 1981 — 801
- FY 1982 — 1,351
- FY 1983 — 1,024
- FY 1984 — 1,750

**Texas Tick Eradication in FY 1984**

A total of 9 new infestations were found in Texas in FY 1984. There were 8 in the quarantined area and 1 outside the quarantined area.

Apprehensions of Mexican livestock down from Fiscal Year 1983. There were 172 head apprehended. Ninety-nine head of cattle with 31 infested. Seventy-three equine with 8 infested.

**Psoroptic Cattle Scabies**

A total of 76 herds or lots were reported as infected during the year.

- Colorado — 19
- Iowa — 3
- Idaho — 3
- Kansas — 7
- Nebraska — 8
Dr. Gary P. Combs, USDA, APHIS, Veterinary Services, Puerto Rico, presented a detailed report on the Puerto Rico Tick Eradication Program as prepared by Dr. Charles B. Suthern and Dr. Combs.

Dr. R. O. Drummond, ARS, USDA, Kerrville, Texas, advised the committee that the pesticide (pyrethroids) impregnated eartags are no longer effective in the control of horn flies, particularly in the southern states. As suspected, we now have large populations of horn flies that are resistant to the two pyrethroids commonly used in the treated eartags.

Dr. Norvan Meyer, Assistant Deputy Administrator for International Operations, USDA, APHIS, Veterinary Services, advised this committee of the serious consideration being given to a cooperative program to continue the screwworm eradication program by gradually moving the “free” area southward until all areas north of the Panama Canal area are screwworm-free. At the present time, the free area buffer zone is at the Isthmus of Tehuantepec, Gutierrez, Mexico.
PROBLEMS ASSOCIATED WITH
LETHAL AVIAN INFLUENZA ERADICATION

Gerald J. Fichtner, Director, Northern Region, USDA, APHIS,
Veterinary Services, Scotia, New York

In retrospect, successful eradication of Avian Influenza in Pennsylvania required demonstration of the absence of all evidence of viral activity. This was achieved through the sacrifice of over 15.5 million birds in 390 flocks, the expenditure of $60.0 million public funds, and the heroic teamwork of federal, state, and industry people working toward a common goal. The known epidemic began in April, 1983 and was concluded when the final flock was depopulated in September, 1984.

To appreciate the significant problems faced during the campaign requires an understanding of the major phases of the entire program. These phases, although overlapping, are based on:

1. Genetic change of the AI virus,
2. absence of initial complete knowledge of the disease or this epidemic,
3. need to demonstrate nationally and internationally that all serologic evidence of viral activity had been removed, and
4. the need to allow this industry to survive. Other basic problems were associated with either the environment or with logistics. Major problems, therefore, included:

**Agenda** — the entire campaign can be divided into four phases:

1. **Pre-Task Force Phase.** Avian influenza was introduced into poultry and spread to at least 25 diagnosed flocks. The disease was reportedly mild in nature. Inoculation of test birds did not produce 75% mortality and thus did not satisfy the accepted definition of “Highly Pathogenic Avian Influenza.” Industry biosecurity efforts, although traditionally lacking, were strengthened by limited educational efforts. State authority was obtained to quarantine premises infected with this “Low-Path” disease. Other eradication activity was not initiated. It was later estimated by the task force that this “Low-Path” disease spread to 80–100 flocks between April and October, 1983. Most people believed that the disease would disappear with limited regulatory control. Instead, a genetic change occurred resulting in a virus causing devastation in the poultry house and killing 75% or more test birds. This phase of the campaign ended in October 1983, with both high and low pathogenic viruses spreading in a susceptible population and with the declaration of an extraordinary emergency.

2. **“Highly Pathogenic (HPAI) Phase” Eradication Phase.** The USDA focus of this initial eradication phase was to eliminate HPAI and to contain the disease within the original quarantine area. Federal authorities and resources were marshalled toward these goals. State and industry objectives, however, included regulatory action to combat “Low-Path AI” as well as “HPAI.” Test and slaughter activities resulted in the depopulation of 249 flocks. Flocks were declared positive based on clinical, epidemiologic and/or laboratory results. Case his-
tories, supported by results of test bird inoculations available at a later date, supported the belief that it was not possible to correlate the events happening in the poultry house with the definition of HPAI. One hundred and one (41%) of 249 isolates from these declared flocks did not kill 75% of the test birds in the laboratory. Quarantines were extended four times due to extensions of the disease. No regulatory actions other than quarantine were taken on the 51 flocks identified by laboratory test as having “Low-Path HI” during this phase. This phase of the campaign ended in January, 1984 when it was determined that all AI virus infections must be eliminated in order to eradicate the disease and prevent further extension of the disease into neighboring poultry concentrations.

3. “Lethal AI” Eradication Phase. The objective of this phase was to eliminate any flocks with clinical, epidemiologic, and/or laboratory evidence of AI. Acutely and subacutely infected flocks (37) were declared infected without demonstrating virus at the time of diagnosis but isolations of virus were subsequently attempted. The identified “Low-Path” flocks (51) carried over from the previous phases of the campaign initially required reisolation of AI virus prior to positive diagnosis. Virus was reisolated from 6 of these 51 flocks. The remaining flocks (45) were eventually redocumented based on laboratory serologic evidence and were depopulated. Up to 75% of the birds tested in these 45 flocks disclosed antibody up to one year after initial virus isolation. During this phase, an additional 50 flocks were detected with serologic evidence of AI through intensive surveillance on the entire population but no action was taken unless AI virus could also be isolated. Case histories documented previous clinical and epidemiologic evidence of influenza. State and federal resources and authorities were directed toward the same agenda at this time.

4. Lethal AI (Sero-positive) Phase. Because it could now be shown that antibody levels remained high in recovered flocks from which virus had been isolated as much as one year previously, it was decided that all sero-positive flocks should be considered for depopulation. Of the approximately 75 flocks accumulated in this category, 51 were declared positive based on significant clinical, epidemiologic and/or laboratory serologic evidence and were depopulated. Continuing surveillance has resulted in the near conclusion of this phase without significant evidence of viral activity.

Without question, an agenda with four distinct phases represented the most significant problem in attaining the ultimate eradication goal. Amending task force policies, acquiring the necessary resources, reeducating both the task force and industry, and maintaining momentum and confidence required participation in, and sensitivity to, change.

Population Surveillance

During the Pre-Task Force Phase, surveillance was based on owners and service representatives observing sick flocks and submitting birds to the
local laboratory, where AI was diagnosed. Those who had now observed AI became “Good Samaritans” and helped their neighbors in making a diagnosis with limited regard to biosecurity. This type surveillance probably resulted in the spread of AI virus within the community.

The Task Force depended upon the telephone exclusively during the “HPAI” Phase. This represented an owner-industry controlled surveillance and was complicated by rising egg prices. There was some tendency to wait and see if the disease had a mild syndrome or was acute—thus allowing virus to be spread prior to reporting by telephone. Surveillance was further complicated by a large religious community without electricity or telephones. “Dead-bird” pickup and egg serology limited projects were initiated during the HPAI phase.

In the “Lethal AI” Phase, surveillance was finally extended to the entire population. AI virus isolations were attempted from weekly submission of swabs from normal mortality birds. Several flocks were detected which had acute or recently acute evidence of disease. The shedding of virus for a short period of time, the effect of putrefaction on virus, and developing hot weather limited the effectiveness of dead-bird pickup as a surveillance tool. Procedures were developed to extend egg and blood serology as a more sensitive method of surveillance.

During the final “Sero-Positive” Phase, egg surveillance and slaughter surveillance, supplemented by dead-bird pickup of turkeys and pullets proved to be the most sensitive method to determine presumptive evidence of avian influenza within the quarantine area.

There were significant limitations to all forms of surveillance utilized throughout the campaign. The telephone led to approximately 320 of the 390 cases. Dead bird pickup resulted in 10 cases. Egg-serology led to the remaining cases but only after repeated sample submissions from the same flocks. For these reasons, population surveillance continued for 6 months following quarantine release.

**Diagnosis**

“Pre-AITF” diagnosis was indicative of mild disease. Clinical signs, mortality rates, and drop in egg production was demonstrated. Following a mild syndrome these flocks recovered but a high percentage of birds with antibody remained in each flock.

During “HPAI” phase, most flocks exhibited dramatic clinical and post mortem evidence of an acute respiratory, digestive, and systemic disease. Forty-one percent of the flocks diagnosed as HPAI did not kill 75% of test birds. Several flocks diagnosed clinically as not HPAI disclosed virus that killed 75% of the test birds or 3, 4, or 5, of 8 test birds. Finally, diagnosticians gave up trying to distinguish HPAI from other AI infections based on the 75% criteria.

In the “Lethal” AI phase, the main problem was attempting to reisolate virus from recovered flocks. Finally, diagnosis was made based on clinical and epidemiologic evidence supported by serologic documentation of earlier virus isolation.
Finally, in the “Sero-Positive” Phase, diagnosis was made based on documentation of clinical and epidemiologic history by examination of owner records and outbreaks within the immediate area. Blood serology supported final diagnosis in each case. Despite exhaustive culture attempts, AI virus was not isolated from these flocks. Special problems occurred with a few flocks which had vaccinated but these were subsequently owner-depopulated without indemnity if there was no evidence of field strain infection.

Depopulation

The removal of 15.5 million birds in the shortest possible time following declaration as infected presented problems which were not associated with program phases. Rather, environmental and logistic constraints remained throughout the campaign. These birds represented approximately 62 million pounds of organic material which is 75% water. Renderers refused the birds due to fear of spreading the disease and lack of a satisfactory marketable product. Burning would be expensive due to the high water content. The only acceptable and efficient alternative was to bury. Burial presented unique problems. Average topsoil in the area was two feet and no EPA approved sites were ever found. A sanitary landfill contracted to bury all birds under 8–10 feet of refuse. Repeated attempts to isolate AI virus from effluent from the landfill were negative. The remaining problems with depopulation were logistic. Crews of chicken catchers took birds from the barns to CO2 filled trash containers. The birds were transported 10–30 miles and buried.

Cleaning and Disinfection

The disposition of manure and subsequent disinfection was the responsibility of the owner of the premises. A reimbursement schedule between the industry and the state later relieved the cost of this activity. Two basic practical problems occurred. The problem of what to do with the manure was never resolved satisfactorily. Alternatives included: 1. Composting, if the manure was not too wet. Some producers used this option but virus was demonstrated within compost piles. 2. Digging a shallow trench, lining with plastic, filling with manure, and covering with plastic was allowed but seldom utilized. 3. Chisel-drill high moisture manure into the soil was possible but few had moisture content high enough to employ this option. 4. Spread manure on fields and plow under the same day. Climatic conditions could not guarantee that the plowing would occur the same day. In reality, many owners spread the manure on the fields and plowed it under as soon as conditions permitted. Virus was isolated from wet manure in a barn up to 105 days following depopulation. Spring warmth and the sun’s rays probably inactivated more virus than any other method.

The second cleaning and disinfection problem involved the definition of clean, prior to disinfection. Traditional industry standards were initially in conflict with the task force definition of “clean.” Rotation of task force
personnel further confused the standard. Assignment of a single person to interpret the regulatory standard combined with a reeducation of the industry resolved this problem. Following disinfection, virus was found through extensive biased sampling on two of over 400 poultry houses—an indication that C&D was generally successfull.

**Transmission and Biosecurity**

During the “Pre Task” Force phase, biosecurity was lax throughout the industry but probably similar to other sections of the country. Transmission was possible through movements of live and dead birds, contaminated equipment and vehicles, and through human contact. Initial attempts to increase biosecurity through industry and state educational efforts proved to be of minimal benefit.

At the beginning of the “HPAI” Phase, projects were initiated to demonstrate AI virus transmission. Isolates from eggs, flats, dust, flys, exterior of dead chickens, exhaust fans, nearly any location around an infected premise, emphasized the need for reeducation of the entire industry in biosecurity procedures. Lectures on biosecurity were given by the task force to anyone who would listen. Media attention was focused on prevention of disease entry. This work proved to be one of the most important contributions to success in the program. Regulatory restrictions on movement, although severe and generally well enforced, were instrumental in further limiting the spread of virus. Traffic on and off farms was well enforced by the industry.

During the final two phases of the campaign, biosecurity remained intense with the result that there was little spread of disease during these periods. Decontamination of the environment and hot weather further reduced virus concentration within the quarantined areas.

**Research/Field Studies**

Prior to the activation of the task force, knowledge of avian influenza was based on previous outbreaks throughout the world. Understandably, when the virus became a highly pathogenic strain, there was little knowledge about the properties of this virus, its action on this poultry population, under this set of husbandry and marketing conditions. For example, it was soon learned that the definition of HPAI (75% death of test birds) was not consistent with field findings. Technical answers to an endless list of questions which could modify existing eradication policies were needed. Here was an opportunity for practical field studies under conditions that could never be duplicated in a laboratory. The task force undertook many field studies. Some studies assisted in changing existing eradication policies. But there were many problems with this approach. 1) Rotation of personnel resulted in some projects not being completed. 2) The background of the project leaders was often regulatory rather than research-oriented. 3) Resources were to be directed toward eradication efforts and not pure research or in depth field studies.

In retrospect, a parallel group of scientists working with, but inde-
dependent of the task force, would have been more productive in obtaining knowledge about the virus, the nature of the disease and the epidemic.

**Resources**

Ideally, it would be beneficial to have adequate resources throughout an eradication campaign. Because the effort had four separate phases, there was some disruption in the availability of necessary resources.

1. State and Federal authorities and/or policy to regulate movement and conduct the eradication program were amended four times, each amendment resulting in some delays and reeducation.

2. Federal funds were requested three times and State funds twice during the campaign. Some interruption in activity was experienced but industry was generally satisfied with indemnities and other funds provided for overhead and C & D.

3. Personnel provided were extremely dedicated but initially lacked knowledge of the poultry industry and poultry diseases. Continuous training was provided.

4. Knowledge of this AI virus, the course of the disease in the host and methods of transmission of this virus were unknown when the campaign started, but the Task Force members learned enough to detect the disease, eliminate the virus and prevent infection of the remaining population.

**Summary**

The major problems in AI Eradication in Pennsylvania were due to phases of the program, environmental constraints, and logistics. Traditional eradication actions were essential but there were natural limitations to these basic policies. This campaign was successful because there was little opposition to new ideas and few self-satisfying needs. Resistance to change was minimal. Wisdom and reason by our advisors; prudence, understanding and fortitude by the task force; and an industry that truly wanted eradication in the shortest possible time, were the ingredients that, in spite of these identified problems, made eradication a reality. On a personal basis, I have never seen a more dedicated and inspiring performance by any state, federal, industry team anywhere, at any time. It was my life’s greatest privilege to see so many beautiful people working together for agriculture and for their country.
AVIAN INFLUENZA TASK FORCE
DEPOPULATION CONCERN IN VIRGINIA

A. J. Roth, DVM, State Director
Virginia Avian Influenza Task Force

Exotic diseases will be a constant threat to the Virginia livestock and poultry industry. Previous outbreaks of VVND, Pseudorabies and now Avian Influenza in Virginia illustrates this point. The modern means of transporting animal, poultry or equipment will serve as a vehicle in spreading these and other diseases within counties and states. The origin of Virginia's Avian Influenza outbreak probably come from dirty equipment contaminated with Avian Influenza virus that was brought to a Virginia farm from out of state. Shortly after the use of this contaminated equipment the Virginia turkey flock disclosed symptoms of Avian Influenza much similar to the earlier cases in Pennsylvania.

The Virginia poultry industry, the Governor, members of his staff, legislators, the Commissioner of Agriculture, and the Virginia animal health officials did not want to live with this disease. Thus, these groups worked towards federal assistance to eradicate Avian Influenza in Virginia as quickly as possible. Federal assistance was made available on January 27, 1984 when approximately 2,700 square miles of the Shenandoah Valley of Virginia was placed under State and Federal quarantine.

Immediately after the State and Federal quarantines were issued a State/Federal task force was established at Harrisonburg, Virginia. The quarantined area was comprised of three counties and areas of two other counties. Within the quarantined area there were 683 commercial and approximately 860 backyard flocks. The total number of birds at risk were 21,000,000. Another factor relating to the total picture is that Virginia is one of the major poultry industries in the nation.

The majority of the commercial flocks in the quarantined area are broilers, 49%; meat turkeys, 25%; breeder flocks, 15%; pullets, 7%, and laying flocks, 4%.

There were 65 flocks positive via virus isolation and four (4) additional flocks were found to be serologically positive. Prior to the establishment of the task force four (4) other flocks were found to be infected via virus isolation. Thus, from December 13, 1983 to July 28, 1984, 73 flocks were depopulated. The infected flocks were classified into the following categories: 43 meat turkey flocks, 5 turkey breeder flocks, 17 chicken breeder flocks, 3 chicken laying flocks and 5 broiler flocks.

The number of infected flocks per month were as follows: 3 in December 1983, in 1984 7 outbreaks occurred in January, 12 in February, 30 in March, 12 in April, 8 in May and 1 in June.

Keep in mind that one of the major goals of the task force was to make every effort for the prompt disposal of AI infected flocks. The prompt disposal of infected flocks was necessary in order to destroy the virus.
factory as soon as laboratory confirmation was obtained. Because of burial problems, we were unable in several cases to meet our goal of having the infected flock in the ground the same day of confirmation or by the following day. In some cases, it took up to 11 days to have an infected flock buried.

The various means of disposal of solid wastes in the quarantine area will be described in the remainder of this report.

**Incineration**

This means of disposal of infected flocks was not practical because of size and method of burn of the Harrisonburg city incinerator. This incinerator was set up to burn paper and depended on the burning of very inflammable material to destroy that type of waste.

**Rendering**

A modern, well-managed facility was available in the quarantined area; however, the plant was never used to dispose of infected flocks because of the refusal of the poultry industry outside of the quarantine area to buy meal that was processed from flocks that AI virus had been isolated. If a virus infected flock had been rendered, the industry would have refused to purchase any meal from the plant. The industry people outside of the quarantined area were concerned about the possible contamination of the end product at the plant with AI virus.

**County Landfill**

Thirteen infected flocks were disposed of by this method; however, the Rockingham County Board of Supervisors, Harrisonburg, Virginia, concerned that we would fill their landfill and possibly contaminate water wells in the community, forbid the Task Force to use their facility after April 3, 1984.

**Burning**

This method of disposal has not been used extensively in outbreaks of animals and poultry diseases in the United States. However, due to problems encountered in finding suitable burial sites in the quarantine area it was decided to carry out a test burn of part of an infected flock. Prior to the test burn, the Virginia Environmental Agency was consulted. The test burning was conducted at the Rockingham County landfill on Saturday, May 19, 1984. Trench burning in conjunction with the air curtain blower—(*DriALL Air Curtain Destructor) was the method selected as opposed to above-ground burning.

The trench measurements were as follows:
- 10 feet deep on the side where the air curtain blower was located
- 8 feet deep on the opposite side that was used for dumping material
- 15 feet wide and 200 feet long
The burning platform was constructed in the bottom of the trench using green hardwood logs 18–24 inches in diameter cut in 3 to 4 foot sections. Three rows of these logs 5 feet apart constituted the base of the burning platform. Fifty bales of grass hay were laid between these rows. Several rows of smaller green hardwood logs (10–12 inches in diameter) were placed across the bottom row of logs. Than smaller green hardwood logs 4 to 5 inches in diameter were laid across these. On top of this, wooden pallets and scrap lumber were placed. Then 11.6 tons of Big Chunk Kentucky Coal was placed on the pallets. Measurements of the final platform including the coal was 12 feet wide, 15 feet long and 4-1/2 feet high.

Approximately 2,500 leghorn hens (10,000 pounds) were then placed on top of the platform directly on the coal. Approximately 8 gallons of diesel fuel was poured on the bales of grass hay and ignited. The weather was clear, temperature was in the low 80’s and the wind was gusty.

Details of the Burning Operation

The fire started very well and was burning intensely within a few minutes. The blower was being used at “normal” speed (1900 RPM) but was not effective due to inability of air to circulate properly.

Progress of the Burn:

8 hours: the fire was burning vigorously and all birds were at least partially burned.

19 hours: the fire was still burning well and most of the chickens were completely burned.

45 hours: the fire was almost out, but there was a sizeable bed of very hot coals. Ninety-nine percent of the chickens were reduced to ash and the remaining few were easily powdered with the toe of a boot.

Costs of the Burning Operation:

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.6 tons of Coal</td>
<td>$1,180.00</td>
</tr>
<tr>
<td>50 bales of grass hay including transportation</td>
<td>122.50</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>$1,302.50</strong></td>
</tr>
</tbody>
</table>

$1,302.50 divided by 2,500 = $.52 which was the cost to burn a bird in this study vs. $.27 to bury a bird at an infected premise.

The cost to burn these birds does not include task force personnel time or the charges made by the disposal company in hauling the chickens from the farm to the landfill.

Observation and Comments:

1. The chickens would have been burned at a faster rate if the fire had been stirred by some means after the first few hours and with less fuel if there had been proper ventilation.

2. The fire may have done as well on level ground as in the trench.
3. Probably twice as many chickens could have been burned if the coal had been spread in a thinner layer.

Conclusions:
1. Burning as a means of disposing of poultry carcasses is viable and can be carried out successfully.
2. Disposal of carcasses by burning is expensive and time consuming.
3. Burning of large numbers of poultry carcasses would generate considerable amounts of smoke which may provoke unfavorable reactions from agencies concerned with environmental protection as well as citizens in the vicinity of the burn.

Burial Sites

From the very start of the Avian Influenza Eradication Program in Virginia, a rapport was established between the Task Force and the regulatory agencies responsible for solid waste disposal. The agencies responsible for approval of burial pits were the Virginia Health Department, Water Control Board and Soil Conservation Service. In the early part of the program, if the burial site on the infected premises was not approved, such infected flocks were buried in the county landfill. The burial of flocks at approved sites on farm premises was working well until the Health Department required the use of plastic liners in all burial pits. The use of plastic was required so that all burial sites could be approved on the infected premises and to prevent contamination of the underground water table. Shortly after the use of plastic in the pits, the owners complained of strong offensive odors and cracks in the burial pits in which fluids were oozing to the surface of the ground. With the oozing of fluid to the surface came the fly problem. The task force attempted to control the flies at these problem pits, however, poultry owners had fears that the fluids would contain the live Avian Influenza virus which could be spread to neighboring poultry flocks. They felt that such spread of the disease could make it impossible to achieve our mutual eradication goal.

These adverse conditions at burial pits prompted the Task Force to request assistance from an authority on solid waste management. Conferences were held with this person as an advisor and the various Virginia regulatory agencies to establish procedures and proper burial pit design. The Task Force Advisor explained that the anaerobic decomposition liquifies the dead birds within 10 days, creating an unstable condition in the burial pit. The liquid is unable to support the soil which soon cracks, settles and forces leachate to the surface.

Further evaluation of problem pits revealed other factors than the plastic liners that helped to create the burial pit problems. These are:
1. Carcasses were placed in the pit to a depth of 4 to 6 feet.
2. Diversion ditches in some cases were not established at time of burial to prevent surface water running into the burial pit.
3. Porous material was not used at the base of the pit to absorb liquids.
4. No one had been assigned from the Task Force to see that the burial site construction specifications were being complied with at the time of design of the pit or during the burying process.

The following burial pit procedures were approved by all waste management agencies and immediately implemented at all new burial sites.

**Approved Burial Pit Procedures**

1. The site shall be approved by the Health Department, Water Control Board and Soil Conservation Service. The site will be free of fissured limestone and highly porous soil and with a minimum of 5 feet from the base of the pit to ground water.

2. Excavate the pit to a depth of 8 to 10 feet with side slopes of about 1/2:1 and ramp one end for access. During excavation, keep top soil separate for capping the finished pit and clay for the compacted base.

3. Limit the pit width to 20 feet. If additional capacity is needed, parallel pits can be constructed.

4. Place 1 foot of soil (at least 30% clay content) in base of pit and against the sides of the pit to the expected height of the carcass layer.

5. Place porous material over the clay to 50% of depth of birds such as hay or straw to absorb fluids in the burial pit.

6. Place carcasses to a uniform depth of no more than 2 feet.

7. Fill remainder of pit with excavated soil minimizing compaction. In filling the pit compensate for the carcass layer plus 10% of the loose fill depth.

8. Cover the cap with 6 inches of top soil.

9. Upslope runoff water must be diverted around the pit so it won’t run into the pit. Runoff from the cap should be managed.

10. An inspector shall be on site during all phases of pit excavation and backfilling to assure that the recommended procedures are followed.

11. Completed burial pits should be inspected on a weekly basis for at least four (4) weeks to assure that integrity is maintained.

**NOTE:** Innoculation of the carcasses with lactobacillus before covering the pit would significantly lower the pH of the decomposing carcasses. This would speed inactivation of the avian influenza virus. It would also inhibit activity of methane forming bacteria and reduce gas formation.

With the corrected procedures for burial pit design and management, we did not experience any further problems when birds were buried. The following slide shows the approved burial pit design.
Remedial Procedures for Problem Disposal Pits

The pit cap should be reshaped to retain surfaced fluids on the cap. Surfaced fluids should be covered with an absorbant material such as woodshavings, woodchips, sawdust or bark and capped with 6 to 12 inches of soil. The volume of porous material should be 3 times the estimated amount of fluids that may ultimately rise to this level. The absorbing material will supply energy to soil microorganisms to quickly decompose and stabilize the leachate.

Digester-Fermenter

The AI Task Force in Virginia tested an alternate method for disposing of poultry carcasses through the use of a digester fermenter. The initial test was on spent hens.

The process is similar to that used for producing silage. A fermentation process lowers the acidity, killing bacteria and viruses in the product.

The procedures were as follows. The birds are ground and 20 to 30 percent corn mash is added along with 1 percent lactobacillus in whey to start the fermentation process. The resulting anaerobic fermentation reduces the pH to 3.5 in several days. The product smells like silage. It can be dried and fed directly to livestock, used as fertilizer or rendered. The entire process takes about 3 to 10 days depending on atmospheric temperature.

The equipment for this process was assembled for the task force on a flatbed trailer to make it mobile.

The process was developed at the University of Georgia under the leadership of Dr. Charles Dobbins, Head of the Veterinary Extension Service.

The equipment worked satisfactorily although feet and heads of birds were not always ground as it was hoped they would be. The present equipment needs to have the capability to process more birds in a day's
time than the present design. I have been advised that the present design is being revamped to increase the speed of the process as well as improving the grinding operation.

**Private Land**

Because the county landfill or rendering was not available to us, it was obvious we needed private, state or federal land to bury infected flocks when such flocks could not be buried on the infected premises. Immediately we investigated the feasibility and the availability of state-owned land, land in George Washington National Forest and private-owned land. We were very fortunate to find several tracks of private land that were used to bury 13 flocks. The state land we examined in the county only had a very small area of land approved for burial, but was never used. Extensive efforts were made towards the use of the National Forest land. This small track of land, approximately 3 acres, in the National Forest was finally approved but was never used because of the cost to develop and maintain, adverse public reaction, the decrease in the number of infected flocks disclosed at that time and the availability of private land.

I am sure the cost to design a pit and the total cost of burial would be of interest to you. These averages were from cost figures for 36 farms. The average cost for excavation of a burial pit was $2,395. The average cost for disposing of poultry and materials in the pit was $3,324, for a total cost of $5,719 per pit. The average cost to revamp a cap was $600 per pit.

**County Ordinance**

The task force planned to bury an infected flock in an adjacent county but still in the quarantined area. Before this occurred the county involved passed the following ordinance:

4-3.1 Importation of diseased fowls and carcasses of diseased fowls prohibited.

It shall be unlawful for any person knowingly to import into Augusta County diseased fowls or carcasses of diseased fowls, nor shall such diseased fowls or carcasses of diseased fowls be knowingly hauled or transported into or through Augusta County without being properly permitted by the Department of Health and/or the Virginia Department of Agriculture and Consumer Services and with the concurrence of the Augusta County Board of Supervisors.

4-3.2 Disposal of diseased fowls off site prohibited.

It shall be unlawful for any person knowingly to deposit, dump or buy diseased fowls or carcasses of diseased fowls on property located within Augusta County unless the property is the site where said fowls were originally maintained and kept at the time the disease was detected.

This ordinance was never challenged by the State because of the availability of private land.
Summary:

The following methods were used to dispose of AI infected flocks in Virginia:

- 13 flocks were buried in the county landfill
- 55 flocks were buried at the infected premises or in private land
- 1 flock at the beginning of the outbreak was sold to slaughter prior to laboratory confirmation
- In 1 flock part of the birds were burned and the remainder were buried.

Serologically positive flocks were disposed of as follows:

- 1 serologically positive flock was sent to slaughter
- 3 serologically positive flocks were rendered

General Comments

This disease experience was financially devastating to the poultry owners and had a great impact emotionally to both the task force and poultry owners.

There were times that flock owners and poultry company personnel lost sight of the emotional effect this mission had on all task force personnel. Task force personnel numbering in the hundreds assumed this assignment taking them away from their distant homes and families for periods of weeks at a time.

It was impressive to witness and to be part of a dedicated group of both state and federal personnel. Virginia benefited from outstanding efforts towards the eradication of this devastating outbreak of avian influenza.

Because of a successful campaign the quarantine was lifted on September 14, 1984.

Conclusion

The following recommendations should be considered in future task forces when birds or animals must be disposed of as rapidly as possible to prevent further spread of the disease.

Burial:

Follow recommendations as outlined for burial pit design and locate immediately private land if other methods of disposal cannot be utilized.

Solid Waste Disposal Systems:

In placing the quarantine, if at all possible have a rendering plant, incinerator, and landfills in the quarantined area that could be used to dispose of infected flocks or herds.

Rendering:

The temperature achieved in the rendering process is sufficient to
destroy the AI virus. The Task Force directors and many authorities consider rendering as the best means of disposal of infected flocks. I feel that the burial pit problems as well as the time element in destroying flocks would not have been a concern in depopulation if rendering were used as the means for disposal of infected flocks from the very start of the AI outbreak as was used in the VVND outbreak in California several years ago.

It is imperative that studies by APHIS, with coordinating States and allied industries be conducted immediately on rendered products to determine if the products are free from contamination by viruses or other infectious agents via normal plant operations. If these studies determine that contamination is not a problem, then we need to inform the industry and animal health officials in all States.

There are portable rendering equipment systems that need to be evaluated as to practicality and if they meet the acceptable standards of the rendering process.

We also need further studies on the fermentation process to determine if the process is applicable when large numbers of animals or birds need to be disposed of in a short period of time.

All of these studies need to be completed as rapidly as possible if we are going to be successful in the eradication of future outbreaks of exotic animal or poultry diseases in these United States.
Introduction

Eradication of a poultry disease such as lethal avian influenza is not easily accomplished. Success requires an understanding of the disease process, an availability of sufficient resources, and most importantly, the support of the affected industry. In the 1983–1984 lethal avian influenza outbreak, there was an additional ingredient for success and that was a true commitment to cooperation. This was especially apparent as individuals, representing various interests and viewpoints, gathered to discuss the current and future program policies and procedures. Even when the outcome was not favorable to all, a special bond was evident as demonstrated when the decision was fully supported in order to achieve a common goal—eradication.

History

The first diagnosis of avian influenza in this outbreak was made in two layer flocks near Lancaster, Pennsylvania, on April 22, 1983. The signs were clinically mild with moderate loss of production and usually less than 10 percent mortality. This was similar to the influenza often reported in turkeys in recent years in other States. The virus was classified at the National Veterinary Services Laboratories (NVSL), Ames, Iowa, as an H5N2 serotype of nonpathogenicity. The Pennsylvania State officials immediately embarked on a control program considering information developed during the First International Symposium on Avian Influenza.\(^1\) In addition, Pennsylvania State University provided diagnostic support and the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), provided the services of an epidemiologist.

Soon, with increased mortality (as high as 1,000 birds per premises per day), the involvement of layer and broiler flocks and the isolation (October 26, 1983) of a highly pathogenic form of the virus, the Pennsylvania Department of Agriculture and the affected industry, recognizing the seriousness of the disease, requested Federal participation. After much discussion between all interested parties and a full evaluation by a group of technical experts (consultants),\(^2\) USDA agreed to declare an Extraordinary Emergency, to activate the Northern Regional Emergency Animal Disease Eradication Organization (READEO), to pay 100 percent of the appraised fair-market value of birds destroyed (the Economic Research Service, USDA, established a table of fair-market values for all classes of poultry according to age and class) and to eradicate this highly pathogenic form of avian influenza. The State of Pennsylvania agreed to participate fully with available manpower and resources while the affected industry agreed to bear the cost of cleaning and disinfecting the infected premises.
A similar agreement was reached with the State of New Jersey (November 24, 1983) when a highly pathogenic H5N2 virus was isolated in a layer flock near Willow Grove, New Jersey. This virus was supposedly introduced by a contaminated feed truck which originated in the Pennsylvania quarantine area. In this case, the affected premises was immediately depopulated, and shortly thereafter, cleaned and disinfected. After completion of an indepth surveillance program with no evidence of disease spread, the State/Federal quarantine in New Jersey was released (March 6, 1984).

In Pennsylvania, the disease continued to spread. Mortality as high as 70 to 90 percent of a flock was noted. The poultry industries from the States of New York and New Jersey expressed concern and requested that the quarantines be strengthened to prevent the interstate movement of live poultry for slaughter from such areas. This was implemented.

In late November 1983, a poultry huckster apparently spread the disease into Virginia. The huckster supposedly hauled turkeys from several premises in the Shenandoah Valley to slaughter. These birds were transported in trucks and coops apparently contaminated by previous association with affected fowl originating in the outbreak area. Such coops and trucks apparently contaminated the premises and infected the numerous multiple age turkeys remaining. Earlier, this same individual was involved in bringing birds from the affected area of Pennsylvania into Maryland (these two premises were immediately depopulated by the State of Maryland) and into Franklin County, Pennsylvania. Although subsequent outbreaks were basically limited to southeast Pennsylvania and the Shenandoah Valley of Virginia, the disease did enter northeast Maryland one more time. This was an extension of area spread from Pennsylvania and, as in the past, the State officials of Maryland responded promptly. Federal indemnity was used in this instance and there were no additional findings of lethal avian influenza in their State.

Upon serological confirmation of avian influenza by the State of Virginia (Harrisonburg, Virginia) and the isolation of a nonpathogenic H5N2 virus by NVSL, the State of Virginia and affected industries immediately decided to eradicate the disease via depopulation of the infected flocks. When the potential magnitude of the outbreak was fully realized, they also requested Federal participation. In this, the technical experts concurred and emphasized the importance of eradicating all forms of H5 associated with the 1983–1984 Pennsylvania outbreak. Although APHIS agreed to expand the program operation to include the Virginia outbreak, they decided not to declare an extraordinary emergency in Virginia, but rather to work in essence under Virginia’s laws and regulations while providing funds for indemnity as provided by the emergency authority of the Act of 1962. (A similar arrangement was implemented during the last case in Maryland when Federal funds for indemnity were provided.)

**Program Activities**

Once a flock of birds was declared infected, it was appraised for indem-
nity purposes, humanely destroyed by the use of carbon dioxide gas, and buried either on the farm or, because of inappropriate soils, in a landfill or other appropriate site. (Unfortunately, rendering of infected birds was not feasible in this outbreak.) The repopulation of the premises was only permitted after the task force had approved the owner's cleaning and disinfection process, had received negative virus isolation results on environmental swabs and had been assured that the premises had been kept vacant of birds for a minimum of 30 days.

As the eradication program progressed, we learned more about how the disease spread. Biosecurity training keyed on premises security and the movement of people, equipment, and trucks as well as egg flats and cartons.

When the influenza virus was isolated from within the egg, it was decided that (considering the movement of eggs) only table eggs would be permitted to move outside the quarantine area. Furthermore, such movement would only be permitted from those flocks sampled on a weekly basis using an approved procedure and found to be negative for avian influenza.

Soon it became evident there was no satisfactory clinical laboratory procedure to differentiate between flocks infected with the highly pathogenic strains of H5N2 influenza virus and flocks infected with H5N2 virus which would not kill laboratory inoculated chickens. In many instances, there seemed to be mixed viral populations. In addition, the clinical disease syndrome in the Virginia Poultry Industry (turkeys, broilers, and later a few layers) was very mild. In fact, the industry in Virginia seriously questioned whether the eradication process was more severe than the disease itself. Therefore, at the request of the Governor of Virginia, USDA appointed a "Blue Ribbon Committee" to review the possibility of using vaccine and/or other options that might be considered as alternatives. This committee recommendation reaffirmed the technical experts (consultants) committee that vaccine should not be used in the current outbreak. Furthermore, they emphasized that use of vaccine could severely interfere with the eradication program.

Other committees were appointed as specific needs were identified. For instance, a special committee was established in Virginia to review the task force and program effectiveness. In addition, a special team was asked to review and critique the epidemiological section of the Pennsylvania READEO (task force) team.

When H5N2 influenza antibodies were found in flocks which were not known to have experienced illness, a drop in production or from which no H5N2 virus could be isolated, it became clear that we needed to address the problem of serologically positive flocks. In a few seropositive flocks, virus was recovered repeatedly over a period of several months before the decision was made to include all forms of H5 associated with the outbreak. In addition, it was questioned whether the 22 countries that had embargoed our poultry and poultry products would in fact ever recognize the United States as free of lethal avian influenza if serological positive flocks...
still remained. Again, based on the recommendations of the technical experts, USDA decided it was in the best interest of the poultry industry to depopulate all flocks within the quarantined areas containing birds with antibodies against lethal avian influenza virus.

**Laboratory Support**

Both State and Federal laboratories shared the workload in providing laboratory support. NVSL served as the reference center and processed most of the specimens requiring virus isolation including diagnostic investigations, dead bird surveillance, and specific studies including collections of insects and environmental swabs. In total, approximately 200,000 samples consisting of cloacal swabs, tracheal swabs, and/or necropsy tissues were submitted to NVSL for virus isolation. In addition, approximately 24,000 blood and/or egg yolk samples were submitted for serology.

In Pennsylvania, the State diagnostic laboratories in New Bolton Center, Summerville, Pennsylvania State University and Delaware Valley College processed over 30,000 blood samples (New Bolton Center only) and over 350,000 egg yolk samples for serology. In Virginia, the State diagnostic laboratory in Harrisonburg, Virginia, processed over 80,000 blood samples and 26,000 egg yolk samples for serology. Also, St. Jude Children’s Research Hospital in Memphis, Tennessee, processed over 20,000 samples including 7,600 samples for virus isolation and 4,700 blood samples for serology collected by the Southeastern Cooperative Wildlife Disease Study (SCWDS), Athens, Georgia, from wild, migratory birds.

**Wildlife**

Historically, influenza outbreaks in poultry have usually been attributed to sources in wildlife. Since the initial outbreak was in an area noted for farm ponds and the presence of numerous migrating waterfowl, it was decided to check waterfowl in the area for the avian influenza virus. SCWDS and St. Jude Children’s Research Hospital, Memphis, Tennessee, sampled over 4,000 wild birds during the first few weeks of the program. In all of these samples, the highly pathogenic avian influenza virus was only isolated from one pen-reared chukar and one pheasant found dead on a roadside. Antibodies, however, against H5 influenza A virus were present in 39 percent of the migratory waterfowl sampled. Based on these results, it was concluded that wild, migrating, free-flying birds were not a source of virus which if present would likely spread the disease. We did not discount, however, that the wild, migrating waterfowl could have been a source of disease introduction.

Laboratory studies conducted by Dr. Robert Webster also disclosed that the poultry pathogenic H5N2 virus from Pennsylvania would not replicate in ducks to the extent they could serve as reservoirs and vectors for this virus. This information indicated the antibodies in free-flying waterfowl were likely due to a different strain of this virus than the one causing losses in poultry, but confirmation required isolating the H5N2 virus from waterfowl. To date, this has not been possible.
Specimens collected by the SCWDS from juvenile mallards on the Middle Creek Game Management Area since August 8, 1984, have repeatedly yielded avian influenza viruses. Studies, however, indicate that these viruses are H6N2, H4N2, and H1N2, but not H5N2. The isolates are not pathogenic for poultry and fail to kill chick embryos in 72 hours; whereas, poultry pathogenic strains typically kill chick embryos in 48 hours.

Statistics

The last flock from which a virus was isolated was depopulated in Pennsylvania on March 31, 1984, and in Virginia on July 1, 1984. The last flock with a serological response was depopulated in Pennsylvania on September 12, 1984, and in Virginia on July 27, 1984. A total of 448 flocks with 17,023,095 birds were depopulated with Federal participation for a total of $41,641,292 paid in indemnity. The area quarantine in Virginia was released on September 14, 1984, and in Pennsylvania all except for seven premises quarantines were released on October 4, 1984. At that time, two of the seven still needed to be cleaned and disinfected and all seven needed to fulfill the requirements of environmental sampling as well as the 30-day period of time without birds on the premises. As of October 10, 1984, direct Federal costs including indemnity and support costs totaled $61,845,419.

Surveillance

Although the eradication process is complete and the State/Federal quarantines released, surveillance continues. A formal surveillance program in the previously quarantined areas will be funded for at least 6 months from the date the areas were released from quarantine. In addition, many other States, and especially the Delmarva Poultry Industry, are continuing with a sound surveillance program established early in the eradication program.

Program Assistance and Support

The successful eradication of lethal avian influenza required the assistance and support of numerous State, Federal, and/or local organizations. For example, the Food Safety and Inspection Service (FSIS) continued to assure the wholesomeness of the poultry slaughtered in the quarantined areas while strictly enforcing the biosecurity measures necessary to prevent disease spread. The Agricultural Research Service answered many of the important questions raised concerning the disease agent and possible means of treatment and/or control. (Additional work in this area still needs to be supported.) When we consider the needs of keeping the industry informed, the Extension Service was most valuable. In addition, Pennsylvania State University, the University of Maryland, and Virginia Polytechnic Institute provided assistance in many ways. With the added support of the many State and local organizations, you can begin to understand why the eradication program succeeded effectively.
Military

Considering the magnitude and scope of the effort necessary to eradicate this outbreak of lethal avian influenza, it is reassuring to know that the military is willing to assist. The military provided over 200 Department of Defense (DOD) employees, of which over 40 were veterinarians with expertise in areas such as diagnostics, pathology, and epidemiology. In addition, the military provided trucks, office equipment, field equipment, analytic equipment, as well as communications equipment so vital to an operation of this size. It is obvious with this kind of support that the military is interested in protecting our poultry industry from the ravages of disease.

Impact on State/Federal Cooperative Programs

We must also consider the impact of the lethal avian influenza program on our continuing State/Federal cooperative programs nationwide. With approximately 1,083 employees, or over 40 percent of the work force of Veterinary Services, APHIS, detailed for varying periods to the Pennsylvania and Virginia task force operations, we know the impact has been severe. Fortunately, the States have been supportive and recognize the need to redirect resources under such circumstances as those dealt with in this particular outbreak.

Economic

Although the eradication process was expensive and many producers suffered severe financial losses not covered by indemnity, the costs of living with such a disease could have been devastating. In fact, a recent Economic Research Service study estimates that if an eradication program were not in place, the potential additional costs to the consumer could have been as high as $5 billion or more. (This cost was established considering the potential spread in the poultry industry east of the Mississippi River during the first 6 months of an outbreak). If this is true, it is important that we remember what we have learned—the importance of divorcing the poultry industry from any association with wild, migrating birds, the importance of strict premises biosecurity, and the importance of controlling the movements of dirty coops, egg flats, and/or cartons. Our efforts today will assure a prosperous tomorrow. In this case, a prosperity based on the health and well-being of our most valued poultry industry.

1. Proceedings of the First International Symposium on Avian Influenza, Beltsville, Maryland, April 22–24, 1981
2. Technical Consultants
   a. Dr. H. E. Goldstein, Chairman, Ohio State Veterinarian, Columbus, Ohio
   b. Dr. R. A. Bankowski, University of California, Davis, California
   c. Dr. Charles Beard, Southeast Poultry Research Station, Agricultural Research Service, Athens, Georgia
   d. Dr. Frank R. Craig, Perdue Farms, Inc., Salisbury, Maryland
e. Dr. Bernard Easterday, University of Wisconsin, Madison, Wisconsin
f. Dr. L. C. Grumbles, Texas A&M University, College Station, Texas
g. Dr. Frank Hayes, University of Georgia, Athens, Georgia
h. Dr. Richard M. McCapes, University of California, Davis, California
i. Dr. B. S. Pomeroy, University of Minnesota, St. Paul, Minnesota
j. Dr. Robert G. Webster, World Health Organization Influenza Center, St. Jude Children’s Research Hospital, Memphis, Tennessee

3. Blue Ribbon Committee
a. Dr. Kenneth Hook (Executive Officer), Animal and Plant Health Inspection Service, Washington, DC
b. Dr. James Arthur, Hy-Line International, Des Moines, Iowa
c. Dr. Max Brugh, National Poultry Research Center, Agricultural Research Service, Athens, Georgia
d. Mr. Monte Frazier, Arbor Acres, Glastonbury, Connecticut
e. Dr. John Newman, University of Minnesota, St. Paul, Minnesota
f. Dr. Duane Olsen, Foster Farms, Puyallup, Washington
g. Ms. Annie K. Prestwood, University of Georgia, Athens, Georgia
h. Dr. Stan Vezey, University of Georgia, Athens, Georgia
i. Dr. Gary Waters, DeKalb Agriculture Research, DeKalb, Illinois
j. Mr. Carl Weston, Hubbard Farms, Inc., Walpole, New Hampshire

4. Virginia Avian Influenza Task Force Review
a. Dr. Max Brugh, National Poultry Research Center, Agricultural Research Service, Athens, Georgia
b. Mr. Harold Ford, Southeastern Poultry and Egg Association, Decatur, Georgia
c. Dr. Lonnie King, Animal and Plant Health Inspection Service, Hyattsville, Maryland
d. Dr. Jack Lamont, California Department of Food and Agriculture (Retired), Sacramento, California
e. Dr. William D. Miller, Virginia Department of Agriculture and Consumer Services, Lynchburg, Virginia
f. Dr. John Wolford, Virginia Polytechnic Institute, Blacksburg, Virginia

5. Pennsylvania Avian Influenza Task Force Epidemiological Review
a. Dr. Max Brugh, National Poultry Research Station, Agricultural Research Service, Athens, Georgia
b. Dr. William Utterback, Animal and Plant Health Inspection Service, Sacramento, California
c. Dr. Bernard C. Zecha, Animal and Plant Health Inspection Service, Cheyenne, Wyoming
Avian Influenza
Quarantine Area in Pennsylvania

- Original quarantine area—11/4/83
- Extended quarantine #1—11/16/83
- Extended quarantine #2—11/21/83
- Extended quarantine #3—12/27/83
- Extended quarantine #4—2/10/84
Avian Influenza

New Jersey Quarantine Area

Positive premises
Quarantine:

- Original—11/23/83
- Reduced—12/30/83
Avian Influenza
Federal Quarantine Area
in Maryland
Avian Influenza
Federal Quarantine Area in Virginia
Avian Influenza

Number of Positive Flocks by Date of First Sick: Pennsylvania

Weekly Accumulation

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1983-1984 AVIAN INFLUENZA OUTBREAK
Avian Influenza

Positive Flocks by Date of First Sick: Virginia

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<td>1 442</td>
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<td>Mar. 4</td>
<td>10</td>
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Lethal Avian Influenza
Chronology

April 22, 1983 — Mild form of avian influenza diagnosed in two Lancaster County, Pennsylvania, layer flocks (H5N2).

October 8, 1983 — Increased mortality (30%), drop in egg production noted, broilers and layer flocks involved.

October 26, 1983 — Laboratory criteria standardized to meet April 22–24, 1981, criteria established by International Symposium on Avian Influenza.

October 27, 1983 — Mortality criteria for highly pathogenic avian influenza (HPAI) met at the National Veterinary Services Laboratories (NVSL), Ames, Iowa. Reisolation pending.

October 31, 1983 — Criteria for HPAI met at NVSL, with reisolation complete.

October 31–November 1, 1983 — Meeting of avian influenza experts as technical collaborators. Recommended declaration of emergency, research, State regulatory officials make avian influenza to be a reportable disease, immediately establish a Federal quarantine around the affected area, and State-Federal officials take every step to safely dispose of all dead and diseased birds. Also, recommended continued use of this committee as a collaborating body.


November 4, 1983 — Meeting with industry officials.


November 4, 1983 — Task force activated to enforce Federal quarantine.


November 9, 1983 — Office of Management and Budget (OMB) approved $12.5 million.

November 12, 1983 — First HPAI infected flock depopulated.


November 21, 1983 — Pennsylvania Federal quarantine expanded further.


November 23, 1983 — Federal quarantine imposed in New Jersey due to layer flock declared positive.
November 24, 1983 — HPAI flock in New Jersey depopulated.
December 6, 1983 — Technical collaborators met.
December 8, 1983 — OMB approved additional funding of $15.2 million, to total $27.7 million.
December 8, 1983 — Regulations imposed prohibiting interstate movement of live poultry, hatching eggs, and embryonated eggs out of Federally quarantined area.
December 27, 1983 — Pennsylvania Federal quarantine expanded further.
December 30, 1983 — New Jersey Federal quarantine reduced from 400 square miles to approximately 12 square miles. No evidence of spread from initial positive flock.
January 25, 1984 — OMB approved additional funding of $34 million, to total $61.7 million.
January 25, 1984 — Extraordinary Emergency in Pennsylvania and New Jersey amended to include all forms of H5 Avian Influenza (Type A) associated with the outbreak (lethal avian influenza).
January 25, 1984 — 9 CFR, Part 53 regulations amended to include avian influenza at 100 percent Federal indemnity level. This is necessary for program activities to be initiated without additional Declarations of Extraordinary Emergency in States other than Pennsylvania and New Jersey.
January 25, 1984 — Last infected flock in Maryland depopulated.
January 27, 1984 — 9 CFR, Part 81 regulations amended to Federally quarantine portions of Maryland and Virginia, as well as to include lethal avian influenza.
February 29, 1984 — A portion of the Pennsylvania Federal quarantined area (Franklin County) was determined to be free of lethal avian influenza and released.
March 2, 1984 — Regulations imposed providing for interstate movement of table eggs only from unaffected flocks, as determined by an organized weekly flock surveillance program, including sampling for virus and/or antibodies.
March 6, 1984 — The remaining portion of the New Jersey Federal quarantine area was determined to be free of lethal avian influenza and released. In ad-
dition, the Extraordinary Emergency provisions in New Jersey were removed.

March 31, 1984 — Last flock with a virus isolation depopulated in Pennsylvania.
April 2, 1984 — Adjusted indemnity rates, retroactive to November 9, 1983, became effective.
April 5, 1984 — The quarantined portion of Cecil County, Maryland, was released.
April 9, 1984 — Depopulation of low-path flocks was initiated.
June 8, 1984 — The quarantined area in Pennsylvania, west of the Susquehanna River, was released from quarantine.
June 15, 1984 — Additional $5 million approved for avian influenza.
July 1, 1984 — Last flock with a virus isolation depopulated in Virginia.
September 14, 1984 — Virginia State-Federal quarantine was lifted.
October 4, 1984 — Pennsylvania State-Federal quarantine was lifted.

Recommendations From Avian Influenza Consultants
February 8, 1984

The committee reaffirms the position that the use of avian influenza vaccine at the present time would jeopardize the national eradication effort.

This Committee decision was predicated upon the following evaluations of advantages and disadvantages involved with the goal of eradicating the lethal avian influenza virus.

Avian Influenza Vaccine

Advantages
1. Reduce disease losses in the vaccinated flocks
2. Reduce shed of virus
3. May decrease susceptibility of flock to infection

Disadvantages
1. Vaccinated flocks can be infected, infected flocks will be difficult to detect, thus infected and shedding flocks will be treated as clean, allowing the virus to spread.
2. Removes serology as a tool to monitor an area for evidence of flu (may be important in removing a quarantine)
3. Will make it difficult to convince importers (international and interstate) that an area is indeed free of the virus
4. Virus shedding flocks when detected are depopulated in a quarantine area, regardless of vaccination history
5. There is a risk of spreading avian influenza virus when vaccine service crews move from flock to flock
6. Killed vaccine (oil emulsion) use is incompatible with broiler production by regulations which require 42 days holding prior to slaughter
7. Use of vaccine results in a relaxation of security and sanitation compliance due to a false sense of security provided by vaccine
8. The only vaccine available for interstate distribution is an experimental vaccine
9. Vaccine use associated with the eradication effort will delay the release of the quarantine by extending the length of the program with masked infection.
MOLECULAR CHARACTERIZATION OF THE
A/CHICKEN/PENNSYLVANIA/83
(H5N2) INFLUENZA VIRUSES

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SUMMARY

In April 1983, an influenza virus of low virulence appeared in chickens in Pennsylvania, USA. Subsequently, in October 1983, the virus became virulent and caused high mortality in poultry. The causative agent has been identified as an influenza virus of the H5N2 serotype. Analysis of the RNA of the viruses isolated in April and October, by gel migration and RNA-RNA hybridization suggested that these strains were very closely related. Polyacrylamide gel analysis of the early (avirulent) isolates demonstrated the presence of low molecular weight RNA bands indicative of defective-interfering (DI) particles. These RNAs were not present in the virulent isolates.

The avirulent virus produced plaques and the HA was cleaved only in the presence of trypsin. In contrast, the virulent virus produced plaques and cleavage of the HA precursor into HA1 and HA2 occurred in the presence or absence of trypsin. The apparent molecular weight of the HA1 from the avirulent virus was higher than that from the virulent virus. The amino acid sequences of the HAs from the two viruses were compared by sequencing their respective HA genes. The nucleotide sequence coding for the processed HA polypeptide contained 1,641 nucleotides specifying a protein of 547 amino acids. The amino acid sequences of the virulent and avirulent viruses were indistinguishable through the connecting peptide region, indicating that the difference in cleavability of the H5 HA is not directly attributed to the amino acid sequence of the connecting peptide. Four of seven nucleotide changes resulted in amino acid changes at residues 13, 69 and 123 of HA1 and at residue 501 of the HA2 polypeptide.

Since there were no deletions or insertions in the amino acid sequence of the virulent or avirulent viruses, the possibility exists that the difference in molecular weight is due to loss of a carbohydrate side chain in the virulent strain.

The results suggest that the original avirulent virus was probably derived from influenza viruses from wild birds and that the virulent strain was derived from the avirulent strain by selective adaptation rather than by recombination or the introduction of a new virus into the population. This adaptation may have involved the loss of defective RNAs, as well as mutations, and thus provides a possible model for a role of defective-interfering particles in nature.
INTRODUCTION

Influenza virus infections of chickens occur relatively infrequently in the United States; an outbreak of disease associated with high mortality occurred in 1924–25 and again in 1929 (Beaudette et al., 1929) and were caused by a fowl plague-like virus. The last highly pathogenic virus outbreak in domestic poultry in North America occurred in turkeys and was caused by A/turkey/Ontario/6632/66 (H5N9). This virus was so pathogenic that the outbreak of disease was self-limiting (Lang et al., 1968). Other outbreaks of disease, with mild respiratory infection and low mortality have occurred infrequently in chickens, — a self-limiting outbreak of disease in chickens in Alabama in 1975 was associated with an H4N8 influenza virus.

In April 1983, an H5N2 influenza A virus appeared in chickens in Pennsylvania, USA and caused low mortality. Subsequently, in October 1983, virulent influenza viruses were isolated from chickens, that caused up to 100% mortality. The virus was spread to Virginia and limited outbreaks of disease occurred in New Jersey and Maryland; mainly chickens and turkeys were infected, but limited numbers of guinea fowl and chukars were also affected. Depopulation of infected poultry by a State, Federal and Industry Influenza Task Force was successful in eradicating the virus by destruction of over 17 million birds at a cost of approximately $61 million.

Of the 13 HA serotypes of influenza virus, only the H5 and H7 viruses are highly pathogenic for avian species (Bosch et al., 1979). Studies on fowl plague virus (FPV) indicate that virulence is polygenic but that the HA gene is a key determinant (Rott et al., 1979). In this report we will utilize the information gained from molecular characterization of the H5N2 influenza viruses in Pennsylvania to provide information on where the viruses came from, and the changes in the viruses associated with acquisition of virulence.

MATERIALS AND METHODS

The viruses used in this study were handled in a P3 containment laboratory at St. Jude Children’s Research Hospital that was approved for such use by the United States Department of Agriculture (USDA).

Viruses and Viral RNA

The avirulent A/Chicken/Pennsylvania/1/83 (H5N2) [Chick/Penn/83] virus isolated from the index case in April 1983 and designated UP8125 by National Veterinary Services Laboratory (NVSL), Ames, Iowa was used as the prototype avirulent virus. The virulent A/Chicken/Pennsylvania/1370/83 (H5N2) virus isolated in October 1983, was used as the prototype virulent virus. Viruses were grown in 11-day old embryonated chicken eggs and were purified by differential sedimentation through 25-70% sucrose gradient in a Beckman SW 28 rotor. Virion RNA was isolated by treatment of purified virus with protease K and sodium dodecyl sulfate, followed by extraction with phenol-chloroform (1:1) as described pre-
viously (Bean et al., 1980). The methods used for RNA analysis and nucleic acid sequencing were described in detail (Kawaoka et al., 1984).

**RESULTS**

**Plaque formation of avirulent and virulent Chick/Penn/83 influenza viruses.**

Studies by Bosch et al. (1979) have demonstrated that the virulence of avian influenza viruses such as FPV can be correlated with their ability to produce plaques in tissue culture systems in the absence of trypsin. The virulent and avirulent Chick/Penn/83 (H5N2) influenza viruses were therefore examined for their ability to produce plaques in tissue culture in the presence or absence of trypsin. The virulent virus (Chick/Penn/1370/83) produced plaques (1-2 mm in diameter) in chicken fibroblast cultures in the presence or absence of trypsin. The avirulent virus, on the other hand, produced plaques only in the presence of trypsin. These results indicate that the highly virulent Chick/Penn/83 (H5N2) influenza virus is similar to FPV in its ability to produce plaques and cause high mortality in chickens. The results also suggest that the virulent virus can cleave the HA precursor into HA1 and HA2 in the absence of trypsin.

**Cleavage of the HA molecule and differences in molecular weight of the HA1 polypeptide between virulent and avirulent Chick/Penn/83 influenza viruses.**

The hemagglutinin of the virulent and avirulent Chick/Penn/83 influenza viruses, produced in cell culture, was examined by radioimmunoprecipitation in order to determine if cleavage of the HA molecule correlated with plaque formation. Cell cultures infected with the virulent or avirulent viruses were labelled with 35S methionine and the cell lysate was immunoprecipitated with a monoclonal antibody specific for the HA molecule of Chick/Penn/1/83. The results of SDS-PAGE analysis (Fig. 1) show that the HA polypeptide of the virulent virus was cleaved in the presence or absence of trypsin and both HA1 and HA2 were detected on the gels (Fig. 1). The avirulent influenza viruses, grown in the absence of trypsin, produced a single band of precipitation corresponding to uncleaved HA, however, when the virus was grown in the presence of trypsin, both HA1 and HA2 were detected. The HA1 polypeptide from the avirulent viruses also showed a reduction in mobility as compared with the HA1 from the virulent virus, suggesting that the molecular weight of the HA1 from the avirulent virus was higher than for the virulent virus. The HA2 polypeptide from the two viruses had identical mobilities.

**Analysis of virus RNAs from A/Chick/Penn/83 influenza viruses.**

Studies by Sriram et al. (1980), have shown that avian influenza viruses possessing antigenically indistinguishable HA and NA glycoproteins can differ significantly in the migration of their RNA segments. The results in Figure 2 show that the RNA segments from the virulent A/Chick/Penn/1370/83 viruses were indistinguishable from the electrophoretic mobilities of the RNAs from the avirulent A/Chick/Penn/1/83
virus. However, the A/Chick/Penn/1/83 showed additional low molecular weight RNAs not found in the A/Chick/Penn/1370/83 virus. The additional low molecular weight RNA segments found in A/Chick/Penn/1/83 may be defective-interfering (DI) RNA species as described by Davis et al. (1980). The similar migration patterns of the virion RNAs in the avirulent and virulent chick/Penn/83 influenza viruses suggests that the virulent strain was probably not derived by reassortment with other influenza A viruses.

To confirm this, oligonucleotide mapping analyses were done on an avirulent (A/Chick/Penn/1/83) and on a virulent isolate (A/Chick/Penn/1370/83). The results (Fig. 3) clearly show that the virulent and avirulent strains are very closely related and suggest that the virulent form of the virus was derived from the avirulent form without the introduction of genes from another virus.

Comparison of the nucleotide and amino acid sequences of the HA from avirulent and virulent Chick/Penn/83 influenza viruses.

The HA genes from both virulent and avirulent viruses were sequenced by the dideoxynucleotide chain termination method (Sanger et al., 1977; Air, 1979). A series of nine synthetic oligonucleotides were used to prime reverse transcription of virion RNAs in a sequential fashion allowing us to "walk" down the gene until the sequences were complete. The 1,641 nucleotide sequence and its deduced amino acid sequences included all of HA1 and HA2 but did not include the signal peptide sequence.

The HA gene of Chick/Penn has the potential to code for a polypeptide of 547 amino acids, and depending on the location of the cleavage site, HA1 would contain a maximum of 324 amino acids and HA2 222 amino acids. Comparison of the nucleotide sequences of the virulent and avirulent HAs showed that there were no insertions or deletions between the two viruses. A total of seven nucleotide changes were detectable between the virulent and avirulent H5 virus sequences, five in HA1 and two in HA2. Three of the seven were third base changes and were silent. The other four mutations resulted in amino acid changes in residues 13, 69 and 123 of HA1 and at residue 501 in HA2. The mutations at residues 69 and 123 of HA1 and 501 in HA2 polypeptides are conservative. The mutations at residue 13, however, is remarkable in that it could alter a potential carbohydrate attachment site.

The amino acid sequences of the connecting peptide regions of these molecules are identical, (Table 1) suggesting that the primary sequence alone does not determine cleavability of the HA. The Chick/Penn HAs do not appear to possess a multi-residue connecting peptide like FPV, but do contain three basic lysine residues at the carboxyl terminus of HA1. Although the sites for HA cleavage in this molecule are unknown, these basic residues could function in a manner analogous to those in FPV i.e., providing appropriate sites for cleavage activation by the sequential action of a trypsin-like endopeptidase and carboxypeptidase B (Garten et al., 1982).
Location of the amino acid changes in the hemagglutinin of Chick/Penn viruses on the three dimensional structure.

To relate the amino acid sequence of the Chick/Penn HA to the three dimensional structure of the H3 hemagglutinin (Wilson et al., 1981), and to locate the changes, the amino acid sequence was aligned as shown in Fig. 4. After alignment with the H3 hemagglutinin, the amino acid changes that occurred at residues 13, 69 and 123 in the HA1 sequence of Chick/Penn are located at positions 23, 78 and 128 in the H3 numbering system.

In the three dimensional structure (Fig. 4), these changes are located in the middle of the stalk (residue 23) in the vicinity of the cleavage site between HA1 and HA2 and on the surface of the globular head (residues 78 and 128). The amino acid change at residue 177 (H3 numbering) of Chick/Penn HA2 was not located, for it lies beyond the carboxyl terminus of H3 (residue 175) derived from bromelain released HA (Wilson et al., 1981). Residue 78 (H3 numbering) is part of antigenic region E on the H3 molecule and residue 128 is in antigenic site B (Daniels et al., 1983). Studies are in progress to determine if the antigenic differences between the avirulent and virulent Chick/Penn viruses detectable with monoclonal antibodies are due to changes in sequence at these sites.

Genetic origin.

To determine the origin of these viruses, a panel of influenza strains was compared with one of the early isolates, Chick/Penn/3/83, by competitive RNA-RNA hybridization using the RNA segments coding for the non-surface proteins. The panel consisted of 20 avian influenza viruses, including three recent avian strains of the H5N2 serotype (Dk/Minn/1545/81, Mallard/NY/189/82, and Ty/Minn/1180/80), two equine and one human virus strain. With each RNA segment, the strains showing closest homology were from either wild or domestic birds from North America. One of the H5N2 strains, Ty/Minn/1180/80, showed closest homology with segments 5 and 7, but its segment 8 showed no competition with that of Chick/Penn. The virus strain with the most closely related "P" genes (RNA segments 1, 2 and 3 isolated as a group) was Gull/MD/5/77. The mammalian strains competed poorly with the Chick/Penn RNAs with the exception of segment 7, and to a lesser extent, segment 8 of equine/Miami.

Experimental infection of ducks.

Since H5N2 viruses have been isolated from asymptomatic infections in wild ducks (Hinshaw et al., 1980) and some of the gene segments of the Chick/Penn influenza viruses were genetically closely related to influenza viruses from ducks, studies were done to determine of the Chick/Penn/83 viruses would replicate in and cause disease in ducks. The avirulent (Chick/Penn/1/83) and the virulent virus (Chick/Penn/1370/83) were inoculated into ducks by the oral-tracheal or rectal routes. After oral-tracheal inoculation, virus was isolated from the trachea of some of the ducks for only one day postinfection (Table 2), and virus was isolated from the feces of one of 12 birds inoculated with the virulent virus. Virus was not
isolated from other organs. The majority of the ducks had a transitory infection, for 90% of the animals seroconverted. After rectal inoculation, virus was recovered only from the bursal tissue. Neither the avirulent or virulent Chick/Penn/83 viruses caused disease signs in ducks.

DISCUSSION

In the present study, we have investigated the changes in the H5 hemagglutinin molecule that occurred during acquisition of virulence by the Chick/Penn/83 virus, always keeping in mind that virulence is a polygenic character in influenza viruses. The studies establish that between April and October 1983, the H5N2 virus acquired the ability to produce plaques in tissue culture without trypsin; the gene coding for the HA accumulated seven nucleotide changes that resulted in four amino acid substitutions in the HA molecule (three nucleotide changes were silent). Additionally, the electrophoretic mobility of the HA1 polypeptide from the avirulent virus was lower than for the virulent virus and this probably resulted from differences in glycosylation.

Alignment of the amino acid sequence of the Chick/Penn influenza viruses with the H3 molecule suggests that two of the substitutions in HA1 (residues 78 and 128) may be in antigenic regions (sites B and E) and that the other substitution in HA1 residue 23 is in a potential glycosylation site in the vicinity of the cleavage site. The fourth amino acid change lies beyond the carboxyl terminus of the bromelain released molecule and could not be located on the three dimensional structure. What do these changes in HA contribute to the virulence of the H5N2 influenza virus? The studies indicate that cleavage of the Chick/Penn hemagglutinin can be correlated with the acquisition of virulence. However, both the avirulent and the virulent strains possess the same sequence of basic amino acids in the connecting peptide region, indicating that the primary amino acid sequence alone cannot explain the cleavability of the HA. Since there are no deletions or insertions in the amino acid sequence of the virulent and avirulent viruses, the difference in molecular weight must be due to loss of a glycosylation side chain in the virulent strain. The amino acid substitution at residue 23 in the vicinity of the connecting peptide is the only potential glycosylation site where a mutation has occurred. The loss of a carbohydrate side chain from this residue may permit access of the endopeptidase to the basic amino acid residues and produce cleavage.

Representatives of each of the 13 hemagglutinin (HA) subtypes and nine neuraminidase (NA) subtypes of influenza A viruses have been isolated from aquatic birds in North America (Hinshaw et. al., 1981), indicating that a large influenza gene pool is maintained in nature. Since H5N2 influenza viruses have been isolated from wild ducks, it seems plausible that ducks may have been the original source of the virus. However, since neither the highly pathogenic virus, nor the earliest isolate of the avirulent form, replicated efficiently in ducks, the virus has apparently undergone sufficient modification to alter its host specificity. The results of the nucleotide mapping showed that the H5N2 viruses are closely related, and
suggest that virulence was acquired by selective adaptation within the population, rather than by the introduction of one or more genes from a different virus. Like the H7N7 seal virus, which also was unable to replicate in the duck intestinal tract, Chick/Penn/83 may be an example of an avian virus strain which was fortuitously introduced into a new species and underwent a period of adaptation before it became pathogenic.

The presence of small RNAs in the avirulent strains of Chick/Penn/83 and the lack of these RNAs in the virulent strain is particularly interesting since this may at least partially explain the sudden emergence of the virulent strain. Although there is a considerable body of evidence about the molecular biology of DI particles of influenza viruses, their role in nature has not been elucidated (Nayak and Sivasubramanian 1983). The interference between the low pathogenic and high pathogenic H5N2 viruses demonstrated in the present experiments, supports the contention that defective particles in the original isolate could in part be responsible for the reduced mortality caused by this virus in the field.

Since 1979, there have been at least three outbreaks of influenza in animals and birds that resulted in significant mortality. The first occurred in seals in 1979–80 when up to 20% of these animals died of primary viral pneumonia associated with H7N7 (Webster et. al. 1981). In 1981–82, an H4N5 influenza virus was associated with up to 15% mortality in seals (Hinshaw et. al. 1984). These viruses, together with the H5N2 virus from chickens in Pennsylvania, all appear to have originated from the avian influenza virus gene pool present in wild birds. It also seems likely that the H5N9 virus that caused high mortality in poultry in Ireland (Dennis Alexander, personal communications) also originated from this source. These incidents emphasize the potential importance of this gene pool as a future source of viruses for humans and other species. The transmission of H5N8 virus that caused high mortality in poultry in Ireland (Dennis and recent outbreaks of highly pathogenic influenza in domestic poultry suggest that it is only a matter of time before a highly pathogenic virus appears in humans.

ACKNOWLEDGEMENT

The authors would like to thank USDA for cooperation in these studies, both APHIS staff, the personnel in the Avian Influenza Task Force and Drs. James Pearson, NVSL, Ames, Iowa and Robert J. Eckroade, University of Pennsylvania for providing the virus strains.

This work was supported by U.S. Public Health Research Grant AI 08831, AI 02649, and AI 20591 from the National Institute of Allergy and Infectious Diseases, Cancer Center Support (CORE) Grant CA 21765, and American Lebanese Syrian Associated Charities.

The authors wish to thank Michael T. Berton for many helpful discussions and Lisa Newberry who provided excellent technical assistance.

REFERENCES

1. Air, G. M. (1979). Nucleotide sequence coding for the “signal peptide” and N
terminus of the hemagglutinin from an Asian (H2N2) strain of influenza virus. *Virology* 97:468-472.


Table 1. Comparison of the amino acid sequences of the connecting peptide flanking regions of representative influenza virus HAs.

<table>
<thead>
<tr>
<th>Virus</th>
<th>HA1&lt;-&gt;HA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSN/33 (H1)</td>
<td>Gly Leu Arg Asn Ile Pro Ser Ile Gln Tyr - - - - Arg Gly Leu Phe</td>
</tr>
<tr>
<td>SEAL/MASS/1/80 (H7)</td>
<td>Met Val Glu Asn Pro Lys Thr - - -</td>
</tr>
<tr>
<td>FPV/34 (H7)</td>
<td>Met Lys Val Glu Pro Ser Lys Lys Arg Glu Lys</td>
</tr>
<tr>
<td>CK/PENN (H5 avir)</td>
<td>Met Val Gln Lys Lys Lys - - - -</td>
</tr>
<tr>
<td>CK/PENN (H5 vir)</td>
<td>Met Val Gln Lys Lys Lys - - - -</td>
</tr>
</tbody>
</table>

Only differences in amino acids from WSN strain are shown, the gaps indicate identical amino acid sequence with the WSN strain. A/WSN/33 (WSN) (Hiti et. al., 1981); A/Seal/Mass/1/80 (Naeve and Webster, 1983); A/Fowl Plague/34 (FPV) (Porter et. al., 1979); A/Chicken/Pennsylvania/1370/84 (chicken). The dashes are included to adjust the alignment.
Table 2
Response of ducks to Virulent and Avirulent A/Chick/Penn/83 Influenza Virus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Dose</th>
<th>Route</th>
<th>Trachea</th>
<th>Rectal</th>
<th>Bursal Tissue</th>
<th>Disease Signs</th>
<th>HI Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick/Penn/1/83</td>
<td>103.8</td>
<td>Trachea</td>
<td>1/3(^a)</td>
<td>0/3</td>
<td>NT</td>
<td>0/3</td>
<td>2/3 (80)(^b)</td>
</tr>
<tr>
<td>(Avirulent)</td>
<td>108.0</td>
<td>Oral</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>108.0</td>
<td>Rectal</td>
<td>0/2</td>
<td>0/2</td>
<td>2/2</td>
<td>0/2</td>
<td>NA</td>
</tr>
<tr>
<td>Chick/Penn/8210/83</td>
<td>103.8</td>
<td>Trachea</td>
<td>1/3</td>
<td>0/3</td>
<td>NT</td>
<td>0/3</td>
<td>2/3 (100)</td>
</tr>
<tr>
<td>(Avirulent)</td>
<td></td>
<td>Oral</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>0/6</td>
<td>0/6</td>
<td>12/12 (1400)</td>
</tr>
<tr>
<td>Chick/Penn/1370/83</td>
<td>107.7</td>
<td>Trachea</td>
<td>NT(^d)</td>
<td>NT</td>
<td>NT</td>
<td>0/12</td>
<td>12/12 (1400)</td>
</tr>
<tr>
<td>(Virulent)</td>
<td>108</td>
<td>Oral</td>
<td>1/12</td>
<td>1/12</td>
<td>NT</td>
<td>0/12</td>
<td>12/12 (1400)</td>
</tr>
<tr>
<td></td>
<td>108</td>
<td>Rectal</td>
<td>0/1</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Pekin white ducks [SPAFAS] (5 months) were infected with the above influenza viruses.

\(^a\)Figures give the number of animals yielding virus - or disease signs to number inoculated.

\(^b\)Figures in parenthesis give mean HI antibody titer.

\(^c\)NA = not available, birds killed for virus analysis on day 3 postinfection.

\(^d\)NT = not tested.
Figure 1. *Radioimmunoprecipitation of avirulent and virulent* *Chick/Penn/83 influenza virus* *HA*. The viruses were grown on MDCK cells in the presence (+) or absence (−) of trypsin. Monoclonal antibody specific for the HA (176/6) was used in the RIP assays. The precipitates were run on a 12% polyacrylamide gel (Laemmli, 1970).
Figure 2. Analysis of the RNAs from virulent and avirulent Chick/Penn/83 influenza viruses by polyacrylamide gel electrophoresis. Electrophoresis was done on 3% - 7 M urea gels in Tris-borate-EDTA buffer (pH 8.3) as described by Maxam and Gilbert (1977).
Figure 3. Oligonucleotide mapping of $^{32}$P labelled Chick/Penn/83 RNA. T-1 digests of viral genome RNA and isolated genome segments were done by the method of Pedersen and Haseltine (1980) as modified by Lee and Fowlks (1982). Isolation of viral RNA segments was as described previously (Bean, 1984).
Figure 4. The three dimensional structures of A/Aichi/2/68 (H3) influenza virus hemagglutinin (Wiley et al., 1981). The numbers indicate the amino acid differences between the avirulent and virulent Chick/Penn influenza A viruses.
LABORATORY STUDIES WITH THE PENNSYLVANIA AVIAN INFLUENZA VIRUSES (H5N2)

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SUMMARY

Short-term studies were conducted to address questions which arose during the 1983-84 avian influenza eradication effort. The following results were obtained: (1) A mixture of litter and feces from a broiler house that contained a flock exhibiting signs and mortality from highly pathogenic avian influenza failed to infect SPF chicks 3 days after collection. (2) Moist feces collected from underneath AI-diseased hens in a cage-layer house infected similar SPF chicks when placed on the feces 2 days after collection but not after 4 days or later. (3) Hatching eggs from 4 AI infected field flocks exhibiting severe signs of disease were incubated and hatched. No virus was isolated from any of the dead embryos and none of the 214 hatched chicks yielded virus or developed AI antibodies. (4) Pathotype determinations of the AI virus studied were influenced by the age of chickens and in one experiment by the breed when using prescribed pathotyping procedures. Greater death losses occurred in 3 wk-old chicks than in older chicks with the same dose of virus by the same challenge route. (5) A virus recovered from the layer feces samples and pathotyped as less than highly pathogenic in all but one assay, killed 23 of 25 mature leghorn hens in one experiment and 24 of 26 in another with classical signs of fowl plague. (6) The AGP test was positive as early as five days after experimental inoculation of the chickens. (7) Virus was recovered from a high percentage of the last eggs laid by the experimentally infected hens. (8) Virus in the feces of hens infected with highly pathogenic AI survived longer in moist feces and at 4°C when compared to dry feces at 25°C. (9) Simultaneous cloacal and tracheal swabs were more sensitive in detecting virus in hens killed by influenza virus than either type swab alone.

INTRODUCTION

The unique events of the 1983-84 avian influenza (AI) outbreak that included the transition of the disease from very mild to fowl plague-like provided opportunities to gain important information on these viruses. This report will present an overview of some of the laboratory studies conducted at the Southeast Poultry Research Laboratory (SEPRL) since November 1983.
MATERIALS AND METHODS

Viruses All three avian influenza viruses were H5N2. The highly pathogenic virus was #1370 and the non-pathogenic virus was #21525. Both were obtained from Dr. Jim Pearson, National Veterinary Services Laboratory, APHIS. A third virus was isolated at the SEPRL from the spleen of a chicken that became infected and died after contact with feces obtained from a poultry house in Pennsylvania. It is termed SEPRL-PA and was characterized as either a pathogenic or highly pathogenic virus depending upon the test results used. Pathogenicity determinations were based on the recommendation of the 1981 International avian influenza symposium in Beltsville, Maryland (1).

Virus assays All virus infectivity determinations were made in 9-11 day old embryonating chicken eggs inoculated in the allantoic sac and held at 37°C. Evidence of virus was the death of the embryo after 24 hours incubation with the egg fluid exhibiting hemagglutination activity which was specifically inhibited by H5 immune serum.

Serology The agar gel precipitin test was done as previously described (2) but using plastic, triangular double diffusion plates with projections on the plate cover to form wells in the finished gel (Alpha Gamma Laboratories, Inc.).*

Chickens White rock and white leghorn chickens obtained from the SEPRL specific pathogen free flock were used at the ages described. They were free of antibodies against Newcastle disease virus, avian influenza, Mycoplasma gallisepticum and Mycoplasma synoviae.

RESULTS AND DISCUSSION

Soon after the AI outbreak in Pennsylvania included the signs of fowl-plague-like disease, task-force personnel sent feces, litter and eggs to the SEPRL for evaluation.

The first submission was five pound plastic bags containing litter and feces removed from a broiler house in which broilers were dying at a high rate with marked signs of AI. Beginning on the third day following collection of the litter, four 3-week-old SPF broilers were placed on the litter in modified Horsfal-Bauer isolation units. Feed was withheld for that first day, encouraging the consumption of edible material in the litter. On every other day thereafter for a 2-week period, additional isolation units were used to expose chickens to other bags of litter that had been stored unopened in the units at 25°C. None of the chickens placed on the broiler litter showed signs of disease or developed antibodies against AI virus. Although the litter had been collected only 3 days earlier from a broiler flock experiencing severe losses to AI and shipped in an insulated container, it failed to transmit the disease to the susceptible chickens placed on it.

Bags of moist feces containing no shavings were submitted from underneath cages of commercial layers experiencing severe mortality from AI. Arriving at the laboratory in an insulated container on the second day
following collection, one bag of feces was placed in each of 12 isolation units at 25°C. On that same day and on every second day thereafter, four susceptible 3-week-old chickens were placed on the feces removed from the bag in each isolation unit. On the sixth day following the introduction of the chickens into the first isolation unit, 3 of 4 chickens showed signs of disease. On the next day, 2 of 4 were dead with 3 of 4 dying by the 8th day. Tissue samples of the dead birds yielded the virus which was termed “SEPRL-PA” isolate in subsequent studies. The fourth chick in the first isolation cage never showed any disease signs nor developed AI antibodies. None of the chicks placed on the feces in the other isolation units developed disease signs or sero-converted. These results indicated that AI virus could survive in moist feces for several days at 25°C but did not persist for 2 additional days as evidenced by the negative AI status of chicks in all but the first isolation unit.

Eggs collected from one white rock and three white leghorn breeder flocks were collected at the height of severe signs of AI. The National Veterinary Services Laboratory subsequently isolated highly pathogenic virus from these flocks. The eggs were shipped to this laboratory and immediately incubated. During incubation, all dead embryos or non-fertile eggs were assayed for AI virus and found to be negative. Eggs were transferred after 18 days of incubation into isolation units containing heaters for hatching, each flock's eggs being held in separate isolators. A total of 214 chicks were hatched from eggs of the four flocks. None of the chicks that died yielded virus, none of the remaining chicks showed signs of disease and when examined 2 weeks later none had developed AI antibodies. Thus, this limited study on a small number of eggs from 4 infected breeder flocks, provided no evidence of vertical transmission of the AI virus.

Determinations of pathotype were made on 3 viruses: the April 1983 Pennsylvania virus, the SEPRL feces isolate and the October 1983 Pennsylvania virus. Using 5-week-old chickens, the April 1983 virus (#21525) killed none of the birds and thus (Table 1) was classified as non-pathogenic in both white leghorn and white rocks. The October 1983 virus (#1370) was classified as highly pathogenic, killing 8/8 white leghorns and 7/8 white rocks in two trials. The SEPRL-PA isolate killed 8/8 white leghorns and either 3/8 or 4/8 white rocks. The SEPRL-PA isolate could, therefore, be classified as highly pathogenic AI virus using leghorns and a low pathogenic AI virus using white rocks.

To explore the response of white leghorns to inoculation with AI virus as influenced by age and time of observation, an additional trial was conducted (Table 2). Ages of leghorns ranged from 3 to 8 weeks of age and the mortality results calculated at 8 and 15 days. There was a clear indication of increasing resistance with age regardless of the day of tabulation of results. Although the proposed definition of a highly pathogenic AI virus stated that chickens 4 to 8 weeks of age could be used, it was clear from these studies that the age within the prescribed range could influence the pathotype determination of some isolates.
To compare the susceptibility of both white leghorn and white rock chicks at different ages to the SEPRL-PA isolate, chicks that ranged from a few days of age to 5 weeks of age were inoculated intranasally (IN) and in the conjunctival sac (IO) with 10^6 ELD_{50} of virus (Table 3). Although the mean death time gradually increased for chicks up to 4 weeks of age, there was little difference in their death rate. Both white leghorn and white rock chicks had less mortality at 5 weeks of age than at 4 weeks of age. The mean death time was 2.8 and 2.2 days for the very young chicks and 8.5 and 8.0 days for the 5 wk-old chicks. It was clear from these studies that age could influence death patterns when chickens are exposed by more natural routes.

When the SEPRL-PA isolate was instilled IN and IO into 25 mature white leghorn hens in individual layer cages, 23 of them died (Table 4). They exhibited severe depression on the 3rd day, profuse watery green diarrhea and some soft or thin shell eggs among the 14 eggs laid. Feces sampled on the third day had AI virus levels that exceeded 10^9 ELD_{50}/gram. On the fourth day, 4 were dead and 2 of the 3 eggs laid were soft shell membrane eggs. Eight hens died on the 5th day. No eggs were laid beyond the 4th day and mortality continued to day 20 with a MDT of 6.5 days. The hens exhibited the classical disease signs and gross lesions previously described for fowl plague (3). Virus was recovered from 12 of 14 eggs laid on the third day but not from any of the 25 eggs laid on the two preceding days (Table 5). Both yolks and albumen contained virus in some of the positive eggs with virus levels exceeding 10^4 ELD_{50}/ml.

When six hens that died of AI were cloacally swabbed on the day of death, three yielded virus (Table 6). After storing the carcasses at 4°C for 4 to 7 days, they were swabbed again, using both tracheal and cloacal swabs. Five tracheal and 3 cloacal swabs yielded virus. It was clear that both tracheal and cloacal swabs were a more sensitive sampling method than cloacal swabs alone for recovering virus from dead hens and that storage at 4°C for 4-7 days did not diminish recovery of virus.

The experiment was repeated in a similar group of mature white leghorn hens. Twenty-four of 26 hens died before day 18 with a MDT of 6.1 days. The last egg was laid on day 3. The two survivors at day 18 were sick when killed on that day. Following inoculation with the SEPRL-PA isolate, 8 of 12 hens were AGP-positive on day 5 and 8 of 8 were positive on day 7. Therefore, 67% of the hens were AGP positive only 3 days after first signs of illness.

Forty-eight hens were inoculated by the IN and IO routes with 10^5 ELD_{50} of the non-pathogenic April 1983 Pennsylvania virus. All developed respiratory signs on the fifth day. Several hens exhibited blue tips on the combs and one bird was unable to stand. On the sixth day respiratory signs continued, the “downer” bird got back up and egg production was off 50%. On day eight, 6 of 6 hens bled were positive for influenza antibodies on the agar gel precipitin test. One of the hens died on day 14 but no AI virus was recovered from the carcass. On day fourteen 23 of 24
hens were AGF-positive for AI antibodies. On day 82, 12 of 45 (27%) were AGP-positive. Following challenge with highly pathogenic virus at 82 days, 34 of 44 hens remained AGP-negative 15 days after rechallenge indicating that they were refractory to reinfection with another H5N2 AI virus.

To determine the influence of temperature and moisture on the stability of AI virus in feces from infected hens, feces were collected on postinoculation day 3 from underneath hens infected with the highly pathogenic #1370 isolate. The watery feces were thoroughly mixed and distributed among forty screw cap vials. Twenty vials were placed with or without caps at either 4°C and or 25°C to simulate moist and dry feces. The vials stored at 25°C yielded viable virus through 2 days in the closed vials and through 1 day in the open vials (Table 7). At 4°C the wet feces yielded viable virus through the 35 days. The open vials yielded virus through day 9. There was an apparent but unproven crossidentification of the day 14 dry samples. It was clear from the results of this experiment that the virus in feces from infected chickens survived longer at the colder temperature in the moist condition. Even the moderately warm temperature of 25°C was deliterous to virus survival regardless of moisture level.

Because of the 1983-84 outbreak in Pennsylvania, avian influenza is going to be the subject of increased fundamental and applied research in that the potential of the virus to severely affect poultry has been forcefully demonstrated. Hopefully continuing research will yield information to prevent or reduce future losses from the ubiquitous virus.

REFERENCES


*No commercial endorsement implied.
Table 1—Pathotype determinations* of three PA AI isolates in 5-wk-old white rock (WR) and white leghorn (WL) chickens.

<table>
<thead>
<tr>
<th>Apr. 83 Virus</th>
<th>SEPRL (PA)</th>
<th>Oct 83 Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>W L W R</td>
<td>W L W R</td>
<td>W L W R</td>
</tr>
<tr>
<td>- -</td>
<td>- 4/8</td>
<td>- 7/8</td>
</tr>
<tr>
<td>0/8 0/8</td>
<td>8/8 3/8</td>
<td>8/8 7/8</td>
</tr>
</tbody>
</table>

* 0.2 ml of $10^{-1}$ Dilution of AF by caudal air sac route.

- Not done
Table 2--Pathotype determinations* of SEPRL-PA AI virus in different age leghorn chickens.

<table>
<thead>
<tr>
<th></th>
<th>3 WK</th>
<th>4 WK</th>
<th>5 WK</th>
<th>6 WK</th>
<th>7 WK</th>
<th>8 WK</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAY 8</td>
<td>7/8</td>
<td>4/8</td>
<td>5/8**</td>
<td>3/8</td>
<td>2/8</td>
<td>3/8</td>
</tr>
<tr>
<td>DAY 15</td>
<td>7/8</td>
<td>5/8</td>
<td>5/8</td>
<td>4/8</td>
<td>2/8</td>
<td>3/8</td>
</tr>
<tr>
<td>MDT</td>
<td>6.3</td>
<td>8.0</td>
<td>6.4</td>
<td>8.8</td>
<td>8.5</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* 0.2 ml of 10⁻¹ dil of AF by caudal air sac route.

** This value was 8/8 in previous expt.
Table 3--Influence of age on chick susceptibility to avian influenza virus (SEPRL-PA).*

<table>
<thead>
<tr>
<th></th>
<th>1-3 Days</th>
<th>1 WK</th>
<th>2 WK</th>
<th>3 WK</th>
<th>4 WK</th>
<th>5 WK</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. Rock</td>
<td>12/12</td>
<td>12/12</td>
<td>12/12</td>
<td>10/12</td>
<td>12/12</td>
<td>6/12</td>
</tr>
<tr>
<td>W. Leghorn</td>
<td>12/12</td>
<td>12/12</td>
<td>12/12</td>
<td>10/12</td>
<td>10/12</td>
<td>8/12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MDT (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. Rock</td>
<td>2.8 5.1 5.9 7.1 8.6 8.5</td>
</tr>
<tr>
<td>W. Leghorn</td>
<td>2.2 4.6 5.4 7.2 7.9 8.0</td>
</tr>
</tbody>
</table>

* 0.1 ml IO and 0.1 ml IN (10^6 ELD_{50}).
Table 4--SEPRL-PA avian influenza isolate * in 25 mature white leghorn hens.

<table>
<thead>
<tr>
<th>Post Inoc. Day</th>
<th>Clinical Response</th>
<th>Number of Hens</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Depressed</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Depressed</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>Dead</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Dead</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>23/25</td>
<td>14 eggs</td>
</tr>
<tr>
<td></td>
<td>MDT</td>
<td>6.5</td>
</tr>
</tbody>
</table>

* *10^6 ELD_{50} instilled IN and IO.
Table 5—Recovery of avian influenza virus from eggs (surface, yolk, albumen).

<table>
<thead>
<tr>
<th>Days eggs laid post infection</th>
<th>Virus positive eggs/eggs assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/12</td>
</tr>
<tr>
<td>2</td>
<td>0/13</td>
</tr>
<tr>
<td>3</td>
<td>12/14* (1SS)</td>
</tr>
<tr>
<td>4</td>
<td>3/3 (2SS)</td>
</tr>
</tbody>
</table>

* Details of Egg results:
- Surface swab 11/14 positive
- Albumen swab 11/14 positive
- Yolk swab 9/14 positive

Some contained >10^4 ELD50/ml
Table 6—Recovery of AI virus from hen carcasses stored at 4 C.

<table>
<thead>
<tr>
<th>Day of death</th>
<th>Swab</th>
<th>Days at 4 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>C*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>T**</td>
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<tr>
<td>11</td>
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* Cloacal swab
** Tracheal swab
### Table 7—Stability of highly pathogenic AI in feces

<table>
<thead>
<tr>
<th>Storage Days</th>
<th>25 C Wet</th>
<th>25 C Dry</th>
<th>4 C Wet</th>
<th>4 C Dry</th>
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<tbody>
<tr>
<td>0</td>
<td>+</td>
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<tr>
<td>1</td>
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<td>2</td>
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<tr>
<td>35</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Collected under hens on Day 3 PI.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY AND OTHER AVIAN SPECIES

Chairman: R. A. Bankowski, California
Vice Chairman: E. T. Mallinson, Maryland

W. E. Adams, GA; T. B. Angel, KY; R. E. Baer, OH; C. W. Beard, GA; S. B. Clubb, FL; M. S. Cover, MO; F. R. Craig, MD; H. M. Ghori, AR; H. E. Goldstein, OH; L. C. Grumbles, TX; D. A. Halvorson, MN; R. Hannessian, MD; R. L. Hogue, IN; I. H. Kahan, PA; D. D. King, MD; D. J. Ligda, IN; R. H. McCapes, CA; M. Meyers, DC; D. A. McMartin, CA; T. R. Mickle, GA; C. D. Murphy, IL; M. S. Newman, OK; T. D. Njaka, WV; H. M. Optiz, ME; J. E. Pearson, IA; I. L. Peterson, MD; E. I. Pilchard, MD; B. S. Pomeroy, MN; S. S. Richeson, MD; R. Schar, MD; J. A. Smiley, ME; J. W. Thomas, SC; H. W. Towers, Jr., DE; D. N. Tripathy, IL; W. T. Tramel, MS; C. R. Weston, NH.

The committee met at 1:30 p.m. on October 22 and again on October 23, 1984. A total of eighteen members and 29 guests attended.

NEWCASTLE AND OTHER DISEASES OF IMPORTANCE

Newcastle Disease

Outbreaks of exotic Newcastle disease occurred in cage birds believed to have originated from birds entering the United States illegally on seven occasions, five originating in birds from California, one originating in Alabama, and one in birds from Texas. The total cost of these infections during FY 1984 were reported to be $264,308.

These outbreaks resulted in isolations of exotic Newcastle disease on 18 premises as follows: California (3), Missouri (1), Utah (2), Oklahoma (1), Florida (4), Georgia (1) and Alabama (6).

According to Dr. Samuel S. Richeson, Chief Staff Veterinarian, Import Birds and Poultry, Import/Export Animals and Products Staff, there were eight lots of commercial cage birds that were refused entry due to exotic Newcastle disease during FY 1984. Four of these lots were diagnosed as velogenic, and four were diagnosed a velogenic viscerotrophic Newcastle disease.

Pulorum-Typhoid and Other Salmonellas

There were 50 isolations of Salmonella pullorum and no isolations of S. gallinarum reported during calendar year 1983. There were 12 states and territories involved in these infections. Almost 50 percent of the isolations were reported from one state.

During January through September 1984, there were 33 isolations of S. pullorum and no isolations of S. gallinarum reported to the National Poultry Improvement Plan staff. Five states reported pullorum isolations. One state reported 29 of the 33 isolations. One outbreak in a commercial broiler operation in that State resulted in six of the isolations. This outbreak was believed to have resulted from contaminations from an
infected flock on the same premises in the previous year. Small numbers of backyard-type turkeys were involved in three of the infections reported. One isolation was obtained from a flock of 12-week-old chukar partridges raised for gun clubs.

In view of the high incidence of *Salmonella* infections this committee joined the committees on salmonella of the USAHA in their resolution to adopt the five statements relating to control and reducing the incidence of *Salmonella* in domestic animals.

This committee also joins the committee on salmonella recommending that an epidemiologist be added to the staff of the National Veterinary Services Laboratory.

**MYCOPLASMOSIS**

A test kit of 25 sera is available from NVSL for calibration of the HI test for *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS) and *M. meleagris* (MM). Mycoplasma-ELISA has been shown to be specific, sensitive and adaptable to testing of serum, yolk, or dried whole blood. Standardization of the test procedure would be desirable. Testing problems, cross-reaction with heterologous serotypes, nonspecific reactions and low sensitivity are still occasionally observed with plate antigens.

Applied research is needed. Further documentation of the economic benefits of eradication versus use of bacterin versus MG controlled exposure in white and brown egg-type replacement pullets under different management systems and studies of the significance of MS infection in commercial laying flocks are needed. Investigations of the effect *M. Iowae*, a common infection in turkeys, on infertility are needed as well as studies on the significance of other serotypes of mycoplasma as a cause of nonspecific serologic reactions and clinical disease.

**PARAMYXOVIRUS**

The paramyxovirus (PMV) evaluation subcommittee which was formed last year is still in the process of gathering information on the significance of PMV on clinical disease other than Newcastle disease virus (NDV).

A report from North Carolina indicated that there has been a problem in the past with PMV-3 infections, usually in November, that caused up to a 55% drop in egg production in turkey breeder flocks. There was also about a 12% increase in poor quality eggs plus a 3% drop in fertility. An inactivated vaccine has been used and there has been no subsequent problem with PMV-3 infection. A similar clinical disease problem was reported by Dr. Saif at the American Veterinary Medical Association in July, 1984 on breeder turkey flocks in Ohio.

There is an inactivated vaccine against PMV-3. It is licensed only on an experimental basis and available to be used under a special permit.

As part of the avian influenza surveillance, PMV-2 was isolated at the National Veterinary Services Laboratories (NVSL) from turkey flocks in Minnesota, Virginia, West Virginia, and from chickens in Pennsylvania.
and Virginia. None of the isolates were reported to be associated with a disease problem.

PMV-3 was isolated from the following admissions of pet birds submitted to NVSL for NDV or Chlamydia psittaci isolation: 5 lovebirds, 3 finches, 1 parrot, 3 cockatiels and 3 parakeets. In addition, PMV-2 was isolated from a parrot. Dr. Susan Clubb of Miami, Florida, reported a disease syndrome in 3 lots of African grey parrots from which PMV-3 as well as reovirus were isolated. An isolate from a duck was submitted from the University of Minnesota which was identified as PMV-6.

An extensive survey was conducted in 174 California turkey flocks for PMV-3 antibody by Dr. M. Ianconescu et al (submitted for publication in Avian Diseases) using the enzyme-labeled immunosorbent assay technique. Antibody was first detected at 5-8 weeks in meat flocks and 10-12 weeks in breeder flocks. Of the 2,037 birds tested, 95.2% over 11 weeks of age had antibodies against PMV-3. There was no clinical disease associated with infection.

It appears from clinical reports that PMV-3 can cause a drop in egg production in susceptible turkeys. However, further experimental data is needed before the complete impact of PMV infections can be evaluated.

The committee discussed the appearance of an unusual strain of Newcastle disease virus. An apparently new paramyxovirus type 1 infection in racing and feral pigeons has existed in Europe for several years. The introduction of the virus into the United Kingdom (U.K.) has resulted in serious losses to pigeons. Although of the same type 1 serotype as Newcastle disease, studies utilizing monoclonal antibodies clearly show the virus to be distinguishable from the usual Newcastle disease viruses of poultry. The virus did spread from pigeons to the chicken industry in the U.K. via contaminated feed ingredients. Severe poultry losses resulted. Most outbreaks in chickens were traceable to the pigeon contaminated feed. Reports in the USA indicate that these or similar viruses have caused losses in pigeons in New York.

AVIAN INFLUENZA

During the past year, (October, 1983 - September, 1984) the outbreak of lethal avian influenza (AI) by type H5N2 in Pennsylvania, Virginia, Maryland and New Jersey in egg-type and broiler chickens and turkeys was the most extensive and costly outbreak of an infectious disease in the history of the poultry industry in the U.S. Accounts of outbreaks of AI and the effort expended by the Federal State and industry cooperative eradication program are described in detail in the 5 presentations given in the general sessions and published elsewhere in these proceedings.

Since the report of the subcommittee at the 1983 USAHA meeting, the diagnosis of avian influenza in turkeys has been made in six states (California, Colorado, Minnesota, as well as Pennsylvania, Maryland and Virginia). (See Table 1).
Avian influenza caused by H5N3 AI virus appeared in California turkeys in the Spring of 1984. The same serotype was isolated from infected turkey flocks in the Spring of 1979.

The predominant signs of disease was a mild respiratory infection with a marked drop in egg production. The isolate which was inoculated into young chickens at the National Veterinary Service Laboratories in Ames, Iowa resulted in no illness or death. In the field, the infection occurred on five individually owned breeder flock premises that were under supervision of a single commercial production company. The premises were widely separated from each other in the central portion of the state.

Diagnosis of the disease by virus isolation was made in four adult breeder flocks on four premises. The outbreaks occurred over a period of 51 days (March 6 through April 26, 1984). Three of the four flocks were immediately destroyed and buried on the recommendation of the poultry industries of California with the concurrence of the California Department of Food and Agriculture. Economic losses associated with the destruction of the flocks were remunerated by the combined contributions from the California state poultry industries, owners of the flocks and funds from the State of California. The fourth flock was spared for reasons of difficulty in arranging burial on the premises, low risk of spreading the infection and economic considerations.

A fifth flock, which was younger and non-laying, located about 25 miles from the first infected premise, was diagnosed serologically in retrospect and no virus was isolated from the birds after a number of attempts.

On one of the depopulated premises housing layers, a second younger replacement flock located in a brooder house was spared and later vaccinated. No signs of infection was detected prior or after depopulation of the laying hens.

Turkey flocks on premises surrounding the five infected premises were vaccinated with an oil emulsion inactivated vaccine produced with the homologous virus in a local vaccine production laboratory.

To determine the pathogenicity of the H5N3 isolate for adult laying chickens, a group of 26, 34-week-old leghorn laying hens proved to be highly resistant to inoculation intratracheally and intraocularly with 0.25 ml of the California H5N3 isolate having an ELD₅₀ of 10⁶.5/ml. Resistance was demonstrated by the lack of any clinical symptoms of disease, including any effect on egg quality or production, and seroconversion in only 3 of the hens 14 days following inoculation. Furthermore, there was no anamnestic response following a reexposure 26 days following the first inoculation. The isolate was also nonpathogenic for 4 week-old chicks and poults. No other outbreaks of AI were reported in California during the fiscal year 1983-1984.

H9N2 was identified in turkey breeders in November, 1983, in which
approximately 60,000 birds were involved. The infection spread to grow-out flocks involving over 1,000,000 birds. Vaccine for H9 was used in breeder replacements. The isolates were classified as nonpathogenic at NVSL.

**Minnesota**

In the fall of 1983, two turkey market flocks were identified with H5 infection based on serology. No economic loss was associated with the infection. Samples were collected at the time of marketing. No other flocks in the multi-farm operation were identified as infected.

In June of 1984, two turkey market flocks containing 40,000 birds on the same farm were identified infected with H6N8. The economic loss was estimated at $60,000. In another part of the state one turkey market flock was identified with H2N3 on samples collected at the time of marketing. Two other flocks on the same farm remained negative.

The third foci of infection involved 100,000 turkeys on two farms belonging to the same operation. They were infected with H4N6. The outbreak was complicated with other infections such as aspergillosis and fowl cholera. The fourth foci involved one flock of 12,000 turkeys and was found infected with H4N6. Two other flocks on the same farm remained negative. None of the four outbreaks appeared to be associated with each other. All isolates were pathotyped at NVSL and found nonpathogenic.

Beginning in July, 1984, a systematic collection of blood samples from each market flock at the various turkey processing plants in Minnesota was initiated by the Board of Animal Health. Nine thousand-one hundred samples were tested by AGP test and positive samples were submitted to NVSL. Two positive flocks were identified at the time of marketing out of 455 flocks tested. One flock was serologically found exposed to H4N6 and the second flock with H2N3.

During the past fiscal year (FY 84), serotypes identified in Minnesota in turkeys on six farms were: H5N?, H2N3, H4N6, H6N8.

**Use of Influenza Vaccine**

Influenza killed vaccine was used in California (H5), Colorado (H1, H9), Iowa (H1, H4, H5, H6), Minnesota (H1, H4, H5, H6), Missouri (H1), North Carolina (H1). Approximately 1,115,000 doses were used in approximately 200 turkey flocks.

It is to be noted that the National Veterinary Services Laboratories have prepared avian influenza isolates for each hemagglutinin antigen that may be requested for use in production of killed vaccines. In some instances an autogenous vaccine is prepared from the specific serotype isolated from an outbreak such as in California (H5N3).

**National Veterinary Services Laboratories (NVSL)**

Dr. E. Pearson reported that between October 14, 1983 and October 1, 1984, the NVSL received 33,941 virus isolation cases resulting in 142,418 samples processed. In addition, about 40,000 serum samples were tested.
H5N2 influenza virus was isolated from 605 submissions from Pennsylvania and 89 from Virginia. Pathotyping of the isolates was done from October through January with the following results:

- Nonpathogenic (did not kill chickens) 104 (26%)
- Pathogenic (1 to 5 chickens died) 166 (41%)
- Highly pathogenic (6 or more chickens died) 133 (33%)

Dr. Pearson also submitted a report of isolations made from birds in the Import Quarantine Stations. The results are summarized in Table 2.

**Avian Influenza as a Reportable Disease**

The AI Technical Collaborating Committee, VS-APHIS-USDA recommended that states make AI as a reportable disease. The delegates to the 1984 National Plans Conference passed a resolution recommending states to make AI as a reportable disease.

The subcommittee made a survey of states to determine the current status of AI as a reportable disease.

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of states contacted</td>
<td>40</td>
</tr>
<tr>
<td>Replies received from states</td>
<td>26 or 65%</td>
</tr>
<tr>
<td>AI Reportable Disease</td>
<td>17</td>
</tr>
<tr>
<td>AI Reported as Exotic Disease, dangerous disease, or highly pathogenic type.</td>
<td>3</td>
</tr>
<tr>
<td>AI not specifically listed as reportable disease</td>
<td>6</td>
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Dr. E. C. Sharman presented a status report on the eradication of avian influenza in Pennsylvania and Virginia. Dr. Sharman reported that the State-Federal Quarantines in both Pennsylvania and Virginia were already lifted. He further presented a preliminary report of an analysis of the economic impacts associated with the outbreaks of avian influenza. The report by the agriculture economists in the Animal Products Branch of the National Economics Division has estimated the cost of AI in Pennsylvania, Virginia, Maryland, and New Jersey to be $80 million to the federal government and poultry industry. The cost of the outbreak to the U.S. consumers was estimated at $350 million because of higher prices of beef, pork and poultry products. If the disease had spread to the eastern part of the U.S., it was estimated that the cost to the poultry producers would have been over $500 million and to the consumers of the U.S. over $5 billion in increased expenditures for red meat and poultry products.

The committee discussed other aspects of the eradication program and wished to urge the USDA to support laboratory personnel and facilities which would contribute to a better understanding of the nature of avian influenza virus in poultry populations. In light of the need for such information it is believed that a second Avian Influenza International Symposium be seriously considered in the very near future.

Based upon the experience and complexities encountered in the eradication program, the Committee on Transmissible Disease of Poultry and
other Avian Species urged the establishment of a subcommittee to review and develop guidelines for the control and eradication of avian influenza. Industry, research and regulatory people should be represented on the subcommittee. This committee would report back its recommendations at the 1985 meeting of the USAHA.

The committee also believed that it is essential that each state would activate or maintain an existing industry-state-federal task force that is concerned with the biosecurity of the poultry industry against AI, VVND and other highly infectious, contagious, disruptive avian diseases. Furthermore, the committee recommended that the respective state livestock officials take immediate steps to activate the highest level of containment of flocks in which avian influenza is suspected or diagnosed.

CERTIFIED VVND NEGATIVE FLOCKS

The full committee commends the subcommittee for the arduous and successful efforts for developing, as well as the USDA for accepting the concept of the VVND Negative Flock Certification program. The latter was incorporated into the 1984 revision of the Exotic Newcastle Disease Eradication Guide. Since the objectives of the subcommittee were achieved the committee was dissolved.

MODEL STATE PROGRAM FOR PET BIRDS

The subcommittee on the Model State Program for Pet Birds met in several locations during 1984 to further its efforts in modifying and seeking support for the proposed National Cage and Aviary Bird Improvement Plan (NCABIP). The plan has been published in the 1982 proceedings of USAHA. Much input was obtained from outside invited representatives of various interested groups. Several written inquiries were also received about the plan which contained pertinent questions. Some modifications of portions of our plan were made as the result of these discussions.

The reception of the basic NCABIP Plan continues to be favorable in both the public and private sectors. Congressional enablement legislation still remains to be enacted, however, there is evidence of increased bipartisan Congressional interest in our program.

The subcommittee has evolved a tentative table of organization for carrying out the plan once it goes into existence. This will be available in the near future for consideration of interested parties. This past year has been most productive and undoubtedly the coming year will see our efforts come to fruition. This is necessary particularly in light of the recent legislation passed in one of our eastern states banning the import of exotic birds. Such steps can be avoided through the adoption of NCABIP.
SANITATION

Dr. E. Bryant raised the subject of transmission of diseases of poultry thru contaminated poultry hauling trucks and crates. He suggested that this committee recommend and add a regulation to the Federal Poultry inspection act requiring that all trucks and crates be properly washed and cleaned before leaving each and every federally inspected poultry processing plant. After considerable discussion on the complexities of the problem, it was the consensus to appoint a subcommittee to study the various factors involved in the management of live haul procedures and equipment and report its findings and recommendations at the 1985 USAHA meeting.

The following subcommittees were formed:

AVIAN INFLUENZA: R. A. Bankowski; C. Beard; F. Craig; D. King; D. Halvorson; J. E. Pearson; I. Peterson; and B. S. Pomeroy, Chairperson.

MYCOPLASMOSIS: D. Johnson; D. McMartin; E. T. Mallinson; B. S. Pomeroy; I. Peterson; W. Towers; R. Yamamoto; and H. O. Opitz, Chairperson.

MODEL STATE PROGRAM FOR PET BIRDS: T. Angel; R. E. Baer; H. Goldstein; S. Clubb; D. J. Ligda; E. T. Mallinson; M. Myers; R. Schar; and H. Kahan, Chairperson.

PARAMYXOVIRUS EVALUATION: C. Beard; I. H. Kahan; D. King; C. Weston; R. A. Bankowski; and J. E. Pearson, Chairperson.

MANAGEMENT OF LIVE HAUL PROCEDURES AND EQUIPMENT: W. Baisely; E. Bryant; I. Peterson; R. McCapes; C. Weston; S. Vezey, Chairperson.

REVIEW AND DEVELOP GUIDELINES FOR CONTROL AND ERADICATION OF AVIAN INFLUENZA: R. A. Bankowski; C. W. Beard; W. B. Chase; J. Ghazikanian; H. E. Goldstein; R. Goode; E. T. Mallinson; R. H. McCapes; H. A. McDaniel; J. A. Newman; J. E. Pearson; I. L. Peterson; P. Poss; A. J. Roth; J. W. Thomas; H. W. Towers; T. Tramel; M. A. Van Buskirk; S. A. Vezey; R. Webster; C. R. Weston; F. R. Craig, Chairperson.
### TABLE 1
AVIAN INFLUENZA SEROTYPES ISOLATED FROM TURKEYS, CHICKENS AND OTHER DOMESTIC FOWL IN THE U.S. (1964–1984) OR BASED ON SEROLOGY

<table>
<thead>
<tr>
<th>STATE</th>
<th>Year First Identified</th>
<th>Hemagglutinin Antigens Identified</th>
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<tbody>
<tr>
<td><strong>Turkeys</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>California</td>
<td>1964</td>
<td>H5, H6, H9</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1965</td>
<td>H6</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1965</td>
<td>H5, H6, H9</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1966</td>
<td>H1, H2, H3, H4, H5, H6, H7, H8, H9, H10</td>
</tr>
<tr>
<td>Washington</td>
<td>1967</td>
<td>H6</td>
</tr>
<tr>
<td>Oregon</td>
<td>1970</td>
<td>H6, H7</td>
</tr>
<tr>
<td>Iowa</td>
<td>1971</td>
<td>H1, H4, H5, H6</td>
</tr>
<tr>
<td>Colorado</td>
<td>1972</td>
<td>H1, H5, H9</td>
</tr>
<tr>
<td>Ohio</td>
<td>1975</td>
<td>H1</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1976</td>
<td>H5, H7</td>
</tr>
<tr>
<td>South Dakota</td>
<td>1978</td>
<td>H1</td>
</tr>
<tr>
<td>Texas</td>
<td>1979</td>
<td>H5, H7, H9</td>
</tr>
<tr>
<td>Missouri</td>
<td>1980</td>
<td>H1</td>
</tr>
<tr>
<td>Kansas</td>
<td>1980</td>
<td>H1</td>
</tr>
<tr>
<td>North Dakota</td>
<td>1981</td>
<td>H5</td>
</tr>
<tr>
<td>Arkansas</td>
<td>1981</td>
<td>H1</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1982</td>
<td>H1</td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H5, H10</td>
</tr>
<tr>
<td>Maryland</td>
<td>1983</td>
<td>H5</td>
</tr>
<tr>
<td><strong>Chickens</strong></td>
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</tr>
<tr>
<td>Alabama</td>
<td>1975</td>
<td>H4</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1978</td>
<td>H6</td>
</tr>
<tr>
<td>District of Columbia</td>
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<td>H1</td>
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<tr>
<td>Pennsylvania</td>
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<tr>
<td>Maryland</td>
<td>1983</td>
<td>H5</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1983</td>
<td>H5</td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
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</tr>
<tr>
<td><strong>Other Species</strong></td>
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<tr>
<td>Pennsylvania</td>
<td>1969</td>
<td>Ducks NA</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1974</td>
<td>Geese NA</td>
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<td></td>
<td>1974</td>
<td>Guinea Fowl NA</td>
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<td></td>
<td>1980</td>
<td>Pheasants H3, H7</td>
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<tr>
<td>New York</td>
<td>1978</td>
<td>Ducks H3, H4, H5, H6, H11</td>
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<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>Guinea Fowl, Quail H5</td>
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<tr>
<td>Maryland</td>
<td>1983</td>
<td>Ducks, Geese, Guinea Fowl, Pigeons H5</td>
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NA — NOT AVAILABLE
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<tr>
<th>Specimens</th>
<th>1983</th>
<th>1984 thru Sept 15</th>
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<tr>
<td>Lots—Private Facilities</td>
<td>261</td>
<td>257</td>
</tr>
<tr>
<td>Specimens:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private facilities</td>
<td>39,412</td>
<td>32,306</td>
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<tr>
<td>USDA facilities</td>
<td>4,391</td>
<td>3,931</td>
</tr>
<tr>
<td>Lots of HAV</td>
<td>79</td>
<td>64</td>
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<tr>
<td>HAV isolates</td>
<td>776</td>
<td>927</td>
</tr>
<tr>
<td>Influenza positive lots</td>
<td>0*</td>
<td>*</td>
</tr>
<tr>
<td>PMV-2 percent of HAV isolates</td>
<td>57*</td>
<td>*</td>
</tr>
<tr>
<td>PMV-3 percent of HAV isolates</td>
<td>13*</td>
<td>*</td>
</tr>
<tr>
<td>VVNDV positive lots—private facilities</td>
<td>**</td>
<td>6</td>
</tr>
<tr>
<td>VVNDV positive lots—USDA facilities</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>VVNDV positive lots—confiscated birds</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lentogenic NDV positive lots—</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>USDA facilities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentogenic NDV positive lots—</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>private facilities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAV—Hemagglutinating viruses other than</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newcastle disease virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VVNDV—Velogenic viscerotropic Newcastle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disease virus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*—Only 246 of the 776 1983 and some of the FY84 HAV isolates have been typed.
**—Isolates were only typed as velogenic.
In early 1983, communication between the National Pork Producers Association (NPPC) and USDA/APHIS laid the foundation for the PRV Pilot Projects. For the 1983 financed year NPPC contributed $100,000 and USDA $400,000 to begin the PRV pilot project program. The NPPC requested that the pilot projects be designed to meet the following guidelines.

— That the pilot projects must be designed to determine the practicality of eradicating the disease from an area.
— That the projects must be designed to provide a definite answer to the question whether the disease can be eradicated from an area.
— That the government and the producers must be prepared to accept the results and modify their approaches accordingly.
— That there be a scientific or technical committee assembled to decide the technical question in the design of the project plans.

Because of the epidemiological characteristics of PRV, and because of past experiences with the disease in Europe, conclusions were drawn by regulatory officials and swine industry personnel that: PRV poses an increasing threat to efficient swine production in the USA; complete control cannot be achieved by means of vaccines and restriction of stock movement; PRV has a high potential for an area wide eradication; potential future costs due to PRV could far outweigh the costs of eradication.

In view of this feeling, and since previous field studies had indicated that in most cases eradication of PRV from herds of swine was both possible and practical, the USDA and NPPC combined forces to bring the pilot projects which have been designed to fulfill the objectives described earlier.

In FY 1983, a $500,000 budget was divided between project areas in Iowa and Illinois. An additional $1.8 million has been budgeted by USDA for FY 1984 and FY 1985. It is planned that project funding will cease on August 31, 1985. It was expected that federally funded projects will have met their objectives by that date.

In late 1983, and in the first months of 1984 four additional federally funded projects were initiated. These are being undertaken in Wisconsin, Pennsylvania, North Carolina and Ohio.

Illinois
Swine producers in Macoupin and Pike counties expressed enthusiastic support for a PRV pilot eradication program. Projects were initiated in areas of these counties in May 1983 and since then have expanded from the
other township areas into several surrounding townships. Principal features of the Illinois project include:
1) All testing is performed “on farm”.
2) Testing is performed by regulatory personnel.
3) Positive herds are quarantined.
4) Approved control procedures include test and removal, depopulation and repopulation and offspring segregation.
5) Vaccination is not approved in PRV negative herds.
6) An evaluation of the PRV skin test is being undertaken in the test areas.
7) State has approved $70,000 for indemnity for herds in the pilot area (until October 1984).

By September 1984, in Pike County 67 herds have been tested. 19 were found PRV positive at the first test. Three additional herds were determined positive on retests. This gives a total of 22 PRV infected herds. In Macoupin County 78 herds have been tested. 22 were positive on initial test and one additional on maintenance testing.

In September 1984 the status of the herds in which positive animals were identified were as follows:

<table>
<thead>
<tr>
<th>Pike County:</th>
<th>22 herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 false positive</td>
</tr>
<tr>
<td></td>
<td>4 positive from vaccine</td>
</tr>
<tr>
<td></td>
<td>3 cleared by test &amp; removal</td>
</tr>
<tr>
<td></td>
<td>1 sold out</td>
</tr>
<tr>
<td></td>
<td>7 under quarantine (in programs)</td>
</tr>
<tr>
<td></td>
<td>1 undetermined</td>
</tr>
</tbody>
</table>

Total 22

<table>
<thead>
<tr>
<th>Macoupin County:</th>
<th>22 herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 false positive</td>
</tr>
<tr>
<td></td>
<td>1 positive from vaccine</td>
</tr>
<tr>
<td></td>
<td>2 cleared by test &amp; removal</td>
</tr>
<tr>
<td></td>
<td>1 sold out</td>
</tr>
<tr>
<td></td>
<td>16 under quarantine (in programs)</td>
</tr>
<tr>
<td></td>
<td>1 undecided</td>
</tr>
</tbody>
</table>

Total 23

Principal findings of the Illinois project to date include:

— More positive herds were encountered than had been anticipated — 45 out of 145 herds tested (30%).

— On the initial test, six herds had single SN positives. Two herds have had single positives on maintenance tests. No other positives have been found in these herds.

— There was more infection in small herds never previously tested than had been anticipated. Fifteen of the 25 positive herds contained less than 60 head of breeding swine; nine contained fewer than 25 head of breeding swine.
— At present the skin test is not a reliable herd diagnostic test.
— For the most part, excellent producer cooperation has been received. Project participation is entirely voluntary as far as the producer is concerned. Only six individuals in Pike County and three in Macoupin County to date have not indicated willingness to participate.
— It is still too early to determine if the area, and cleaned-up herds can be maintained infection-free.
— Three herds have been picked up or maintenance tested. This was to be expected.
— 44% of herds initially positive are still under quarantine.

Iowa

In July 1983 the Iowa project officially began. The project area was designated as Marshall County. Initially, approximately 98% of the pork producers in the area indicated their willingness to participate.

Principal features of the Iowa project include:
— All testing is performed “on farm”.
— Testing is performed by local practitioners on a fee-basis.
— Herds identified as PRV positive by project testing are not quarantined.
— Approved control procedures include test and removal, depopulation and repopulation, offspring segregation and vaccination.
— Vaccination is an approved control procedure for use in PRV seropositive and seronegative herds.
— If available, the subunit vaccine will be tested in an evaluation.

The project is divided into two phases:

Phase 1

**Determination of the PRV status** of all cooperating herds in the area by an area wide “on-farm” testing program.

Phase 2

**Selection of control programs.**

**PRV negative herds**

All herds determined to be negative or probably not infected have the opportunity to establish either: a qualified negative herd or a PRV vaccinated negative herd.

Testing will continue in these herds for certification as required by state and/or federal regulations.

Herds initially considered not infected, and where enrollment in either (i) or (ii) would not serve any useful purpose to either the herd owner or the pilot project, may select a modified plan in which the herd is monitored every six to nine months.

**PRV positive herds**

Herds that have been diagnosed as infected with pseudorabies in
Phase I of the project have three options from which to choose in an effort to eliminate the disease: depopulation-repopulation; test and removal; or controlled vaccination.

Herds with clinical symptoms, or herds that have been classified as infected on the basis of serology, may continue to vaccinate at program expense, but for not more than six months before attempting to eliminate the disease from the herd according to one of the established infected herd plans.

The current status of herds in the Iowa project are:

<table>
<thead>
<tr>
<th>Status</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herds classified clean</td>
<td>178</td>
</tr>
<tr>
<td>Herds classified infected</td>
<td>31</td>
</tr>
<tr>
<td>Undetermined</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total herds tested</strong></td>
<td><strong>211</strong></td>
</tr>
</tbody>
</table>

The current status of the 31 infected herds is:

<table>
<thead>
<tr>
<th>Status</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Now PRV free</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>PRV probably eliminated</td>
<td>7</td>
<td>41.9%</td>
</tr>
<tr>
<td>PRV probably controlled</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>PRV still cycling</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
<td></td>
</tr>
</tbody>
</table>

Principal findings of the Iowa project to September 1984 include:

— A killed pseudorabies vaccine has been or is being used in 71 herds in the pilot project area. A total of 16,624 doses of vaccine has been administered to mostly breeding age animals in 71 herds that are in the infected, undetermined or high-risk categories.

— Seventeen vaccinated infected herds in the project area have been successful in getting all negative tests on two or more groups of progeny.

— Six of the above herds that had an apparent low incidence of infection on the initial test, eliminated the seropositive animals, vaccinated the balance of the herd, and have been successful in getting negative tests on all progeny.

— Of the 29 swine producers in the project area that buy and finish feeder pigs, only three have been found to be infected. Two of the three receive pigs from a known infected feeder pig source within the project area.

— Seventeen qualified negative herds in the area have continued to maintain their status and ten new herds have qualified for that status at this time.

— One herd that was originally believed to be negative has become infected during the past year.

— The general feeling of the Iowa officials is that under their format a minimum of three years will be needed to achieve area clean up. It is therefore very questionable whether cleanup can be achieved before federal funds terminate.
Pennsylvania

A state federal pilot project was officially initiated in October 1983. The basic elements of this plan include the following:

1) Surveillance, including testing of all boars and sows slaughtered in Pennsylvania.

2) Trace back and epidemiologic investigation of all positive animals.

3) Quarantine of infected herds and premises until all infected animals are removed. Permits for movement are restricted to movements of feeder pigs to approved quarantined feedlots and movements direct to slaughter.

4) Permits for movements from quarantined herds contingent upon implementation of an approved herd plan for elimination of PRV within eight months.

5) Movement of breeding stock is restricted to negative animals or animals originating from qualified PRV negative herds.

6) Utilization of DNA fingerprinting for epidemiology purposes.

There continues to be excellent industry cooperation regarding depopulation procedures, although there is mounting pressure for industry to assist owners of infected herds. By traceback they are identifying 89% of positive swine. However in only 7 of 26 cases have positive swine been identified back to a positive herd.

Wisconsin

In February 1984, a statute was enacted in Wisconsin relative to PRV eradication in the state. The statute has three major provisions:

1) The Department of Agriculture is required to conduct a PRV surveillance program on Wisconsin swine (slaughter testing of breeding swine).

2) The legislation calls for eradication of PRV. Each infected quarantined herd must institute a clean up program based on the recommendations published by the Livestock Conservation Institute.

3) Indemnity is paid for the removal of breeding stock from a quarantined herd undergoing approved “clean up”.

As of August 1984, 14 quarantined herds have been identified in Wisconsin. An analysis of the pilot programs economic impact is also being planned.

North Carolina

In February 1984, a PRV pilot eradication protocol was approved. The program is based upon slaughter testing of identified breeding swine. Recommended control and eradication procedures are similar to those used in Pennsylvania. As of August 1984 35 quarantined herds had been identified in North Carolina. As of that date 18 had been cleaned by test and removal and 1 was out of business. The remainder were on Test and Removal plans or yet to establish a plan.
Initial information on the Illinois Pseudorabies Pilot Project was presented to this Committee in October, 1983, and appears in the Proceedings book on pages 438–444 inclusive. This paper will be an update on the project from October, 1983, through September, 1984.

The Pike County portion of the project was expanded southward from Detroit, the initial township, into Montezuma and Pearl townships. The project area encompasses approximately 70 square miles. There were 71 swine herd owners in the project and buffer zone area, of which 68 have participated by allowing blood samples to be collected from all or a predetermined representative portion of their herds. Positive animals have been disclosed in 21 herds on initial test and 1 on monitoring test. Six of these herds, generally with low SN positive titered swine and only one positive animal per herd, have been determined not to be infected through additional testing and epidemiology. In 3 additional herds the positive titers have been confirmed as due to prior pseudorabies vaccination. This leaves a total of 13 infected herds in the 68 herds tested, for an infected herd percentage of 19%.

A total of 34 retests have been conducted in the positive herds during the project, as well as 93 tests for maintenance of qualified pseudorabies-negative herds (there are ten in the area) or monitoring of previously negative herds. One new infected herd was disclosed on monitoring testing. This herd of 100 head of breeding swine had an initial negative test of 28 head. Nine of ten animals on the monitoring test conducted in June, 1984, had SN titers. Investigation revealed that this herd had been established in mid-1981 through purchase of negative gilts from a qualified herd about 90 days after the owner had disposed of a breeding herd that was suspected of being infected with pseudorabies. The owner had maintained a continuous feeding operation on another premise. Spread to the breeding herd occurred through the interchange of equipment and personnel between the feeding and the breeding herds. This infected herd was depopulated in August, 1984.

The three-township area in Pike County and the number of herds tested and number found infected, as follows:

- Detroit Township and buffer zone: 39 herds, 10 infected
- Montezuma Township: 16 herds, 2 infected
- Pearl Township: 13 herds, 1 infected

The Macoupin County portion of the project was expanded from the original 1½-township area to 4½ townships, located in the northwest corner of the county. This project area encompasses approximately 160
square miles. There were 84 breeding swine herd owners in the area, of which 78 have been participants in the project. Eight of nine persons in the area who only finish feeder pigs have agreed to selective blood testing. This is being done during the month of October. On the initial test, positive animals have been disclosed in 23 herds. Two of these herds have been determined not to be infected through additional testing and epidemiology. Previous administration of pseudorabies vaccine, resulting in low dilution titers, has been confirmed in one herd. This leaves a total of 20 infected herds in the 78 herds tested, for an infected herd percentage of 25.6%. Thirteen of the infected herds were already under quarantine at the start of the project. All were found to still be infected, through testing conducted during the period of the project.

Thirty-two positive herd retests were conducted. Twenty maintenance tests in the two qualified herds in the area or monitoring tests of previously negative herds have been conducted. All were negative.

The 4½-township area in Macoupin County and the number of herds tested and number found infected, as follows:

<table>
<thead>
<tr>
<th>Township</th>
<th>Herds</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barr Township</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>North Palmyra Township</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Scottville Township</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>South Otter Township</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>South Palmyra Township</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

Transparency #1 summarizes testing to date in the two project areas.

Transparency #2 provides the present status of all herds in which positive swine have been disclosed.

The Illinois Legislature, in late May, and with the support of the Illinois Pork Producers Association, appropriated State indemnity funds for voluntary depopulation of infected herds in the project area. Breeding swine were eligible for an indemnity of $25.00 per head, in addition to their market slaughter value. The appropriation applied during the State fiscal year which ended on June 30, 1984. The following protocol was developed: (1) Breeding swine must be identified, herd count taken, and a depopulation agreement signed by June 30; (2) Breeding swine must be sent to slaughter no later than August 15; (3) Feeder swine and nursing pigs do not qualify for indemnity and must be sent to slaughter or be transferred to a feedlot or another premise currently under pseudorabies quarantine, on permit from the Department, no later than December 1; and (4) Premises must be cleaned and disinfected, under supervision, following removal of swine. Each owner of an infected herd was contacted. Due to the short period in which to make long-range plans, credit considerations and anticipated higher hog prices in the fall, only three owners indicated a definite interest and two actually depopulated. We feel that five to seven more owners would have depopulated if indemnity funds had been available throughout the entire year, however.

The field trial of the capsular intradermal antigen (skin test) was discontinued in March, due to less than acceptable correlation with the SN
test. Sixty-two herds in the project areas, plus 1 large, known infected, herd outside the area, were utilized in the field trial; 1,281 swine were tested — 1,222 negative, 59 positive. Data on the field trials of the intradermal antigen was submitted to Special Diseases Staff, Veterinary Services, in April, 1984.

Some of our conclusions derived from the project, to date, are as follows:

1. More infected herds were disclosed than had been anticipated.
2. A number of small herds were found to be infected. Pseudorabies was evidently causing no problems or else clinical signs were disregarded by the owners.
3. Single SN positives in otherwise negative herds occur and must be evaluated epidemiologically.
4. Herds can be cleaned up through removal of positives and continued retesting.
5. Results on maintaining cleaned up areas are promising. Only 1 new infected herd has been disclosed, to date, in the 35 monitoring tests in Pike County and 14 in Macoupin County shown in this report. An additional 10 herds monitored during the month of October have all been negative.
6. The intradermal antigen (skin test) has not proven to be reliable as a diagnostic aid for detecting infected herds, at the present time.
7. Some type of indemnity to swine herd owners for herd depopulations must be available in any widespread eradication program.
## Illinois Pseudorabies Pilot Project

**Initial Herd Tests**
- **Negative Herds**: 49  
- **Positive Herds**: 19

**Swine Tested on Initial Herd Tests**
- **Negative**: 1,369  
- **Positive**: 100

**Positive Herd Retests**
- **Swine Tested on Positive Herd Retests**: 655  
- **Negative**: 593  
- **Positive**: 62

**Monitoring -- Maintenance**
- **Tests -- Herds**: 93  
- **(Tests in Qualified or Previously Negative Herds)**

**Swine Tested on Monitoring --**
- **Maintenance Tests**: 1,650  
- **Negative**: 1,637  
- **Positive**: 13

**Total Number of Swine Tested**
- **Negative**: 3,774  
- **Positive**: 175

---

<table>
<thead>
<tr>
<th></th>
<th>Pike County</th>
<th>Macoupin County</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SN Test</strong></td>
<td>Cumulative Total</td>
<td>Cumulative Total</td>
</tr>
<tr>
<td><strong>Initial Herd Tests</strong></td>
<td>68</td>
<td>78</td>
</tr>
<tr>
<td><strong>Negative Herds</strong></td>
<td>49</td>
<td>55</td>
</tr>
<tr>
<td><strong>Positive Herds</strong></td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td><strong>Swine Tested on Initial Herd Tests</strong></td>
<td>1,469</td>
<td>1,690</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>1,369</td>
<td>1,271</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>100</td>
<td>419</td>
</tr>
<tr>
<td><strong>Positive Herd Retests</strong></td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td><strong>Swine Tested on Positive Herd Retests</strong></td>
<td>655</td>
<td>937</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>593</td>
<td>843</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>62</td>
<td>94</td>
</tr>
<tr>
<td><strong>Monitoring -- Maintenance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tests -- Herds</strong></td>
<td>93</td>
<td>20</td>
</tr>
<tr>
<td><strong>(Tests in Qualified or Previously Negative Herds)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Swine Tested on Monitoring --</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maintenance Tests</strong></td>
<td>1,650</td>
<td>171</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>1,637</td>
<td>166</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total Number of Swine Tested</strong></td>
<td>3,774</td>
<td>2,798</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>3,599</td>
<td>2,280</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>175</td>
<td>518</td>
</tr>
</tbody>
</table>
ILLINOIS PSEUDORABIES PILOT PROJECT

Status of the positive herds located in the two counties is as follows:

<table>
<thead>
<tr>
<th>Status Category</th>
<th>Pike County</th>
<th>Macoupin County</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determined not to be infected</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Titers were due to PRV vaccination</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Following Herd Plan 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Released from quarantine</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Still under quarantine</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Following Herd Plan 1A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Released from quarantine</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Still under quarantine</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Following Herd Plan 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Released from quarantine</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Still under quarantine</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Following Herd Plan 3A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Still under quarantine</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Using vaccine, at present</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Undecided</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>22</strong></td>
<td><strong>23</strong></td>
</tr>
<tr>
<td>Herds still under quarantine as of this date</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>
Pseudorabies Committee Report

Chairman: M. H. Lang, Des Moines, IA
Vice Chairman: P. E. Bradshaw, Griggsville, IL

Fred Alderink, MD; George Edwards, NC; D. D. Gingerich, IA; Howard T. Hill, IA; L. W. Hinchman, IN; Donald Hoogestraat, SD; C. L. Kanitz, IN; J. P. Kluge, IA; C. W. Monsees, MO; K. E. Myers, IA; J. P. Quigley, Jr., GA; Carson Rogers, NE; L. W. Schnurrenberger, MD; P. L. Spencer, IL; Thomas Thurber, NE; Willard Waldo, NE.

The Pseudorabies Committee of USAHA convened on Tuesday, October 23, 1984 at 1:30 p.m. in the Scott Room of the Hyatt Regency Hotel, Fort Worth, Texas.

The committee chairman first asked for a report of the pilot projects currently underway in Illinois, Iowa, Wisconsin, Pennsylvania, and North Carolina. For the purpose of this report, the status of these projects have already been presented in a paper presented by Dr. David Thawley preceding this report and will be submitted for publication in the Proceedings of the 1984 Annual Meeting of the USAHA.

Dr. Harry Goldstein reported that Ohio has adopted rules that require a change of ownership test on all breeding swine offered for sale and that a traceback program is being considered for all sows and boars entering slaughter channels. A state indemnity program is also being considered.

Dr. Jack Flint reported that Minnesota has enacted rules to tighten controls on spread of Pseudorabies. There are 263 quarantined herds in Minnesota at the present time and since 1982, 36 herds, or 12% of the state's seed stock producers have gone out of business because of pseudorabies infection. To assist quarantined producers a rapid screen test has been made available to local veterinarians for herd clean-up efforts. The test kits have met with very little demand or interest so far.

Additional comments were presented from South Dakota and Hawaii industry that they recognize that PRV exists in their states and plans are being considered or underway to effect some control measures.

Dr. L. W. Schnurrenberger reported on the most recent slaughter serum survey that indicated that the incidence of PRV nationwide seems to be stabilized somewhat from the last survey conducted in 1980–1981 when the incidence was 8.4%. The current incidence as reported is 8.78% with 11.26% positive in the eight major hog producing states of the Mid West to 3.05% positives for the remainder of the States. Dr. Fred Alderink reported that an economic survey on the costs associated with PRV are being conducted in all five pilot project areas and it is hoped that the data collected will provide the swine industry and regulatory officials with the information necessary to decide on the feasibility of an eradication program.

Dr. Howard Hill, Iowa State University, reported the genetic differences
seen in a study conducted on five different breeds of swine immunized for PRV.

Dr. Sandy McGregor, University of Wisconsin, repeated a paper presented earlier to the AAVLD general session as a rapid test for detection of PRV antibodies called the Latex Agglutination Test. A very sensitive test that can be set up and read in 8-10 minutes.

Dr. Rick Sharpe, Norden Laboratories, reported on their progress with the development of a sub-unit vaccine. The product is in the process of being licensed and developed for commercial production. This product was first developed by Dr. Kenneth Platt, Iowa State University and information on the vaccine presented to this committee a year ago.

The role of feral swine in spread of PRV was discussed following a report by Dr. Victor Nettles on his findings while studying diseases in wild swine populations in 18 states, Puerto Rico and the Virgin Islands. Seropositive animals were detected in 16 of 27 sites where feral swine exist. The prevalence rate for the 16 sites was 28.6% with an average titer of 1:16. (Range 1:4 to 1:512).

That concluded the technical papers to be presented.

The chair recognized a motion from the floor that some states do not accept ELISA test results for interstate movement of swine. The motion called for all states to enact the necessary enabling legislation or rules to allow for officially tested negative swine to move in interstate commerce. The motion was seconded and passed.

A second motion called for this Committee to work with APHIS, NPPC and LCI to develop and draft minimum standards for PRV Qualified Negative States or Areas. The final proposal to be presented at the next USAHA annual meeting for consideration by the full Pseudorabies Committee a year from now.

Motion seconded and passed.

The meeting was adjourned.
The Iowa Pseudorabies Eradication Pilot Project

The Iowa pseudorabies eradication pilot project has been in operation for approximately 15 months. The organization and status of the project are shown in the following figures.

**Figure 1.** The project is organized in Marshall County in the heart of the hog raising industry of Iowa. It was selected not at random but as representative. The project objective of enrolling every hog raiser in the county has been nearly achieved and the project goal is to achieve county wide control.

**Figure 2.** Case finding was based on serotesting statistical samples of all enrolled herds. On each seropositive herd, a plan for elimination was worked out between the farmers and program veterinarians. A total of 31 seropositive herds have been identified.

**Figure 3.** Depopulation and repopulation on herd bases have been planned on 4 farms. All are currently in progress in herd depopulation.

**Figure 4.** Test and removal of seropositive pigs have been planned on 4 farms. None had over 3 positive animals detected in complete testing of breeding stock. Three have been reclassified as free at this time and 1 is still in post-removal testing.

**Figure 5.** Offspring segregation with rotation of breeding stock and controlled vaccination have been planned for the majority of farms, 23 in all. Implementation has been successfully completed on 3 farms and they have been reclassified free, 4 farms are probably free but require one more serological testing, 8 farms are still rotating out seropositive breeding stock, and 11 farms still have seropositive progeny as well as breeding stock, though in this last group, virus transmission appears to be stopped on 3.

**Figure 6.** This table summarized the present status of the 31 herds in which seropositive pigs have been identified. These herds are monitored by serological sampling every 3 months.

**Figure 7.** A total of 178 enrolled farms have been seronegative throughout the program. Eight farms are qualified negative while 95 farms are modified qualified negative in that they are serologically monitored on the statistical sample basis based on risk level. Five farms are controlled vaccinated negative while 68 farms are modified controlled vaccinated negative with seromonitoring on the statistical sample basis based on risk level.

The following trends and observations have been made as of this time.

**Figure 8.** Killed vaccine only has been used since the start of the project and principally in infected and high risk herds to protect the pigs against clinical disease. A total of 57% of the uninfected farms are not vaccinating.

**Figure 9.** In comparison to the initial outbreak of pseudorabies in Hardin County a decade ago in which no vaccines were used, prompt use of vaccine upon occurrence of the disease in Marshall County was correlated with a 61% reduction in disease in breeding stock and a 48% reduction in
deaths in baby pigs.

**Figure 10.** In further comparison of the outbreaks of pseudorabies in Hardin and Marshall Counties a decade apart, the clinical syndromes in unvaccinated pigs appeared identical. In Marshall County, where 18 infected herds were detected on serological bases only, 15 of these were being vaccinated prior to the appearance of pseudorabies in the area. None of the herds in which clinical outbreaks occurred were vaccinated prior to appearance of the disease.

**Figure 11.** Neutralizing antibody titers 3-6 months postvaccination in infected and uninfected herds are compared with those in unvaccinated infected herds in this figure. In infected pigs vaccinated with MLV vaccine, 85% of pigs were positive with mean titers of 1:20; in those vaccinated with killed vaccine, 78% were positive with mean titers 1:22; in unvaccinated infected herds, 49% were positive with mean titers 1:12. In uninfected herds vaccinated with MLV vaccine 24% were positive with mean titers 1:16; in those vaccinated with killed vaccine 19% were positive with mean titers 1:4. Vaccination of infected pigs evokes additional seroresponse. In uninfected pigs vaccinated with killed vaccines, titers are 4-fold lower and are shorter lasting than if vaccinated with MLV vaccine.

**Figure 12.** Serological testing conducted so far in the program has provided evidence that the statistical sampling procedure being used in adequate to detect seropositive herds.
Figure 1.

MARSHALL COUNTY, IOWA PSEUDORABIES PROJECT

- 590 FARMS
- 211 HOG FARMERS JUNE 1983
- 209 FARMS ENROLLED IN PROGRAM
- 31 FARMS CLASSED AS INFECTED
- 22 HOG FARMS OUT OF BUSINESS
- SINCE START OF PROGRAM
SEROLOGICAL TESTING SAMPLE SIZE

Statistical sampling based on a 95% probability of detecting infection in a herd in which 10% are positive animals.

Sample size required: Up to 100 head: Test all pigs up to 25
100-200 head: Test 27 pigs
Over 200 head: Test 28 pigs

HERD PLANS - INFECTED HERDS

DEPOPULATION - REPOPULATION - PLAN 3A

Currently 4 farms

All in progress in depopulation
3 Feeder pig operations 1 Farrow to finish
3 Semi-confinement 1 TOTAL CONFINEMENT
Figure 5.

HERD PLANS - INFECTED HERDS

CONTROLLED VACCINATION WITH OFFSPRING SEGREGATION - PLAN 3C

CURRENTLY 23 FARMS

3 HERDS RECLASSIFIED FREE (1 CONFINEMENT, 2 SEMI-CONFINEMENT)
4 HERDS PROBABLY FREE (4 SEMI-CONFINEMENT)
8 HERDS WITH NO EVIDENCE OF VIRAL TRANSMISSION BUT POSITIVE
  HOGS STILL ON FARM (3 CONFINEMENT, 4 SEMI-CONFINEMENT,
  1 PASTURE)
11 HERDS WITH EVIDENCE OF VIRAL TRANSMISSION (4 CONFINEMENT,
  7 SEMI-CONFINEMENT)
ALL 23 FARMS ARE FARROW TO FINISH

Figure 7.

SEROLOGICAL TESTING SCHEDULE IN 178 UNINFECTED HERDS

QUALIFIED NEGATIVE HERDS AND CONTROLLED VACCINATED HERDS

TEST ACCORDING TO CODE OF FEDERAL REGULATIONS, TITLE 9, PART 85

MODIFIED QUALIFIED NEGATIVE HERDS AND MODIFIED CONTROLLED VACCINATED HERDS

HIGH RISK HERDS: 48 HERDS

TEST ACCORDING TO STATISTICAL SAMPLE EVERY 6 MONTHS

LOW RISK HERDS: 130 HERDS

TEST ACCORDING TO STATISTICAL SAMPLE EVERY 9 MONTHS
Figure 9.

CLINICAL COURSE OF PSEUDORABIES WITH AND WITHOUT USE OF VACCINE

<table>
<thead>
<tr>
<th>CLINICAL MANIFESTATIONS</th>
<th>Vaccination not Done</th>
<th>Vaccination Instituted Promptly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER OF HERDS</td>
<td>AFFECTED</td>
</tr>
<tr>
<td>Disease in breeding stock</td>
<td>9</td>
<td>413/495</td>
</tr>
<tr>
<td>Deaths in baby pigs</td>
<td>4</td>
<td>2493/2956</td>
</tr>
</tbody>
</table>
Figure 10.

**PSEUDORABIES OUTBREAKS IN 1973-1974 IN 2 IOWA COUNTIES**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>No clinical disease recognized serologically</td>
<td>18</td>
<td>58</td>
</tr>
<tr>
<td>Clinical disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute disease in pigs</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>Chronic disease in pigs</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>Reproductive disease in breeders</td>
<td>7</td>
<td>54</td>
</tr>
<tr>
<td>Disease in other animals</td>
<td>4</td>
<td>31</td>
</tr>
</tbody>
</table>
Figure 11.
NEUTRALIZING ANTIBODY TITERS IN PIGS

MLV VACCINATED, INFECTED HERDS
384 SAMPLES

KV VACCINATED, INFECTED HERDS
812 SAMPLES

UNVACCINATED, INFECTED HERDS
467 SAMPLES

MLV VACCINATED, UNINFECTED HERDS
628 SAMPLES

KV VACCINATED, UNINFECTED HERDS
1284 SAMPLES
SEROLOGICAL TESTING FINDINGS

Among 41 herd samplings detecting positive breeding stock, greater than 10% were positive in 37 samplings, with a mean of 60% of the animals positive.

Among 18 herd samplings detecting positive progeny, greater than 10% were positive in 16 samplings, with a mean of 65% of the animals positive.

At the present state of the Iowa project it is observed that pseudorabies causes clinical disease in non-immune pigs today similar to a decade ago, but prompt vaccination upon appearance of the disease significantly reduces losses and vaccination prior to exposure prevents clinical outbreaks. Offspring segregation with rotation out of the breeding herd is proving effective and practical in herd cleanup, usually with no down time for the operator. The areawide enrollment of farmers with statistical serosampling of herds is proving effective in locating infected farms. The problem of farms with only 1 or 2 seropositive pigs in an entire herd continues to keep the sample size higher than would otherwise be necessary. Complete herd tests and removal of seropositive animals from such herds is proving successful but is costly.
Wild swine definitely should be considered as a potential reservoir for pseudorabies. Experimental infection of four European wild boar by Tozzini et al. 1982 resulted in no disease but virus was shed in the saliva and nasal discharge and subsequent infection occurred in contact controls. Virus was isolated from tonsil at slaughter. Oral virus shedding lasted 1–2 weeks. Antibody was produced and SN titers ranging from 1:16 to 1:64 at 42 days post infection.

Wild swine populations are present in 18 states, Puerto Rico and the Virgin Islands. The nationwide population probably is about 1 million hogs with Florida, Texas, Hawaii, and California being the leading states. These animals originated from two ancestral pools (1) hogs descendent from domestic stock and (2) hogs introduced for hunting purposes (European wild boar). The animals that we have today are all hybrids to some extent. For hunting, animals that appear as European wild boar are most desirable.

Wild swine eat a wide variety of roots, mast, farm residue, and live and dead animal matter. They are our most prolific big game mammal. Average litter size ranges between four and seven piglets and in warmer areas, two litters a year are possible. Theoretically, even at 80% mortality of young, if adult sows breed twice a year and produce five pigs per litter, the population will increase annually.

Between January 1979 and November 1983, the Southeastern Cooperative Wildlife Disease Study (SCWDS) surveyed wild swine from 27 sites in 11 southeastern states. Serums were tested on 423 animals and 93 (22%) had titers ≥ 1:4 on virus neutralization tests. Positive titers ranged from 1:4 to > 1:1512, with 1:16 being the median and mode for positive readings. Prevalence for reactors for the 16 enzootic sites was 28.6% (93 of 325).

Seropositive swine were identified in the following counties: Clarke County, Alabama; Union County, Arkansas; Franklin, Hendry, Orange, Osceola, Sarasota, and Wakulla counties, Florida; Chatham, Bryan, Liberty, McIntosh, and Telfair counties, Georgia, Pearl River County, Mississippi, and Georgetown County, South Carolina.

Virus isolation and fluorescent antibody tissue section tests were made on 278 of the aforementioned 423 pigs. Pseudorabies virus (PRV) was
demonstrated in spleen and tonsil by both methods in one pig. This animal came from Tosohatchee State Preserve, Orange County, Florida, or Merritt Island National Wildlife Refuge, Brevard County, Florida (mixed shipment). The PRV-infected swine had a purulent metritis and ulcerative dermatitis and a concurrent *Brucella suis* infection. The animal's virus neutralization titer was 1:4.

Since the time period mentioned above, SCWDS had opportunity to test 13 wild swine from the Havasu National Wildlife Refuge, Mojave County, Arizona. All were negative serologically. Ten of these animals were also tested for virus and found negative.

Moving further west, SCWDS worked collaboratively with Dr. David Jessup of the California Fish and Game Department on wild swine surveys in that state. Dr. Jessup collected animals from Merced, Monterey, San Luis Obispo, Santa Clara, and Tehama counties with the total number being 174 serums. These serums also were tested by the ELISA test at the National Veterinary Services Laboratory, and there were 10 positive or suspect animals on the ELISA test. However, all follow-up virus neutralization tests were below 1:4.

In Hawaii, studies have been made for pseudorabies on two islands, Molokai and Hawaii. It is doubtful that Molokai had infected wild swine although 2 of 127 (1.6%) had virus neutralization titers (1:4 and 1:8). Virus isolation attempts on nine pigs were negative from Molokai. Recent serologic studies on the Big Island of Hawaii yielded titers in 53 of 116 (46%) seropositives by the ELISA test. These animals were strongly reactive and it is likely that pseudorabies is enzootic on the Big Island since disease has been observed in hog catching dogs.

In investigating a great many cases of acute poisoning in cattle caused by various chemical agents over the last thirty years, it has been observed that many cattle herds experienced bacterial infections either concomitant with poisoning or within a very few days following the apparent correction of the poisoning condition. It has been observed that many cattle harbor a number of latent bacterial pathogens that can become active pathogens when changes within the animal body provide conditions favorable to their growth and in many cases, invasion into the animal body proper. A number of pathogens such as *Corynebacterium* species and *Pasteurella* species may be found in the upper respiratory tract of healthy cattle in which growth and invasion is suppressed by healthy mucous membranes and the animals' body defenses. A number of enteric pathogens including *Salmonella* species are also harbored in the gastrointestinal tract without causing any apparent signs of illness probably because conditions for optimum growth are lacking. It has been well established that many cattle harbor various *Clostridium* species in various tissues of the body in the form of inactive spores thought to have been carried into the body by internal parasites and their larvae. The spores lie dormant and will not vegetate under normal tissue conditions requiring necrotic tissue under anaerobic conditions in order to vegetate and to grow.

In acute poisoning in cattle resulting from strong necrotizing agents such as a number of the different heavy metals and metalloids and fertilizers containing potash and ammonium salts or ammonia nitrogen compounds, it has been observed that many that die have a concurrent clostridial septicemia and toxemia. Death in these cases may often have been the result of the clostridial toxemia rather than the ingested poison. The *Clostridium* species found in cases of this nature have been *Clostridium chauvei*, *Clostridium septicum*, *Clostridium sordelli*, and *Clostridium novyi* and either one or more of the species may be involved. It was also observed that poisoned cattle that had been immunized against the various clostridia or had been treated with antibiotic did not have a concurrent clostridial septicemia and toxemia.

There are a number of cases of acute poisoning in which concurrent clostridial septicemia and toxemia were found that could be presented; however, one case involving two cattle herds that were poisoned by tin compounds will be provided to exemplify heavy metal poisoning in which a concurrent clostridial septicemia and toxemia occurred.

Two herds of cattle were pastured in separate fields adjacent to a tin manufacturing plant. One herd consisted of grade Angus cattle, and the other consisted of very fine Hereford cattle. It was reported that the tin

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*Bacteriological Infections as a Sequelae to Poisoning*

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Winchester, Kentucky 40391
plant had been releasing volumes of chemical effluent into the air that was reported to be quite irritating and obnoxious to those living near the tin plant. Symptoms of poisoning began to occur in the grade Angus herd and a few days later in the Hereford herd.

The symptoms of poisoning in the grade Angus herd included incoordination, muscular trembling, impaired vision, central nervous system signs including extreme ferocity to the point of charging personnel and any vehicles moving in the pasture. After a few hours, those with more advanced symptoms were unable to stand. Two were taken alive to the state laboratory for autopsy, humanely put to death and immediately autopsied.

The autopsy findings on gross examination revealed severe liver necrosis and septicemia with bacillary forms identified by fluorescent antibody technics as *Clostridium novyi* and a necrotizing hemorrhagic gastroenteritis. The gross pathology provided the characteristics of a heavy metal type of poisoning and secondary *Clostridium novyi* septicemia and toxemia. The veterinarian was advised to institute antibiotic therapy in both herds, and did treat the Hereford herd. The owner of the Angus herd chose not to institute any therapy because all were showing symptoms, the danger in handling them and a lack of good facilities for treating cattle of this temperament. However, a few of the cattle that were not so vicious as the others were administered an antibiotic. All of the Angus cattle died. Those autopsied that had not received antibiotic therapy were heavily infected with clostridial organisms. Those autopsied that had received antibiotic therapy did not reveal the presence of clostridia in their tissues. Tin poisoning was later confirmed.

There have been numerous cases of poisoning in cattle over the years in which the causative agent was revealed and removed, and the cattle were improving well under therapy. About a week after the initial poisoning in which there were no further losses and the cattle appeared to be returning to normal health, the cattle developed a severe diarrhea, became dehydrated and lethargic. In the latter course of the illness, the mucosal lining of their intestines would be expelled along with the severe diarrhea. In such cases, the owner believed that his animals were again being poisoned until the disease condition was defined, and the cattle were placed under corrective therapy. The condition as described was the result of a sudden massive growth of *Salmonella* species. In a number of cases, the personnel handling the cattle during therapy also became ill with the *Salmonella* infection.

An example of the numerous cases of this kind that have been investigated is one involving two herds of cattle owned by a farmer located on two different farms about five miles apart. One herd was a Hereford cattle herd located on one farm, and the other herd was an Angus cattle herd located on the other farm. The Angus herd was divided into breeding stock and yearling heifers, all registered stock.

The farmer prepared the feed for his cattle from corn, oats, bran and
ground alfalfa. Following the mixing of new feed, the Hereford herd and the Angus breeding herd became ill following eating the feed. The Angus heifers were being maintained on the remainder of the previous feed mix and were not demonstrating any signs of illness. Nursing calves in the Angus breeding herd and the Hereford herd did not have any signs of illness.

Autopsies conducted on animals that died revealed that the animals had died as a result of ammoniacal poisoning with a great excess of potash and phosphorus as well as ammonium salts in the gastrointestinal contents. Examination of feed samples revealed that the source of the potash, phosphorus and ammonium compounds was in the newly prepared feed. The farmer discontinued feeding the new feed, and the cattle began to return to normal health.

The source of the contamination of the feed was determined as fertilizer residues in the bottom of his corn crib. He had stored his fertilizer in the corn crib for several years and fertilizer from broken bags had accumulated in the bottom of the crib. He never used the bottom layer of corn in the crib when he mixed the feed; however, he had to be away and his two sons mixed the feed using all of the corn including the contaminated corn in the bottom of the crib.

About a week later when the sick cattle seemed to be returning to good health, those on both farms began to have diarrhea, became dehydrated and lethargic, and some began passing intact plugs of intestinal mucosa. Nursing calves remained healthy, and the Angus heifers that were never ill remained in good health. Autopsies and laboratory examinations revealed that the cattle were now suffering from a severe case of salmonella infection in which the causative agent was *Salmonella enteriditis* serotype D. Nursing calves became sick with the salmonella infection a few days later. The losses in the two herds were heavy although most of the sick cattle responded to recommended antibiotic therapy under veterinary care.

During the time that the cattle were under therapy for salmonellosis, the farmer and those helping to treat the cattle became ill with the *Salmonella enteriditis* infection.

A similar case of acute poisoning in cattle in which the poisonous agent was molybdenum followed much the same pattern. A week after poisoning occurred, the herd became ill with salmonellosis in which the agent was *Salmonella enteriditis* serotype D. The cattle owner and his family handling the cattle during therapy also became ill with *Salmonella enteriditis* infection.

In cases of poisoning, it is recommended that proper therapeutic measures be taken to prevent concurrent and possible subsequent infections. In the event that an infectious agent hazardous to man does occur, those handling the animals should be advised of the hazard and precautions to take to prevent possible infection.
REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND ENVIRONMENTAL QUALITY

Chairman: A. J. Roth, Richmond, Virginia

Vice Chairman: Robert H. Singer, Winchester, Kentucky

F. J. Alderink, MD; F. M. Applehans, TN; A. W. Bailey, OK; R. P. Crawford, TX; S. L. Diesch, MN; C. R. Dorn, OH; J. A. Farrar, FL; S. L. Hendricks, MN; W. E. Jennings, TX; J. C. Leightly, MD; E. L. Menning, VA; W. R. Miller, MD; R. L. Parker, SC; J. E. Pearson, IA; J. C. Prucha, MD; D. F. Schwindaman, MD; T. P. Siburt, VA; C. D. Stumpff, KS.

The Committee on Public Health and Environmental Quality met at 1:30 p.m., Wednesday, October 24, 1984, as scheduled. A total of 10 members and 14 guests were in attendance.

The Subcommittee on topics for discussion at next year's annual meeting were reported by Dr. Ed Menning. The subcommittee suggested 11 oral topics. These will be evaluated and those 4 or 5 topics that can be reported will constitute our next year's agenda.

Dr. Morris Potter discussed current and future veterinary activities at CDC and answered questions that committee members had in regard to policies at CDC.

Dr. Wiley Tanner, Texas Department of Health, reported on Tick Borne Zoonosis. He showed many slides on this subject. He discussed in detail Rocky Mountain Spotted Fever and Lyme disease. He discussed the Lone Star tick and the Brown Dog tick in their relationship to transmitting these diseases.

Dr. C. D. Stumpff presented an excellent update on program activities and the number of tuberculosis cases in cattle and buffalo this year. He stressed that more funding was necessary in order to carry the program to a successful conclusion.

Dr. Robert Singer discussed Bacterial Infections in Cattle following Poisoning. Dr. Singer's report was presented to you this morning thus I will not report on his subject matter.

Dr. Paul Siburt presented a report on Ethylene Dibromide in Agriculture. Ethylene Dibromide (EDB) is an insecticidal fumigant used against certain pests of stored products, for the treatment of fruit and vegetables, for the treatment of equipment in flour mills and the treatment of soil against some soil insects and nematodes. It has been used as a soil insecticide since the 1920's.

Ethylene dibromide is also added to all gasolines which contain lead in order to prevent the accumulation of lead in the engine. The lead is converted to volatile-lead bromide and eliminated through the exhaust.

Although EDB has been used as an insecticide for over 50 years, and is highly toxic, very few cases of poisoning of man or animals have been reported following its use on soils.
On September 30, 1983, the Environmental Protection Agency suspended EDB uses for soil fumigation. On February 3, 1984, the EPA suspended all uses of the pesticide EDB for the fumigation of stored grain and spot treatment of grain milling equipment.

The subcommittee on Economic Loss to Industry in Human Productivity Due to Zoonosis was prepared by Dr. S. L. Diesch and these findings were reported to the Committee.

It was reported that very little organized information is available that reflect costs of this nature to industry and the national economy. It is recommended that the U.S. Department of Agriculture take an active role in obtaining this very important information. The subcommittee's report is attached.

October 22, 1984

Report to:

The Public Health and Environmental Quality Committee
by the subcommittee on
Economic Loss to Industry in Human Productivity
Due to Major Zoonoses

Dr. S. L. Diesch, Chairman
Dr. Fred Alderink, Dr. A. W. Bailey,
Dr. J. A. Farrar, Dr. C. D. Stumpff

Information in this report was collected from the members of the subcommittee and Dr. Morris Potter, Center for Disease Control, UPHS, Atlanta, GA.

The following brief abstracts of information indicate the status of economic loss in human productivity. Since there appears to be no uniform criteria for economic loss determination of human productivity due to a zoonosis, each report must be considered separately. Parts or all of reprints of the five diseases discussed are attached indicating how the assessments were made.

Brucellosis

Following a review of available information, the National Brucellosis Technical Commission calculated the cost of a case of brucellosis in a packing plant employee to the industry to be $4,095 in 1977. This figure did not include physicians' fees or hospitalization.¹ (See addendum 1.)

Salmonellosis

Cohen et al² collected information on the total cost of 234 cases which was $151,125 (mean of $645.00): $103,400 (68%) in medical costs; $39,836 (26%) (mean $170.24) in lost income or productivity; and $7,889 (5%) in miscellaneous costs. It is further broken down to patients who saw a physician and were not, or were hospitalized (see addendum 2).

Rabies

The economics of one rabid dog in California was estimated at $105,790
or over $1,500 per person treated. This did not include lost work time and other factors\(^3\). (See addendum 3.)

**Psittacosis**

The cost of an outbreak of psittacosis in twenty-eight employees of a turkey-processing plant in Nebraska was determined. The diagnosis was serologically confirmed in twenty-two of the employees. The cost of the outbreak for reimbursed medical costs, workmen's compensation, and lost wages was $19,000\(^4\). (See addendum 4.)

**Trichinosis**

In Illinois twenty-three persons became ill with trichinosis following eating raw home-made summer sausage. Twelve patients were hospitalized for an average of ten days each. Seventeen of the twenty-three patients submitted information on medical expenses incurred and wages lost because of the outbreak. The costs totaled almost $20,000\(^5\). (See addendum 5.)

Information concerning economic loss to industry in human productivity due to zoonoses is limited. Much of the previous economic assessments were conducted by public health investigators. This committee should discuss and possibly recommend that the United States Department of Agriculture and other agencies further their efforts in the economic assessment of diseases which are transmitted between animals and humans.

**REFERENCES**


CURRENT STATUS OF HUMAN SALMONELLOSIS AND PROSPECTS FOR CONTROL
Scott D. Holmberg and Morris E. Potter
Centers for Disease Control
Atlanta, GA 30333

Salmonella remains a health burden to society. Despite the efforts of state, local, and federal health agencies, the reported incidence of human salmonellosis in the United States has increased in the past 20 years. The number of reported isolates was more than 40,000 in 1983, and it has been shown that for every reported case there are 50–100 unreported ones. Thus, it is estimated that between 2 to 4 million Americans are infected with Salmonella each year.

It is clear that salmonellosis is an economic burden to society. Although it is difficult to assess accurately the economic costs of salmonellosis to society, the patient-related costs for 234 individuals in a Salmonella outbreak in 1976 are illustrative (Figure 1). The average cost per patient was high, even when presented in 1976 dollars. This one outbreak alone was responsible for an estimated $151,000 in direct costs and almost 4 million dollars when both direct and indirect costs for reported and unreported cases were included in the cost estimates. Since 1976, medical costs in all the categories presented in this article have been increasing at a rate of 10%–15% per year. Thus, the costs of this outbreak would be approximately doubled if assessed in 1984 dollars. Extrapolating from this and other examples, the total economic impact of salmonellosis has been estimated to be about 1 billion dollars a year (Dr. D. Houston, Salmonella Symposium, New Orleans, 1984). In addition, great expense is often incurred when commercial items found to be contaminated with Salmonella are recalled, as for example the estimated $5 million lost in recalling of Salmonella-contaminated chocolate candy in 1973. In fact, no matter how one estimates the cost of salmonellosis, they are large.

The epidemiologic characteristics of patients with Salmonella infections have changed little since Salmonella surveillance began in the early 1960s. Outbreak investigations continue to identify foods and products of animal origin as the major vehicles for Salmonella outbreaks in humans. As presented at the International Salmonella Symposium in New Orleans in 1984, techniques of molecular biology have been used to characterize or to "fingerprint" salmonellae. In the last 3 years, we have found that this combination of epidemiologic investigation with techniques of molecular biology has greatly increased our understanding of the epidemiology of salmonellosis. To recapitulate these data briefly, endemic or sporadic Salmonella infections seem to occur as a series of small outbreaks. Organisms that are introduced into a community by contaminated food products appear to have little person-to-person transmission, and then disappear, only to be replaced by new Salmonella species. Recent molecular epidemiologic data emphasize that Salmonella is primarily a foodborne disease with little person-to-person transmission.
Thus, as we see it, there is little meaningful controversy about the health implications, costs, or sources of salmonellosis in the United States. A more important debate centers on where to interrupt transmission of the organism.

Some have advocated that transmission can be interrupted at the consumer end, and that good domestic hygiene will provide an effective barrier to the spread of salmonellae. We do not believe that trying to interrupt transmission of Salmonella at the consumer level will have much impact on salmonellosis for three reasons:

First, educating consumers and changing personal food handling and preparation habits is difficult. In a 1973 Gallup survey of 831 homemakers only a few of the women interviewed understood the sources and significance of Salmonella (Figure 2). Seventy-four percent did not know that Salmonella was a bacterium that causes food poisoning; 67% did not know how to prevent its spread once introduced into the home in a contaminated product; and 39% erroneously believed that poultry and meat in the market had been inspected for the presence of salmonellae. Their performance on the questionnaire underscored that Salmonella can truly be called an "unknown disease of millions."

Second, even if the American public is adequately educated, reasonable techniques of food handling may still not prevent cases of salmonellosis. In a recent study in Great Britain (Figure 3), frozen broilers were marked with an indicator organism, a non-pathogenic E. coli, and food preparers in 60 kitchens were asked to prepare these broilers as they normally would. Subsequent environmental sampling showed tremendous cross-contamination of many areas of the kitchens.

Third, as mentioned earlier, our studies have found that Salmonella is introduced into a community by contaminated foodstuffs, and there is little person-to-person transmission. Most cases seem to derive directly from salmonellae in products of food-animal origin, so stopping further spread to additional persons will not substantially change the incidence of human disease.

The public health significance of these data should be apparent. Intervention strategies relying on consumer education have been and will continue to be only marginally effective in controlling salmonellosis. We need, therefore, to interrupt transmission of Salmonella at earlier points.

Perhaps the most dramatic example of the benefits of intervening at the origins of Salmonella is seen in turtle-associated salmonellosis. Legislation banning the sale of pet turtles has resulted in a significant decrease in the frequency of salmonellosis in the 1-to 9-year-old age group in the United States. Furthermore, a recent turtle-associated outbreak of salmonellosis in Puerto Rico demonstrates that this public health problem would still be widespread in the United States if this contaminated vehicle were still available.
In conclusion, over the last several years the combination of epidemiology and molecular biology has demonstrated repeatedly that, in the United States, salmonellosis is primarily a foodborne disease. Epidemic strains of *Salmonella* are introduced into the community by foods of animal origin, and there is little person-to-person transmission. Since most salmonellosis appears to be caused directly by contaminated foods, and since attempts to educate the public about proper food-handling and preparation have been largely ineffective, we believe that human salmonellosis will continue to increase unless steps are taken to eliminate *Salmonella* from the food chain.
Patient-related costs for 234 surveyed, Salmonella heidelberg outbreaks, Colorado, 1976

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<thead>
<tr>
<th>Cost Category</th>
<th>No. affected</th>
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<th>Cost/person</th>
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<td>Physician office charges</td>
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<td>$9,132</td>
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<tr>
<td>Emergency-room charges</td>
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<tr>
<td>Medication costs</td>
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<tr>
<td>Hospitalization costs</td>
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<td>$88,079</td>
<td>$1,295</td>
</tr>
<tr>
<td>Income or productivity loss</td>
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<td>$39,836</td>
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<tr>
<td>Miscellaneous costs</td>
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<td>$101</td>
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<tr>
<td><strong>TOTALS</strong></td>
<td><strong>234</strong></td>
<td><strong>$151,125</strong></td>
<td><strong>$645</strong></td>
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</tbody>
</table>
Results of Gallup survey of 816 women
National sample, 1973

<table>
<thead>
<tr>
<th>Question</th>
<th>% Who Answered Correctly</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Know what <em>Salmonella</em> is</td>
<td>26%</td>
</tr>
<tr>
<td>- Know how to minimize the spread of <em>Salmonella</em> that may be present in raw meat and poultry that are brought home</td>
<td>33%</td>
</tr>
<tr>
<td>- Believe that raw meat and poultry are inspected for <em>Salmonella</em></td>
<td>39%</td>
</tr>
</tbody>
</table>
Contamination of various objects during the preparation of 60 frozen broilers, contaminated with *E. coli* K12

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<tr>
<th>Object</th>
<th>No. of samples</th>
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<tr>
<td>Cutting-board</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Same, after rinsing</td>
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<td>77</td>
</tr>
<tr>
<td>Plate, dish, strainer</td>
<td>29</td>
<td>90</td>
</tr>
<tr>
<td>Same, after rinsing</td>
<td>32</td>
<td>72</td>
</tr>
<tr>
<td>Grating of sink</td>
<td>54</td>
<td>87</td>
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<tr>
<td>Raised border of sink</td>
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<td>67</td>
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<tr>
<td>Tap</td>
<td>56</td>
<td>82</td>
</tr>
<tr>
<td>Dishcloth</td>
<td>38</td>
<td>74</td>
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<tr>
<td>Kitchen utensils</td>
<td>81</td>
<td>68</td>
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<td>Kitchen table</td>
<td>209</td>
<td>65</td>
</tr>
<tr>
<td>Doorhandle</td>
<td>51</td>
<td>14</td>
</tr>
</tbody>
</table>

From: de Wit JC, Broekhuizen G, and Kampelmacher EH J. Hyg. (Camb) 1979; 83: 27-32
REFERENCES


REPORT ON THE 1984 INTERNATIONAL SYMPOSIUM ON SALMONELLA

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University of Massachusetts
Amherst, Massachusetts 01003
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College of Veterinary Medicine
University of Minnesota
St. Paul, Minnesota 55108


INTRODUCTION

It has long been recognized that salmonellae occur throughout the World, that they commonly infect food producing animals, with or without development of serious disease, and that raw foods of animal origin are frequently contaminated by salmonellae and serve as a major source of human infection. A variety of approaches are being used in different parts of the world to prevent or contain infections in domestic food animals. Marked broad success has been achieved only with the host-adapted species *Salmonella pullorum* and *S. gallinarum* in poultry and to a lesser extent with *S. cholerae suis* in swine.

The large expanding international trade of fresh red-meats, poultry, marine foods and animal by-products for use in animal feeds, as well as of domestic animals, has understandably caused concern by importing countries. It is understandable that import restrictions have been enacted which are sometimes viewed as being unrealistic and which result in restraint of trade.

Although there have been an ongoing series of conferences, committees and commissions in various parts of the world to consider ways to improve control of salmonellae, most of them have considered the problem rather narrowly from either a geographic, product, or species standpoint. It is quite obvious that improvement in control of salmonella must be considered from an international perspective and that all people directly and indirectly involved with control efforts appreciate the full breadth of the problem and the practical capabilities and limitations of control efforts. Those with a primary interest in public health who call for marketing foods and feeds free of salmonella are no more correct in their assessment of practical control methods than food producers who call for solving the threat to human health in the kitchen.

The symposium was initiated by the American Association of Avian Pathologists and was sponsored by an additional 35 federal agencies,
professional organizations, and national, state and private producer organizations. This broad and generous support resulted in funding of almost $45,000 which made the Symposium possible.

OBJECTIVES

1. To review the international salmonella situation in respect to:
   A. Food animal production.
   B. Red meat and poultry processing.
   C. Animal feeds.
   D. Public health.

2. To identify practical methods and approaches attainable with developed techniques to prevent or reduce infections in the major food producing animals and in products derived from them.

3. To assess control of host-adapted salmonellae such as *S. gallinarum* and *S. dublin*.

4. To assess the utility of national and regional regulatory programs for controlling salmonellae including the impact of regulatory action on world trade.

5. To develop a consensus among the participants of the current and expected future situation and practical approaches to the control of salmonellae.

THE PROGRAM

A total of 40 papers by invited speakers and 36 posters were presented by authors from 18 countries. The nearly 200 in attendance from a total of 24 countries represented all groups concerned with salmonella control including public health; poultry and livestock breeding, production, slaughtering and marketing; other food producers; feed manufacturers including those producing animal by-products; and regulatory and research people.

With all the inherent disadvantages of an international meeting of this type, it served admirably to bring together these people who often have rather divergent and sometimes perhaps narrow interests and perspectives, and to allow consideration of the breadth of the salmonella problem.

The sum and significance of the information presented was broadly summarized by the following five statements which were endorsed by the group following a lengthy discussion near the end of the program.

1. The eradication of salmonella in domestic animals is not attainable at this time except for specific infections such as *S. pullorum* and *S. gallinarum*. Serious efforts should be made to control and reduce the incidence of salmonella infections in domestic animals.

2. Methods are available to reduce salmonella contamination of processed feeds to safe levels, but feed contamination remains a widespread and serious problem. Further improvement is necessary.
3. It should be candidly recognized that raw foods of animal origin are frequently contaminated by salmonella and that such contamination levels can not be expected to change greatly in the near future.

4. Trade barriers should not be erected and import requirements in respect to salmonella should not exceed standards attained in the importing country.

5. There should be continuing programs to provide education and information on proper food handling to the consumer and to all those handling foods.

A brief commentary will be attempted in respect to each of the above statements to at least partially explain them. The comments are made with the realization that they must necessarily be fragmentary and that others might make quite different comments.

1. The eradication of salmonella from domestic animals is not attainable at this time except for specific infections such as *Salmonella pullorum* and *S. gallinarum*. Serious efforts should be made to control and reduce the incidence of salmonella infections in domestic animals.

Although *S. pullorum* and *S. gallinarum* are either well controlled or eradicated from poultry in most of the developed countries, *S. gallinarum* is rampant in Mexico and in many countries in Central and South America as well as much of the Near East. *Salmonella dublin* has recently spread through much of the U.S. and also into parts of Western Canada and has long been a problem in much of Europe.

Sweden has for some years had an expensive national program to control the paratyphoid salmonella in their small poultry industry. Slaughter for fresh carcasses is allowed only if the flock is negative or has a low incidence of infection. Our Canadian colleagues are several years into a program to reduce the incidence of salmonella in poultry by a broadranging microbiological control program.

Several poultry breeding organizations have developed salmonella control programs, some of which have been quite successful. An important segment of the turkey industry in Minnesota has been operating a salmonella control program for some years which has demonstrated that salmonella-free turkey flocks can be maintained and slaughtered without carcass contamination.

Most of the poultry and livestock industry of the world makes little or no effort to prevent infection by salmonellae. Infection in herds and flocks is common and may occur with or without serious clinical disease.

Killed vaccines show some promise for aiding in control, at least in turkeys, and “crippled” recombinant live cultures and subunit vaccines are under active investigation by a number of workers.

Colonizing chicks and poults with negative gut microflora has been demonstrated to be effective in containing infection but does not prevent low levels of infection in heavily exposed flocks. The fact that some
antibacterials prolong the salmonella carrier state is presumably a consequence of disrupting the normal complex protective microflora of the gut.

Epidemiological techniques have been further improved and plasmid profiling has been used very effectively in combination with longer established methods by our colleagues in the Centers for Disease Control for tracing transmission from livestock to humans.

With all things considered, one must conclude that salmonella infections remain common in food producing animals throughout the world and that there is no reason to expect that this situation will change greatly in the near future.

2. Methods are available to reduce salmonella contamination of processed feeds to safe levels, but feed contamination remains a widespread and serious problem. Further improvement is necessary.

It should be clearly recognized that any thermal destruction process, including steam pelleting of feeds for salmonella destruction, results in a logarithmic reduction of viable cells. Therefore, zero is never reached and it is inappropriate to call for sterile feeds. Rather a somewhat arbitrary tolerance must be accepted which would result in a product that is not capable of initiating a salmonella infection. Animal feeds in Europe were reported to be well controlled in respect to salmonella. In many parts of the world, including the U.S., feeds are frequently contaminated, and animal by-products constitute one of the more common sources of contamination. It has been gratifying to learn that the National Renderers Association is developing a voluntary program to reduce the level of contamination in products produced by member organizations.

3. It should be candidly recognized that raw foods of animal origin are frequently contaminated by salmonella and that such contamination levels can not be expected to change greatly in the near future.

This statement was intended to clearly recognize current and expected future reality. It was not considered economically feasible to produce salmonella free herds and flocks on a large scale, now or in the near future, which is an essential for producing fresh meats and poultry dependably free of salmonella. Employing the best techniques during slaughter and processing of infected herds and flocks can greatly reduce cross contamination. One major U.S. chicken producer indicated that they are able to hold carcass contamination to about 2% compared to an industry average of perhaps 20% or greater. Cross contamination can also be much reduced by using improved slaughter and processing techniques for red-meat animals.

Irradiation is recognized as an effective method of a pasteurization which produces minimal change in the product. Public acceptance of irradiated foods is questionable and the true cost for large scale use has yet to be demonstrated.
4. **Trade barriers should not be erected and import requirements in respect to salmonella should not exceed standards attained in the importing countries.**

There has long been a perception that salmonella contamination of foods and feeds or of salmonella infections in livestock and poultry has sometimes been used as an excuse to erect trade barriers to serve other purposes. Perhaps at times such trade barriers have been put in place because of lack of knowledge of the true situation in the importing country. Additionally there are legal requirements, including those in the U.S., which mandate that no pathogens be present in foods or in feed. Such legal requirements cannot be broadly enforced without disastrous disruption of food supplies.

Some would have preferred calling for development of acceptability standards agreed to by all trading nations. The statement adopted provides an immediate objective and does not preclude the more desirable longer range development of international standards.

5. **There should be continuing programs to provide education and information on proper food handling to the consumer and to all those handling foods.**

The title of the last paper on food handling was most aptly termed “Good Food Handling — The Final Line of Defense Against Food-borne Infection”. It was emphasized that nowhere can one be assured that salmonella, as well as other pathogens including e.g. *Clostridium perfringens*, *Campylobacter jejuni*, *Bacillus cereus*, *Escherichia coll*, *Vibrio cholera*, *Vibrio parahaemolyticus*, shigella, staphylococci and streptococci, may not have contaminated the food chain. Good food handling is indeed an essential final line of defense against all of the food-borne infections.

These five statements, which represent a concensus of those present, were not intended to indicate that salmonella infections and contamination of foods and feeds should be ignored. Rather they are to indicate that salmonella and salmonellosis constitute a major health problem of both man and other animals and that control requires a long-term broad and comprehensive effort. The magnitude of economic loss in the Federal Republic of Germany in 1977 from salmonellosis in man and domestic animals was estimated to be 251.2 million German marks ($81 million current exchange rate). Of this slightly over half of the economic loss was from infections in food producing animals. West Germany, though a small country, is not atypical and indicates that a similar economic loss probably occurs throughout much of the world.

This is a brief and necessarily superficial report. A proceedings will be published early next year and will include all papers in total and summaries of posters and edited discussions. It may be purchased, at a price yet to be determined, from the American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, Philadelphia, PA 19348-1692.
SALMONELLA AND ARIZONA SEROTYPES FROM ANIMALS AND RELATED SOURCES REPORTED DURING FISCAL YEAR 1983

Kathleen Sutch, BS
and
C. D. Murphy, DVM, MS
The Diagnostic Bacteriology Laboratory
National Veterinary Services Laboratories
Animal and Plant Health Inspection Service (APHIS)

SUMMARY

Serotyping of salmonella cultures from animal disease cases and epidemiologically related sources is reported for October 1, 1982, through September 30, 1983 (FY 1983). A total of 5694 field isolates were serotyped. The most frequently identified salmonella serotypes were Salmonella typhimurium, S. cholerasuis var. Kunzendorf, S. heidelberg, S. typhimurium var. Copenhagen, and S. agona. The most frequent sources of cultures, including both Salmonella and Salmonella-arizona serotypes were turkeys followed by cattle, swine, and chickens.

INTRODUCTION

Data for this report were accumulated at the National Veterinary Services Laboratory, Animal and Plant Health Inspection Services, 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. Most of these laboratories appreciate the importance of accurate data and made a concerted effort to provide quality input. Also, the reports were screened for obvious errors. However, it was impossible to verify each entry and the quality of the total report is a reflection of the cooperative spirit of these laboratories.

The purpose of this report is to make the data available to epidemiologists and others who have a need for it. The data are represented in tables similar to previous reports in order that comparisons can be easily made. For this reason although Arizona isolates are now reported on the basis of their corresponding salmonella antigens, they are separately reported in Table 3.

To illustrate how “Arizona” serotypes are reported: Arizona 7a, 7b:1,7,8 is now identified as Salmonella 18:Z4, Z32 (Arizona). We have continued to use the term “Arizona” in parenthesis following the serotype in order to ease this transition. The most frequently occurring serotype on Table 3 is 18:Z4, Z32 (Arizona). It is usually associated with turkeys, and made up 25% of the serotypes identified in cultures from turkeys. If it were included
in Table 10 of the most frequently occurring Salmonella serotypes it would displace S. anatum as fourth in frequency.

DISCUSSION

Cultures for serotyping were received from 45 states, the District of Columbia, and Puerto Rico (Tables 1 and 3). This was one more state than in FY 82.

The total number of cultures serotyped was 5649. This compares with 5389 serotyped during FY 82. One serotype was identified for the first time in animals in the United States. S. bangkok was isolated from a gray parakeet in Pennsylvania.

One serotype, S. saint paul decreased from 223 in FY 80 and FY 82 to 95 in FY 1983. It ranked thirteenth in frequency of occurrence this year. Previously, it has been among the 10 most frequently identified serotypes since 1970.

S. krefeld increased from 20 isolations in FY 82 to 67 this year. It was one of the most frequently identified serotypes from horses (Table 9). The majority of isolations (61) were from California, three came from Louisiana and three from Minnesota. Thirty-eight of the isolations were from horses, 2 from cattle, 2 from swine, 2 from other animals, and the rest were environmental and miscellaneous. Although this is a large increase over what has been identified in previous years, the majority of isolations came from a single source and do not represent a widespread problem.

S. cerro increased from 35 isolations in 1982 to 80 this year. The most noticeable sources of increases were from turkeys (0-29) and feed (0-10).

One serotype S. broughton, was identified 11 times after not having appeared in FY 80, FY 81, or FY 82. All of the 11 isolates were from California turkeys.

Salmonella 61:1,5 (formerly arizona 26:30) continues to be strongly associated with sheep.
SALMONELLA AND ARIZONA SEROTYPES
TABLE 2. DISTRIBUTION OF MUCOCELLS SECRETIONS OF SOURCES - FEEDS

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<tr>
<th>Source</th>
<th>Sheep</th>
<th>Horse</th>
<th>Moose</th>
<th>Cattle</th>
<th>Duck</th>
<th>Other</th>
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Note: The table continues with more entries and data.
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<tr>
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**Table 2. Continued**

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### Table 7. Cattle--Most Frequently Identified Serotypes in FY83

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Percent morbidity and mortality refer to average values across all identified cases.
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Table 9. Horses—Most frequently identified serotypes in FY83.
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<td>844 (1)</td>
<td>790 (1)</td>
<td>533 (1)</td>
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<td>(KUNZENDORF)</td>
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<td>611 (2)</td>
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<td>117 (10)</td>
<td>61 (13)</td>
<td>39 (17)</td>
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*Number of times the serotype was identified

**Rank beginning with the most common
REPORT OF THE COMMITTEE OF SALMONELLA

Chairman: B. S. Pomeroy, Minnesota
Vice Chairman: G. H. Snoeyenbos, Massachusetts

C. W. Beard, GA; F. D. Bisplinghoff, IN; B. O. Blackburn, IA; T. E. Carpenter, CA; M. S. Cover, MO; M. L. Crandall, MD; W. H. Dubbert, VA; R. D. Glock, AZ; D. A. Halvorson, MN; R. L. Hogue, IN; W. L. Kadel, KY; D. D. King, MD; T. T. Kramer, IA; E. T. Mallinson, MD; C. S. McCain, OK; E. L. Menning, VA; G. W. Meyerholz, D.C.; E. V. Morse, IN; H. J. Olander, CA; I. L. Peterson, MD; M. E. Potter, GA; R. A. Robinson, MN; R. D. Schar, MD; L. D. Schwartz, MI; K. Van Steenbergh, MO; W. T. Tramel, MS; S. A. Vezey, GA; C. R. Weston, NH.

Ex Officio: A. H. Bentley, Canada; W. B. Bixler, MD; W. E. Patterson, GA.

The Committee met at 1:30 p.m., Thursday, October 25, 1984. Sixteen members and 25 guests attended.

Activities of the Committee since 1983 meeting were primarily confined to support of the International Symposium on Salmonella. Dr. G. H. Snoeyenbos, General Chairman of the Planning Committee presented a brief report on the Symposium. A complete report was given as part of the USAHA program and will be included in the proceedings.

Four general reports were presented to the Committee.

1. Dr. C. D. Murphy reported for Dr. B. O. Blackburn, in respect to the changes in the system reporting results from the Salmonella Typing Laboratory. He noted some recent shifts in Salmonella serotypes and distribution and indicated that they were developing capability in plasmid analysis. They expect this service to be available early in January, 1985. Both Drs. Murphy and M. E. Potter (CDC) indicated they would be able to provide training in plasmid analysis. Dr. Murphy's report will be included in the proceedings.

2. In the absence of A. H. Bentley, David Brown reviewed the current progress of the Salmonella Coordinating Unit of Agriculture Canada toward developing a total microbiological control system in the poultry industries as an aid in prevention and reduction of Salmonella and other diseases. He reported that they had developed a series of audio visual aids for an extensive educational efforts. These can be purchased for use in other countries. They have also developed a working paper on "Economic Study of Salmonella Poisoning and Control Measures in Canada." A copy of the Economic Analysis and Visual Aids are available by contacting Andre Trempe, Service Division, Marketing and Economic Branch, Sir John Carling Bldg., Ottawa, Ontario KIA OC5 Canada.

3. Dr. M. S. Cover reviewed the work conducted at Ralston Purina Co., St. Louis, Missouri on the parameters in milling and processing to reduce Salmonella in feeds. They concluded that the conditioner chamber prior to pelleting should be a minimum of 180°F with 200°F
being preferable and a moisture level to 18% for satisfactory results. Under such processing conditions the numbers of Salmonella are greatly reduced, but a guarantee of freedom of Salmonella would not be possible.

4. Dr. F. D. Bisplinghoff, Chairman of the National Renderers Association Salmonella Committee reported extensive activity to organize an effort to reduce the incidence of Salmonella in animal proteins. An animal protein industry meeting was held on September 12, 1984 which was broadly attended by industry representatives from packer-renderers, blenders, National Renderers Association members and independent renderers. A Salmonella Committee composed of members of each group were selected to develop a Salmonella reduction program. The new program will be presented to the Center for Veterinary Medicine–FDA and FSIS–USDA on November 8, 1984. Dr. W. H. Dubbert, FSIS–USDA, indicated interest in working with the group to establish bench mark data. The Committee as a whole was greatly pleased to learn of this developing program.

Subcommittee Reports

1. Regulatory Programs–Production

Dr. I. L. Peterson reported that APHIS–VS has not received any report of isolation of *S. gallinarum* since 1980. The United States is likely to be free of the infection. He also reported that 50 isolations of *S. pullorum* had been made including one outbreak in a commercial broiler operation. The National Poultry Improvement Plan, essentially a voluntary program, is the only regulatory program for the control of Salmonella in livestock and poultry.

2. Regulatory Programs–Processing

Dr. W. H. Dubbert, FSIS–USDA, reported that further work had confirmed the effectiveness of chlorination of wash water up to the level of 200 ppm to reduce the contamination level of carcasses. Work has been initiated to examine other antibacterials as substitutes for chlorine. Officials of FSIS continue to give top priority to Salmonella research and to update their information on the possible use of irradiation for destroying microorganisms in animal products.

3. Industry

Dr. R. D. Gloch, Chairman of the Livestock Industry Subcommittee reported on a meeting of his subcommittee with representatives of the National Pork Producers Council, National Cattlemen’s Association, veterinary medical diagnosticians and epidemiologists. Dr. Gloch indicated that in his opinion, which was supported by epidemiologic information and frequency of identification in diagnostic laboratories, Salmonella was common and was one of the major clinical and economic problems in cattle and swine. He emphasized the need for expansion of research to better understand the variety of expressions of Salmonella in cattle and swine and the need for epidemiologists to be associated with Salmonella Typing.
Laboratory at NVSL. Dr. M. Vorhies, Director of the Veterinary Diagnostic Laboratory at South Dakota State University recommended that processing of diagnostic cases should routinely include screening for Salmonella.

4. Research

Dr. R. A. Robinson, Chairman of the Subcommittee on Research on cattle and small ruminants, reported he was able to make limited epidemiologic investigations of *S. dublin* infections in cattle, but on the whole very little research was being done except for limited studies on the use of vaccines in calves in California.

Drs. B. S. Pomeroy and K. V. Nagaraja reported on their field studies to reduce and eliminate *S. hadar* in turkey breeding flocks associated with two hatcheries. One hatchery had infected breeder flocks on ten farms. Over a two year period utilizing a cleaning and disinfecting and monitoring programs and the use of an experimental oil emulsion killed vaccine, *S. hadar* has been eliminated from the system. In the second hatchery, eight farms were contaminated and over a two year period, using only a cleaning and disinfecting and monitoring programs, the infection was eliminated in breeder flocks on five farms. The program will be continued through FY '85 to make sure the *S. hadar* contamination has been eliminated.

5. Extension-Education

In the absence of Chairman E. T. Mallinson, Dr. S. A. Vezey reported on two meetings of the subcommittee to evaluate visual aids developed by Agriculture Canada and the University of Georgia for educating members of the poultry industry on biosecurity. He exhibited a number of exceptionally fine visual aids developed for this purpose and offered to assist others in developing additional visual aids. The Committee is gathering data for cost benefit analysis of greatly improved biosecurity.

6. Response by USDA to Resolutions Passed at the 1983 USAHA Meeting.

1. Resolution concerned with the development of a National Animal Salmonella Reference Center at NADL resulted only in the current development of ability to perform plasmid analysis.

2. Resolution concerned with dealing with future infection of *S. gallinarum* as an exotic disease has resulted in plans to strengthen the capability of each state to respond through the National Poultry Improvement Plan. The committee expressed its opinion that this section would be inadequate to meet the threat of infection should it occur in a large commercial flock.

7. Committee Action on Two Proposed Resolutions

One resolution deals with the development of capabilities to pursue epidemiologic investigations of Salmonella outbreaks and the addition of an epidemiologist to the Salmonella Typing Laboratory.

The second resolution deals with the five statements developed at the 1984 International Symposium on Salmonella in New Orleans. These resolutions were referred to the Resolutions Committee.
Chairman Pomeroy noted that Dr. E. V. Morse, long time Chairman of the Committee on Salmonella, asked that he be relieved from membership on the Committee. The Committee wishes to commend Dr. Morse for his long term service to the Committee and USAHA and for his major research contributions on Salmonellosis of animals.

**Subcommittee Assignments for 1984**

**A. Diagnostics, Data Collection and Epidemiology**

B. O. Blackburn, Chairman; Carpenter, Glock, Kadel, McCain, Morse, Potter, Robinson and Van Steenberg.

**B. Regulatory Programs**

1. Production, I. L. Peterson, Chairman; Beard, Crandall, Glock, Hogue, King, Mallinson, Menning, Morse, Pomeroy, Robinson, Schar, Snoeyenbos, Tramel and Weston

2. Processing—W. H. Dubbert, Chairman; Crandall, Menning, King and Patterson

**C. Industry**

1. Feed and Feed Ingredients
   M. S. Cover, Chairman; Bisplinghoff, Crandall, Dubbert, Patterson, Peterson, Pomeroy and Robinson.

2. Poultry Breeders
   C. R. Weston, Chairman; Halvorson, Hogue, Peterson, Pomeroy, Schar, Snoeyenbos, Tramel and Vezey.

3. Livestock Industry
   R. D. Glock, Chairman; Kramer, Morse, Olander, Robinson and Van Steenbergh.

**D. Research**

1. Poultry—G. H. Snoeyenbos, Chairman
   Beard, King, Pomeroy and Weston.

2. Swine—T. T. Kramer, Chairman
   Glock, King, Morse, Olander, Robinson

**E. Extension-Education—E. T. Mallinson, Chairman**
   Bentley, Carpenter, Halvorson, Meyerholz, Schwartz, Tramel and Vezey.

**F. Ex Officio—A. H. Bentley (Canada) W. B. Bixler and W. C. Patterson.**
BLUETONGUE VIRUS, SEROTYPE 2: VECTOR TRANSMISSION AND PATHOGENICITY FOR SHEEP

T. L. Barber, DVM, PhD and R. H. Jones, PhD
Arthropod-borne Animal Diseases Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture
P.O. Box 25327, Denver, Colorado 80225

Summary

Bluetongue virus (BTV), serotype 2, was isolated from infected cattle in the United States in 1982, 1983 and 1984. These studies were designed to determine a) the pathogenicity for sheep of the 2 strains of serotype 2 that have been isolated and b) the transmissibility of the 2 strains of virus by Culicoides variipennis, the principal vector of BTV in the United States. Our studies showed that, under laboratory conditions, serotype 2 had moderate pathogenicity in inoculated sheep; the pathogenicity was comparable to that seen with other domestic serotypes under similar conditions. Biological transmission of serotype 2 to sheep by C. variipennis that were infected in vitro was shown; sheep-to-sheep transmission by infected flies was also demonstrated but was not as successful as has been found with other domestic serotypes. The changeable nature of BTV suggests that serotype 2 is likely to become better adapted to C. variipennis and livestock in the U.S. in the future.

Introduction

The first isolation of bluetongue (BT) virus (BTV) serotype 2 in the Western Hemisphere was from samples collected from infected cattle in Ona, Florida in the fall of 1982. Serotype 2 was subsequently isolated from infected cattle at Belle Glade and near Dunnellon, Florida in 1983. The most recent isolation of serotype 2 was from infected cattle at Brooksville, Florida in 1984 (Gibbs EPJ, Greiner EC and Barber TL: unpublished data). Blood from the infected cattle at Belle Glade and Brooksville also yielded BTV serotype 13. Two strains of serotype 2, Ona A and Ona B, were recovered in 1982. The strain differences were seen when the viruses were electrophoretically typed. The strains found in 1983 were identified as the Ona B strain of serotype 2.

The continuing occurrence of this relatively recently introduced serotype in our livestock population has raised some important questions. There are very few sheep in Florida and infections of sheep have not been reported (Gibbs EPJ and Greiner EC: personal communication, 1984). However, serotype 2 in the Republic of South Africa has been a frequent cause of disease in sheep for 25 years (Erasmus BJ: personal communication, 1983). This raises the question as to the pathogenicity of the strain of serotype 2 in the U.S. for sheep. Also, the vector implicated for serotype 2 near Ona, Florida was Culicoides insignis Lutz whereas the principal vector for BTV in most of the U.S. is believed to be C. variipennis (Coquillet). This raises the question as to whether or not C. variipennis
will readily transmit the U.S. strain of BTV serotype 2. This report describes our efforts to answer questions about the pathogenicity of serotype 2 for sheep and the transmissability of serotype 2 by the principal vector for BTV in the U.S.

Materials and Methods

Virus — The 2 strains of BTV serotype 2 used in these studies were obtained from blood samples collected from infected cattle in 1982 at Ona, Florida.¹ The strains were designated Ona A and Ona B and were previously described.²,³ The virus was isolated in embryonated chicken eggs (ECE) and adapted to hamster kidney (BHK-21) cells and to sheep. Approximately 300,000 ECE lethal doses (ELD₅₀) of serotype 2 in infected sheep blood was given to each sheep for immunity challenge. All immunity challenges were done with the homologous serotype and with the homologous strain of serotype 2, Ona A or Ona B. Virus stocks were assayed for BTV titer by intravenous inoculation of ECE or by cocultivation in microplates with L-929 (mouse fibroblast) cells.²

Sheep — The sheep were 12 to 18 month-old Warhill wethers and were from a BT-susceptible flock reared near Laramie, Wyoming.

Insects — A laboratory colony of C. variipennis (Sonora strain) was used for all transmission studies and all procedures were routine.⁵,⁶ After insects were exposed to infection, they were held for 16 to 18 days of extrinsic incubation before their use in a transmission trial, or 14 days before a portion of a group was used to determine an infection rate (%: number positive/number tested). Pools of possibly infected insects were assayed by the intravascular inoculation of 11-day ECE,² while single flies for infection rates were assayed in BHK-21 cells in 96-well microplates.⁷

Serology — Serums from experimental sheep were tested for precipitating (P) antibodies by the BT immunodiffusion (BTID) test and for neutralizing (N) antibodies by a plaque reduction neutralization test.⁸,⁹ Serums were undiluted when tested for P antibodies; serums were diluted 2-fold from 1:10 when tested for N antibodies. Titers of N antibodies in serums were expressed as the final dilution of serum in the serum-virus mixture; the endpoint was that dilution at which 80% of the BTV plaques were neutralized.

Experimental Design and Results

Inoculation of sheep with BTV, serotype 2 — Results of the primary infection of sheep with BTV, serotype 2, Ona A strain are shown in Table 1. Sheep No. 1 and 2 were given pools of washed blood cells from the infected cattle at Ona, Florida. The pools were made from blood samples that were subsequently found to yield BTV when assayed in ECE. BTV was isolated from sheep No. 1 nine days after inoculation (DAI). Although BTV was not isolated from sheep No. 2, the sheep had barely detectable P antibodies on 14 DAI which were not detectable in serum samples collected weekly thereafter; the sheep had detectable N antibodies on DAI 35 and 42 at serum dilution endpoints of 1:10 and 1:20, respectively. Both
sheep were given immunity challenge with 5 x 10⁹ cell culture infectious doses (CCID₅₀) of serotype 2, Ona A strain that was adapted to BHK-21 cells because sheep blood source virus was not available. Sheep No. 1 was resistant to challenge having no detectable viremia, fever or leukopenia. Sheep No. 2 was partially resistant to challenge having viremia detectable only on 11 days after challenge (DAC), fever on 6 and 7 DAC and leukopenia on 7 DAC. The same immunity challenge inoculum elicited 10 to 12 days viremia, 2 to 5 days of fever and 1 to 6 days of leukopenia in fully susceptible sheep (Table 1, sheep No. 3 and 4). Sheep No. 3 through 7 were initially infected with BTV, serotype 2, Ona A strain that was adapted to BHK-21 cells. These sheep all became infected and had clinical signs of illness of variable duration and intensity; all developed P and N antibodies to BTV and were resistant to immunity challenge.

The second serial passage of BTV, serotype 2 in sheep (Table 1) was initiated with infected blood collected from sheep No. 3 on 7 DAI; the blood contained 10⁵.₈ ELD₅₀ of BTV per ml. Sheep No. 8 and 9 had clinical signs that were moderate and were similar to what has been reported from sheep experimentally infected with serotypes of BTV previously found in the U.S.¹⁰ The sheep were resistant to immunity challenge.

The third serial passage of BTV, serotype 2 in sheep (Table 1) was initiated with infected blood collected from sheep No. 8 on 7 DAI; the blood contained 10⁵.₇ ELD₅₀ of BTV per ml. Sheep No. 10 and 11 had moderate clinical responses that were similar to those seen in sheep No. 8 and 9. The sheep were resistant to immunity challenge.

**Infection of sheep with BTV, serotype 2 by vector bites —** *C. variipennis* were fed on donor sheep No. 5 (Tables 1 and 2) on 7 DAI. At the time the flies were fed, the donor sheep had swollen lips, congested and hyperemic oral mucous membranes and a slightly elevated rectal temperature (103.6 F). Blood samples collected from the donor sheep on DAI 5 through 11 yielded BTV, serotype 2. After extrinsic incubation, the flies were fed on a recipient sheep, No. 12; 378 flies took a blood meal. Sheep No. 12 had no clinical response, did not develop antibodies to BTV and was susceptible to immunity challenge that was given 33 days after insect bites (DAIB). The pool of flies that had fed on sheep No. 5 and subsequently on sheep No. 12 were triturated and were assayed in ECE and by inoculation into sheep No. 13. Sheep No. 13 had no clinical response, did not develop antibodies to BTV and was susceptible to immunity challenge that was given 42 DAI. Assay of the pool of flies in ECE was likewise negative (Table 2). Apparently, the viremia in the donor sheep, No. 5, was inadequate to infect the flies that were fed to begin this transmission experiment.

In the next transmission experiment, uninfected *C. variipennis* were fed on donor sheep No. 8 (Tables 1 and 2) on 9 DAI. At the time the flies were fed the donor sheep had a swollen muzzle and lips, congested and hyperemic oral and nasal mucous membranes and a fever (106.2 F). Blood
samples collected from the donor sheep on DAI 5 through 14 yielded BTV, serotype 2; on DAI 9, blood collected from the donor sheep had a titer of $10^{3.7}$ ELD$_{50}$ of BTV per ml. After extrinsic incubation, the flies initially fed on donor sheep No. 8 were fed on a recipient sheep, No. 14; 71 flies took a blood meal. Sheep No. 14 had a mild clinical response (Table 3) but viremia was detectable from 5 through 14 DAIB. The only febrile responses were 12 and 13 DAIB, 105.4 F and 104.8 F, respectively; sheep No. 14 was resistant to immunity challenge that was given 30 DAIB. Because of the late and mild response to the primary infection, flies were not fed on sheep No. 14 but instead, heparinized blood collected on 12 and 13 DAIB was fed to flies through a membrane. The blood contained $10^{4.0}$ and ELD$_{50}$ of BTV per ml. The flies were fed a mixture of 50% freshly collected uninfected sheep blood and 50% infected blood. After extrinsic incubation, the flies were fed on sheep No. 15; 161 flies took a blood meal. Sheep No. 15 had no clinical response, did not develop antibodies and was susceptible to immunity challenge that was given on 42 DAIB. This experiment demonstrated the biological transmission of BTV, serotype 2, Ona A strain by C. variipennis from sheep No. 8 to sheep No. 14.

The next 2 transmission experiments were conducted with BTV, serotype 2, Ona B strain because that strain of virus had been recovered from infected cattle at locations about 100 miles north and south of Ona, Florida. The sheep used in these experiments were housed outdoors in a pen that was double-screened with fine mesh screening. This was an attempt to determine pathogenicity under conditions that were more natural than isolation rooms. For these studies a plaque purified strain of Ona B from infected BHK-21 cells was mixed with freshly collected uninfected sheep blood and was fed to uninfected C. variipennis through a membrane (Table 2). After extrinsic incubation, the infection rate in the infected flies was found to be 2.0%. The remaining flies were fed on sheep No. 16 or No. 17; the sheep received 169 and 246 bites, respectively. The pools of flies that fed on sheep No. 16 and 17 were positive for BTV, serotype 2. The 2 sheep had febrile responses on DAIB 6 through 11 and uninfected flies were fed on both sheep on DAIB 7. The 2 sheep also had viremia, leukopenia, mild mouth lesions and were resistant to immunity challenge that was given DAIB 42 (Table 3). Clinical signs of illness did not appear to be any more severe in sheep in the screened pen than in sheep in indoor isolation rooms.

The flies that first were fed on sheep No. 16 and 17 were fed, after extrinsic incubation, on sheep No. 18 and 19; the sheep received 231 and 169 bites, respectively. The pools of flies that fed on sheep No. 18 and 19 were positive for BTV, but assays of individual flies were negative (Table 2). Blood collected from sheep No. 18 on DAIB 7 yielded BTV serotype 2 but the sheep had a fever (106.8 F) only on DAIB 10. Blood collected from sheep No. 19 yielded BTV on DAIB 9 through 17 and had fever on DAIB 9 and 10 and 12 through 17. Uninfected flies were fed on sheep No. 18 on DAIB 9. Sheep No. 18 had detectable N antibodies to BTV on DAIB 14 and P and N antibodies on DAIB 21. All other weekly bleedings were negative for
antibodies to BTV until after immunity challenge. Sheep No. 18 was susceptible to immunity challenge that was given 42 DAIB. Sheep No. 19 had P and N antibodies from DAIB 14 through the immunity challenge period. Sheep No. 19 was partially resistant to immunity challenge but had a low level but detectable viremia on DAC 7, 9 and 11 (Table 3).

The flies that first were fed on sheep No. 19 were fed, after extrinsic incubation, on sheep No. 20 and 21; the 2 sheep received 107 and 109 bites, respectively. The pools of flies that fed on sheep No. 20 and 21 were negative for BTV (Table 3). The 2 sheep had no clinical response, did not develop detectable P or N antibodies to BTV and were susceptible to immunity challenge (Table 4).

The next experiment was an attempt to repeat the preceding experiment. Uninfected C. variipennis were fed through a membrane on sheep blood that was combined with cell culture source BTV. The infection rate of these flies was 2.0% (Table 2). The flies were fed on sheep No. 22 and 23; the sheep received 100 and 104 bites, respectively. The pools of flies that fed on sheep No. 22 and 23 were positive for BTV. Sheep No. 22 had a fever (105.0 F) on DAIB 8 and uninfected flies were fed on the sheep that day. Although viremia was not detected, sheep No. 22 developed P and N antibodies to BTV beginning 14 DAIB that persisted for the duration of the experiment (Table 4). Sheep No. 23 had a fever (104.2 F) on DAIB 9 and uninfected flies were fed on the sheep that day. Although viremia was not detected, sheep No. 23 developed P and N antibodies beginning 21 DAIB that persisted for the duration of the experiment (Table 4). Both sheep were resistant to immunity challenge that was given 42 DAIB.

The flies that first were fed on sheep No. 22 and 23 were fed, after extrinsic incubation, on sheep No. 24 and 25; the sheep received 44 and 79 bites, respectively. The pools of flies that fed on sheep No. 24 and 25 were negative for BTV as were individual flies that were assayed for infection rate determinations (Table 2). Sheep No. 24 had no clinical response, did not develop detectable P or N antibodies to BTV and was susceptible to immunity challenge. Sheep No. 25 had a fever on DAIB 10 (104.4 F) but viremia was not detected. The sheep did not develop detectable P or N antibodies to BTV and was susceptible to immunity challenge.

Discussion

Both the Ona A and Ona B strains of BTV, serotype 2, were shown to be biologically transmitted, sheep-to-sheep, by C. variipennis. However the transmissability of serotype 2 could not be shown beyond a single sheep-to-sheep passage. It appears that the current strains of serotype 2 are not as transmissible as serotype 17 was previously shown to be by flies from the same colony of C. variipennis. That report described 15 serial sheep-to-sheep transmissions by infected flies over a period of 13 months. The same scientists had previously shown sheep-to-cattle and cattle-to-sheep biological transmission of BTV serotypes 10 and 17 with colonized C. variipennis.
The infection rates of 2.0% that were obtained with serotype 2 (Table 2) were lower than those reported in a series of experiments when serotype 17 was used. In those experiments, flies were fed BHK-21 cell source BTV serotype 17, strain 62-45S, in a blood meal. The infection rates obtained were from 17% to 22% depending on the assay procedure utilized. The low infection rate with C. variipennis and serotype 2 are similar to low infection rates obtained with C. venustus Hoffman and BTV serotype 11. In the study with C. venustus, an infection rate of 0.7% was obtained. This showed the considerable variability among Culicoides species and BTV with regard to biological transmission. Another study showed variability in susceptibility among several C. variipennis field populations. The study also showed that the strain and serotype of BTV made a difference in the infection rate.

Although BTV serotype 2 was not readily transmitted by the principal vector for BTV in the U.S., the changeable nature of BTV suggests that serotype 2 will adapt to C. variipennis and thereafter may become more widespread in the U.S. ruminant livestock population. The pathogenicity of serotype 2 for sheep is also likely to be more severe both under field conditions and as the virus becomes better adapted to vectors that are available in the U.S.

Acknowledgements

The authors thank Mr. Lee Thompson and Mr. Marlin Larson for technical assistance and Mrs. Helen Britton for word processing and editing.

REFERENCES


## TABLE 1 -- Results of primary infection of sheep with bluetongue virus, serotype 2, Oma A strain; sheep were infected by inoculation with bovine, cell culture or sheep origin virus

<table>
<thead>
<tr>
<th>Passage</th>
<th>Virus level</th>
<th>Viremia duration (days)</th>
<th>Fever duration (days)</th>
<th>Leukopenia duration (days)</th>
<th>Lesions duration (days)</th>
<th>Clinical response</th>
<th>Overall rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>bovine C</td>
<td>unknown</td>
<td>1</td>
<td>yes (1)</td>
<td>no</td>
<td>no</td>
<td>subclinical</td>
</tr>
<tr>
<td>2</td>
<td>bovine C</td>
<td>unknown</td>
<td>1</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>subclinical</td>
</tr>
<tr>
<td>3</td>
<td>cell culture</td>
<td>5 x 10^9</td>
<td>1</td>
<td>yes (10)</td>
<td>yes (5)</td>
<td>yes (6)</td>
<td>mild</td>
</tr>
<tr>
<td>4</td>
<td>cell culture</td>
<td>5 x 10^9</td>
<td>1</td>
<td>yes (12)</td>
<td>yes (2)</td>
<td>yes (1)</td>
<td>mild</td>
</tr>
<tr>
<td>5</td>
<td>cell culture</td>
<td>5 x 10^9</td>
<td>1</td>
<td>yes (7)</td>
<td>no</td>
<td>no</td>
<td>mild</td>
</tr>
<tr>
<td>6</td>
<td>cell culture</td>
<td>1 x 10^5</td>
<td>1</td>
<td>yes (10)</td>
<td>yes (8)</td>
<td>yes (3)</td>
<td>mild</td>
</tr>
<tr>
<td>7</td>
<td>cell culture</td>
<td>1 x 10^5</td>
<td>1</td>
<td>yes (10)</td>
<td>yes (5)</td>
<td>yes (3)</td>
<td>mild</td>
</tr>
<tr>
<td>8</td>
<td>sheep No. 3</td>
<td>3 x 10^5</td>
<td>2</td>
<td>yes (10)</td>
<td>yes (6)</td>
<td>yes (3)</td>
<td>moderate</td>
</tr>
<tr>
<td>9</td>
<td>sheep No. 3</td>
<td>3 x 10^5</td>
<td>2</td>
<td>yes (8)</td>
<td>yes (5)</td>
<td>yes (4)</td>
<td>moderate</td>
</tr>
<tr>
<td>10</td>
<td>sheep No. 8</td>
<td>3 x 10^5</td>
<td>3</td>
<td>yes (10)</td>
<td>yes (7)</td>
<td>yes (5)</td>
<td>moderate</td>
</tr>
<tr>
<td>11</td>
<td>sheep No. 8</td>
<td>3 x 10^5</td>
<td>3</td>
<td>yes (7)</td>
<td>yes (2)</td>
<td>yes (10)</td>
<td>moderate</td>
</tr>
</tbody>
</table>

---

*a Dose is in cell culture infections doses (50%) for sheep No. 3 through 7; in embryonated chicken egg (ELD50) lethal doses for sheep No. 8-11.

*b Sheep were housed in insect-free isolation rooms where they were fed and watered daily; clinical responses were possibly diminished under such conditions of care; infections were rated as subclinical, mild, moderate or severe.

*c Pool of washed blood cells from calves in Oma, Florida.
### TABLE 2 -- Summary of transmission experiments with *Culicoides variipennis* and bluetongue virus serotype 2

<table>
<thead>
<tr>
<th>Donor sheep No. (DAI or DAIR)</th>
<th>Recipient sheep</th>
<th>Assay of pool of flies after feeding on recipient sheep</th>
<th>Infection rate (IR)&lt;sup&gt;c&lt;/sup&gt; for flies fed on recipient sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sheep No.</td>
<td>No. of bites</td>
<td></td>
</tr>
<tr>
<td>5 (7 DAI)</td>
<td>12</td>
<td>378</td>
<td>Neg</td>
</tr>
<tr>
<td>8 (9 DAI)</td>
<td>14</td>
<td>71</td>
<td>+</td>
</tr>
<tr>
<td>14 (12 and 13 DAIB)</td>
<td>15</td>
<td>161</td>
<td>Neg</td>
</tr>
<tr>
<td>NA&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16&lt;sup&gt;f&lt;/sup&gt;</td>
<td>169</td>
<td>+</td>
</tr>
<tr>
<td>NA</td>
<td>17&lt;sup&gt;f&lt;/sup&gt;</td>
<td>246</td>
<td>+</td>
</tr>
<tr>
<td>16 (7 DAIB)</td>
<td>18</td>
<td>231</td>
<td>+</td>
</tr>
<tr>
<td>17 (7 DAIB)</td>
<td>19</td>
<td>169</td>
<td>+</td>
</tr>
<tr>
<td>18 (9 DAIB)</td>
<td>20</td>
<td>107</td>
<td>Neg</td>
</tr>
<tr>
<td>18 (9 DAIB)</td>
<td>21</td>
<td>109</td>
<td>Neg</td>
</tr>
<tr>
<td>NA</td>
<td>22&lt;sup&gt;f&lt;/sup&gt;</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>NA</td>
<td>23&lt;sup&gt;f&lt;/sup&gt;</td>
<td>104</td>
<td>+</td>
</tr>
<tr>
<td>22 (8 DAIB)</td>
<td>25</td>
<td>44</td>
<td>Neg</td>
</tr>
<tr>
<td>23 (9 DAIB)</td>
<td>24</td>
<td>79</td>
<td>Neg</td>
</tr>
</tbody>
</table>

<sup>a</sup>Flies were infected either by feeding on sheep previously inoculated with BTV, previously infected by insect bite, or by feeding flies through a membrane (see f below); DAI=days after inoculation; DAIB=days after insect bite.

<sup>b</sup>Pools of flies were assayed for BTV after they were allowed to feed on the recipient sheep; the assay was by intravascular inoculation of embryonated chicken eggs.

<sup>c</sup>Infection rate was determined by assay of individual flies that were removed from the population after 14 days extrinsic incubation; assay was in BHK-21 cells.

<sup>d</sup>NT—not tested.

<sup>e</sup>NA—not applicable.

<sup>f</sup>Transmission was by infected insect bites from flies that had been fed through a membrane on an artificially infected blood meal that consisted of 9 parts defibrinated normal sheep blood and 1 part cell culture source BTV.
TABLE 3 -- Responses of sheep during passage of BTV, serotype 2, Ona A strain, sheep-to-sheep by bites of infected *C. variipennis*

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Fever</th>
<th>Viremia</th>
<th>Leukopenia</th>
<th>Seroconversion</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>Neg</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>15</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

a Colonized *C. variipennis* were fed on a viremic sheep No. 8, (Tables 1 and 2) 9 days after inoculation of the sheep with 300,000 ELD<sub>50</sub> of serotype 2; *C. variipennis* were held for extrinsic incubation and fed on sheep No. 14; heparinized blood collected from sheep No. 14 on DAIT 12 and 13 was fed to *C. variipennis* and these, after extrinsic incubation, were fed on sheep No. 15.
TABLE 4 -- Responses of sheep during serial passage of BTV, serotype 2, Ona B strain by C. variipennis

<table>
<thead>
<tr>
<th>Sheep Passage</th>
<th>No.</th>
<th>No.</th>
<th>Fever</th>
<th>Viremia</th>
<th>Leukopenia</th>
<th>Seroconversion signs</th>
<th>Clinical Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>16,17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>2/2+</td>
<td>2/2+</td>
<td>2/2+</td>
<td>2/2+</td>
<td>Mild</td>
<td>2/2R&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>18,19</td>
<td>2</td>
<td>2/2+</td>
<td>2/2+</td>
<td>Neg</td>
<td>1+; 1±</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>20,21</td>
<td>3</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>2/2S</td>
</tr>
<tr>
<td>Replicate Experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22,23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>2/2+</td>
<td>Neg</td>
<td>Neg</td>
<td>2/2+</td>
<td>Neg</td>
<td>2/2R</td>
</tr>
<tr>
<td>24,25</td>
<td>2</td>
<td>1/2+</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>2/2S</td>
</tr>
</tbody>
</table>

<sup>a</sup>Colonized C. variipennis were fed through a membrane on an artificially infected blood meal, held for extrinsic incubation and were then fed on these 2 sheep.

<sup>b</sup>Both sheep were positive for the factor indicated.

<sup>c</sup>Resistant.

<sup>d</sup>Susceptible.

<sup>e</sup>Partially resistant.
REPRODUCTION OF PERIDUCTILE LYMPHOID INFILTRATION WITH A RETROVIRUS INSOLATED FROM FIRM UDDER DISEASE OF SHEEP

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SUMMARY

Fourteen seronegative ewes were inoculated by intramammary injection with 2 retrovirus isolates and 6 seronegative ewes were mock inoculated with cell culture control medium. Eight months after inoculation 6 virus-inoculated and 2 mock-inoculated ewes were necropsied. Five of the virus-inoculated ewes developed similar mammary lesions as natural cases of firm udder disease. Five became seropositive to caprine arthritis-encephalitis virus (CAEV) and retroviruses were reisolated from 6 of them. The 2 mock-inoculated ewes did not seroconvert nor did they develop the mammary lesions observed in the naturally affected ewes and no virus could be isolated from them. However, the clinical entity of firm udder disease was not reproduced in lambing. Six virus-inoculated ewes, five of their daughters, 4 mock-inoculated ewes and their two daughters will be observed at their next lambing approximately 15 months after experimental inoculation of the ewes.

INTRODUCTION

A clinical condition of ewes in Idaho referred to as “udder induration” is characterized by postparturient hardness of the mammary gland and scant or nonexistent milk production. The condition occurs at a rate of 5 to 10% in some range flocks in Idaho and Oregon and producers cull these ewes because they cannot raise their lambs. The milk collected from the glands is usually bacteriologically sterile and there is no increase in inflammatory cells or other alterations in milk quality. No mycoplasmas or chlamydia have been isolated from the milk.

Recently, this condition has been termed firm udder disease and the microscopic lesions characterized. The most common finding is periductile mononuclear cell infiltrations with lymphoid follicles and germinal centers. Similar lesions have been associated with infection by ovine progressive pneumonia (OPPV).
Six retrovirus isolated antigenically related to caprine arthritis-encephalitis virus CAEV and OPPV have been obtained from the lesions of ewes affected with firm udder disease. This paper described reproduction of the lesions of natural firm udder disease with two of these isolates.

MATERIALS AND METHODS

As previously described, eleven ewes with typical firm udder disease were obtained from four sheep flocks in Idaho and Oregon. Tissue was examined for the presence of virus, bacteria, mycoplasmas and chlamydia. Retroviruses were isolated from the udders of six ewes. Two isolates (hb4 and hb29) were selected for inoculation for the present studies because the ewes from which they came were seropositive to OPPV and CAEV (gp135) by immunodiffusion and had typical lesions of firm udder disease.

The supernatants from cultures containing isolates Hb4 and Hb29 were clarified and transferred to caprine synovial membrane cells in Dulbecco's minimum essential medium containing 5% fetal calf serum. Supernatants were collected 2 times at 7 day intervals after appearance of cytopathic effect. Each pool was filtered (0.22 micron) and frozen in small aliquots at 70°C.

Twenty ewes, 6 Suffolk and 14 Dorset of various ages were aggregated according to age and breed and divided into three groups. Six received an inoculation of medium from uninfected cultures, 7 received isolate Hb4 and 7 received isolate Hb29. One ml of the medium was injected caudal to the teat 2.5 cm into the parenchyma of the left half of each mammary gland. The ewes were all bred to lamb 2 to 3 months from the time of injection. All sheep were seronegative to OPPV and CAEV by immunodiffusion prior to injection.

At parturition all ewes were examined for evidence of firm udder disease. Seven days after parturition the lamb(s) were withheld from the ewes. After 9 hours the ewes were milked dry and the volume in each udder half was measured. The lamb(s) were then returned to the ewes.

Eight months after inoculation, 6 virus-inoculated and 2 mock-inoculated ewes were killed and necropsied. Serum was collected and examined for antibody against the gp 135 of CAEV in immunodiffusion. A portion of each half of a mammary gland was explanted as previously described for virus isolation. For histopathology three blocks from the upper parenchyma and two blocks above the teat cistern from each half of the udder were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Explants of mammary tissue were observed for up to seven passages. When cytopathic effect was seen or when the cultures were terminated, they were trypsinized, cytopspun (Cytospin Shandon-Southern) onto glass slides, fixed in acetone, and examined for viral antigens by immunofluorescence. The immunofluorescent reagent primarily identifies the p28 of CAEV and OPPV.
RESULTS

At lambing no ewe exhibited clinical signs of hard udder disease. Furthermore, there was little, if any, difference in milk production 7 days postpartum between virus and control inoculated ewes.

There were, however, differences in the serology, virology and histopathology between the control and virus inoculated sheep when they were necropsied 8 months after infection (Table 1). No virus could be isolated from the mammary glands nor could antibody against CAEV (gp 135) be demonstrated in the serum of the mock-inoculated sheep. One of the control udders had a few interstitial accumulations of lymphocytes in the alveolar regions but the other one did not. Virus was isolated from all of the udder halves of the virus inoculated sheep. Five of the six virus inoculated sheep had periductile accumulations of lymphocytes and macrophages as well as lymphoid follicles and germinal centers. One of the six only had mild interstitial accumulations of mononuclear cells in those alveolar regions. Five of the six seroconverted in immunodiffusion to the gp135 of CAEV. A seventh virus inoculated ewe was necropsied because she died of an unrelated cause at 40 days postinoculation. Virus was reisolated from both udder halves and similar but less severe periductile lesions were observed. Virus isolation was confirmed by the formation of typical multinucleated cells and the finding of viral specific antigens in the cells by immunofluorescence.

DISCUSSION

The microscopic lesions associated with firm udder disease of sheep are periductile accumulations of mononuclear cells and lymphoid follicles and germinal center formation. This lesion was reproduced in 5 of 6 sheep inoculated with 2 different retroviruses which were isolated from natural cases of firm udder disease.

The lesions produced by these isolates have previously been reported in sheep infected with retroviruses. However, no clinical syndrome of firm udders and agalactia has been associated with the lesions. If our isolates produce clinical firm udder disease in ewes after long term infections, we can assume them to be primary etiologic agents in firm udder disease. If the clinical disease cannot be reproduced, the lesions which appear to be produced by this and perhaps other retroviruses such as OPPV and CAEV are incidental findings at best. Experiments are in progress which we hope will distinguish these two possibilities.
TABLE 1
Results from Ewes Necropsied 8 Months after Inoculation with 2 Retrovirus Isolates

<table>
<thead>
<tr>
<th>Ewe</th>
<th>Inoculum</th>
<th>Virus Recovery</th>
<th>Mammary Lesions</th>
<th>AGID</th>
</tr>
</thead>
<tbody>
<tr>
<td>606</td>
<td>Control</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>398</td>
<td>Control</td>
<td>-</td>
<td>IL</td>
<td>-</td>
</tr>
<tr>
<td>380</td>
<td>hb29</td>
<td>+</td>
<td>IL; Periduct LF, GC</td>
<td>+</td>
</tr>
<tr>
<td>2083</td>
<td>hb4</td>
<td>+</td>
<td>IL; Periduct LF, GC</td>
<td>+</td>
</tr>
<tr>
<td>596</td>
<td>hb29</td>
<td>+</td>
<td>IL</td>
<td>+</td>
</tr>
<tr>
<td>526</td>
<td>hb4</td>
<td>+</td>
<td>IL; Periduct LF, GC</td>
<td>+</td>
</tr>
<tr>
<td>607</td>
<td>hb29</td>
<td>+</td>
<td>IL; Periduct LF, GC</td>
<td>+</td>
</tr>
<tr>
<td>2025</td>
<td>hb29</td>
<td>+</td>
<td>IL; Periduct LF, GC</td>
<td>+</td>
</tr>
</tbody>
</table>

See MATERIALS AND METHODS for description of sheep, inoculae, virology procedures and AGID.

IL = interstitial lymphocytes; Periduct LF, GC = periductile lymphoid follicles and germinal centers.

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REPORT OF THE COMMITTEE ON SHEEP AND GOATS

Chairman: Michele C. Howard, Sacramento, CA
Vice Chairman: J. R. Pitcher, Hyattsville, MD

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The Sheep and Goat Committee met at 1:30 p.m. on Monday, October 22, 1984. There were 17 members and 42 guests, a total of 59 people.

The Committee met as requested by the President of USAHA to consider the business of the committee and submits the following report.

Dr. Lyn Barber of USDA, ARS, Arthropod-borne Animal Diseases Research Laboratory, Denver, CO reported on research involving the transmission and pathogenicity of bluetongue virus, serotype 2. Serotype 2 was isolated in 1982 and 1983 from cattle and Culicoides insignis in Florida. Serotype 2, for 25 years, has been the most frequent serotype recovered from clinically ill sheep in South Africa. The principal vector of bluetongue virus in South Africa is Culicoides imicola; in the United States it is Culicoides variipennis. However, Dr. Barber reported that the serotype 2 strain of bluetongue virus found to date in the United States was not readily transmitted by Culicoides variipennis, yet Dr. Barber feels the threat of serotype 2 to the sheep industry remains, since bluetongue has historically been a changeable virus and is capable of being a severe pathogen of sheep. Dr. Barber reported that regulations for the movement of ruminant livestock into the United States must be strengthened if we are to avoid the accidental introduction of additional serotypes of bluetongue virus.

Dr. Jeffrey Stott, research scientist from the University of California at Davis, brought to the committee an update on bluetongue research in California. Their on-going goal is to develop improved diagnostic techniques.

Monoclonal antibodies have been successfully used to develop several assays for the presence of bluetongue virus proteins. A peroxidase antiperoxidases (PAP) technique has been used to identify bluetongue virus proteins in formalin – fixed parafin – embedded bluetongue virus infected chick embryo tissues. Monoclonal antibodies have also been used in a dot-blot test on nitrocellulose to identify bluetongue virus in infected cell cultures. A western blotting procedure has also proven to be a sensitive assay to identify bluetongue virus in infected cell cultures. Genome seg-
ment 7 of bluetongue virus, serotype 17, has been cloned and successfully used as a diagnostic probe in a dot-blot hybridization system to identify bluetongue virus infected cell cultures.

Studies directed at defining virulence factors for bluetongue virus proteins have also been initiated.

Dr. Jack Pitcher of APHIS discussed the distribution and mode of introduction of bluetongue virus, serotype 2. Since the first bluetongue serotype 2 isolate was confirmed from a sentinel herd of cattle in Ona, Florida in 1982, bluetongue serotype 2 has been isolated from a sentinel herd at Belle Glade in southern Florida, and from a herd of cattle in northern Florida. Recently, sentinel herds at Belle Glade and Brooksville, Florida have seroconverted. No virus isolations have been made at this time from these two herds. Dr. Paul Gibbs, University of Florida, advises that surveillance of these sentinel herds will be discontinued soon because of lack of funding.

Relatively large numbers of Florida feeder cattle are sent to southern California feedlots. These cattle have been monitored by the University of California at Davis and the State of California. Dr. Bennie Osburn reports that antibodies to bluetongue virus, serotype 2, have been detected in these cattle, but that no serotype 2 has been isolated at this time from the cattle in California.

Dr. Pitcher also summarized the scrapie program for the fiscal year 1984. Twenty-five scrapie outbreaks were confirmed in fiscal year 1984: 11 in Ohio, 4 in Michigan, 3 in Wisconsin, 2 in New York, and 1 each in Iowa, Oklahoma, Kentucky, Indiana, and Pennsylvania. This compares with 15 confirmed scrapie outbreaks last fiscal year. Forty animals, 39 Suffolk and 1 Hampshire, were confirmed by laboratory tests as being infected with scrapie.

Approximately 1,055 infected and bloodline animals were depopulated and indemnity funds paid to flock owners. Eight flocks were totally depopulated due to heavy infection and more than one bloodline being involved.

Reporting of scrapie has improved under the bloodline program. Grade flocks, however, pose a problem under the present program, because bloodlines cannot be established or indentified. An agreement has been reached between breed registries (for a fee and with the permission of the owners and APHIS) to provide pedigree information on affected flocks to be used for bloodline tracing.

The study to determine if the scrapie agent is transmitted vertically is progressing under the cooperative agreement between USDA and the International Sheep and Goat Institute, Utah State University. Production of offspring via embryo transfer was completed this fiscal year. The above study has been expanded for fiscal year 1985 to include a project to study the vertical transmission of scrapie in goats.
The role of the laboratory diagnosis in supporting a clinical diagnosis of scrapie in sheep was discussed by Dr. W. D. Taylor from the National Veterinary Services Laboratories. There are currently no lab tests to confirm this diagnosis in the live animal. Dr. Taylor discussed in detail the collection, preparation, and shipment of specimens for the laboratory diagnosis of scrapie, and the problems arising from improperly collected and/or improperly prepared and shipped diagnostic specimens. The routine laboratory histopathology procedures were described and discussed, and Dr. Taylor also discussed the alternative lab diagnosis of mouse inoculation, which can delay confirmation of a positive diagnosis two years or more.

Dr. Scott Adams of Pullman, Washington described a disease of post-parturient ewes, referred to as "hard bag" by producers and veterinarians in Idaho and Oregon. After lambing, ewes with hard bag have large, firm udders which contain normal milk, but very little milk can be milked out through the teat canal. The lesions which are common to most cases include periductile lymphoid cell infiltration and germinal cell formation. A retrovirus related to caprine arthritis-encephalitis virus, and ovine progressive pneumonia virus has been isolated from the lesions and shown to cause the same lesions in experimental sheep. As yet, however, the clinical entity, "hard bag," has not been reproduced. These studies are continuing.

Dr. Al Smith from Oregon State University addressed the subject of footrot vaccines. In an ongoing research in footrot vaccines conducted at Oregon State, two vaccines have been combined, making a broad, polyvalent vaccine containing 8 serotypes. However, Dr. Smith warned that there still could be emergence of disease due to types not included in the vaccine. The University is involved in cooperative work with Iowa, Illinois, Utah, and Colorado. Dr. Smith would like to receive continued support at the current, or at an increased level of funding for at least 3–5 years. The sheep industry recognizes this disease as a high priority.

Also addressing the subject of footrot, Dr. Dave Hird from the University of California at Davis discussed a study done at a ranch in California using the ICI vaccine Footvax. The vaccine is being tested in the areas of prevention, cure, and safety. Results of this study show 19% of non-vaccinates were cured, 53% of vaccinated animals were cured, and 53% of non-vaccinated animals developed disease, while only 9% of the vaccinated animals developed disease. Dr. Hird stressed the fact that vaccine should be used in conjunction with good herd management.

Dr. William A. Knapp, Jr., Executive Secretary of the United Food Animal Association, reported on IR-4-FDA activities. A cooperative arrangement exists between IR4 and FDA, whereby a drug approved by FDA in a major species can be a candidate for approval in a related minor species. Since sheep are not classified as a minor species, they are not benefiting from this program.
The committee was apprised of the current status of last year’s committee resolutions by Dr. Jack Pitcher.

There were 3 resolutions passed by the committee. These resolutions addressed the need for continued research emphasis on Footrot; the continuation and expansion of the Sentinel Herd Program for detection of Bluetongue; and industry’s participation in the decision regarding the possible relocation of the Denver Bluetongue Laboratory.
A major outbreak of Bovine Tuberculosis was disclosed in two bison herds in North Central South Dakota in the spring of 1984.

This outbreak had its beginning in September, 1981 in a herd of elk in South Dakota owned by a person who had purchased elk from Herd Owner A referred to in this report. Tuberculosis was subsequently confirmed in an elk herd owned by Herd A. Herd A also had a buffalo herd apart from his elk operation.

Both of these elk herds were destroyed without compensation in August of 1982 after considerable litigation. This outbreak in elk was reported previously in 1982 and was published in the USAHA proceedings. The exact source of the infection in the elk was never determined. However, Herd Owner A had purchased elk from a number of sources including two zoos located in Iowa and Illinois in 1978. It is believed that these elk were responsible for the introduction of tuberculosis in the elk herds. The significance of this source and possible others will be discussed later in this paper as associated with the tuberculosis outbreak in South Dakota in 1984.

At the time of the elk problem it was recognized that there was danger of transmission to the buffalo herd. The buffalo herd of Herd A was a part of his father’s herd referred to as Herd B in this report. The herds were divided about 1981. The infected elk had been confined on both premises. Elk were on Herd B premises from 1978 to 1981. Elk were on Herd A premises from 1981 to the time of destruction which was in 1982. Herd A also had a herd of about 250 bison. Herd B had a herd of about 300 head of bison. Both herds were kept under ranch conditions. Both herds were restrained by wire fence in agriculture conditions and had some contact with domestic cattle herds.

Tuberculosis in Herd A buffalo first became apparent on December 10, 1982. A caudal fold test performed for sale purposes yielded one suspect in 116 animals tested.

This animal was subsequently classified as a reactor on the comparative cervical test. Gross lesions were disclosed on post mortem and bovine tuberculosis was subsequently confirmed by laboratory procedures. Herd A was quarantined and tested repeatedly until December, 1983. No further TB reactors were found and the herd was released from quarantine in January, 1984. A summary of the herd tests are listed in Figure 1.
Herd B also was extensively tested from 1982 to 1984. This was the parent herd of Herd A. Herd B sent a group of bison for custom slaughter early in April of 1984. Lesions of tuberculosis were observed in one animal and M. Bovis was later confirmed. As the result of this finding, Herd B was placed under quarantine. After further investigation disclosed movements between the two herds, Herd A was also re-quarantined. Eighty-two animals belonging to Herd B, from which the lesioned animal originated was tested. The caudal fold test was used and two reactors were disclosed. One of the reactors was lesioned and bovine tuberculosis was confirmed. The test results of the second group of bison from Herd Owner B resulted in 47 Reactors of 52 tested via the cervical route. 103 animals in the same group were tested via the caudal fold route; 32 additional reactors were found.

A summary of the test results in Herd B is illustrated in Figure 2.

At this point it was decided that further testing in this herd was not advisable and both herds were depopulated in the summer of 1984. The post mortem results of Herds A & B resulted in a combined gross lesion rate of 52%. A summary of the lesions rate for both herds is illustrated in Figure 3. At least 90% of the lesions were located in the thoracic cavity with the left bronchial node most commonly involved lymph node.

**Epidemiology:**

1. Extensive effort was made to determine the source of the infection in these two herds. Both of the herds were involved in selling breeding stock. A major study was made to locate and evaluate other bison and cattle herds that had received cattle from Herds A and B. This effort is incomplete at this time. This report will cover the current status of this investigation.

2. The original South Dakota herds were maintained under the same conditions as domestic cattle. The impression that bisons are wild animals is misleading. Few bison are free to roam and exist in the wild in the United States today. Nearly all bison are confined by fencing, handled and maintained the same as cattle, and often in the same enclosure.

3. The most obvious common source of the infection in both of these herds was from the previous infected elk herd. Other possible sources of infection must also be considered. The possible sources of infection are illustrated in Figure 4.

4. When exposure occurred in the South Dakota herds is still a matter of speculation. Circumstantial evidence would indicate that exposure could have occurred as early as 1978 from the elk purchased or from exposure occurring in 1981 to known infected elk. This view is supported by the fact that the one bison maintained in Herd B after exposure to elk in 1981 was confirmed as infected with Bovine Tuberculosis in 1982. However, when one considers the extensive involvement of Herd A supports the theory that exposure had occurred as early as 1978. Based on this information, it was decided to
investigate sales for the years of 1981 thru 1984. Results of this investigation would then be available to evaluate the question of the importance of 1978 exposure.

Extensive sales were made from both of these herds. Sales were by private treaty and thru national, regional, and local livestock sales. A number of sales, for example, occurred in the years of 1981, 1982, 1983 and 1984 from the Western Livestock Show and Sales, Denver, Colorado. At the present time there are 85 known exposed bison herds involving 338 bison from sales of exposed animals. This has resulted in possible exposure of 2200 additional bison. At present there are 20 states involved plus Canada which have received exposed bison.

At the present time Bovine Tuberculosis has been confirmed in eight states. These states are listed in Figure 5.

States with confirmed M. Bovis herds are as follows:

- Colorado (5)
- South Dakota (2)
- Kansas (2)
- North Dakota (1)
- Nebraska (1)
- Ohio (1)
- Tennessee (2)
- Minnesota (2).

Alabama (1 suspicious herd not confirmed at this time). Investigation of other cases is still in progress at this time.

At the time of the outbreak in South Dakota bison, no federal regulations or indemnity existed for bison. Because of the apparent extensive movement of animals from these herds, the decision was made to include bison under the regulations as for cattle in this particular situation. Such involvement was justified because of the threat of spread to domestic cattle population. Such extenton would seriously hamper the bovine TB eradication program. Approximately 50 exposed cattle herds involving 3600 head of cattle will require investigation because of exposure to tuberculosis. At this time proof of spread into domestic cattle has not been established. However, investigations are incomplete. If by some miracle this has not happened, it will be most fortunate. One of the most beneficial results from this effort will be to reduce the threat to the Bovine Tuberculosis eradication program in cattle. The reduction of the problem in bison herds and reduction of the public health aspect is also of high importance.

The current outbreak has provided evidence that bovine tuberculosis transmits efficiently among bison. In one case the entire herd of 50 bison was condemned for tuberculosis upon Federal inspections. This included 32 purchased exposed bison plus 18 original herd members. In virtually all cases, tuberculous lesions involved the thoracic cavity. Problems have been recognized in bison with the caudal fold tuberculin test. Bison appear to be less sensitive to this test than are cattle. However, this has yet to be completely evaluated.

Procedures of handling exposed herds of bison have followed guidelines established for cattle. If possible, exposed animals and other exposed herds have or will be depopulated. It is anticipated that this will be accomplished in the majority of cases. Exposed animals and herds where depopulation is not possible will be extensively tested and evaluated.
### Figure #1
#### TB Test History
#### Herd A

<table>
<thead>
<tr>
<th>Date</th>
<th>Test Method</th>
<th>No. Animals</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5-83</td>
<td>CF</td>
<td>140 Bison</td>
<td>140N</td>
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<tr>
<td></td>
<td></td>
<td>9 Elk</td>
<td>9N</td>
</tr>
<tr>
<td>11-1-83</td>
<td>CF</td>
<td>209 Bison</td>
<td>209N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 Elk</td>
<td>9N</td>
</tr>
<tr>
<td>11-9-83</td>
<td>CF</td>
<td>49 Bison</td>
<td>49N</td>
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<td>12-13-83</td>
<td>CF</td>
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<td>255N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 Elk</td>
<td>9N</td>
</tr>
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4

### Figure #2
#### 1982–84
#### Herd B

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Date</th>
<th># Tested</th>
<th>Results</th>
</tr>
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<td>CF</td>
<td>11-2-82</td>
<td>47</td>
<td>47N</td>
</tr>
<tr>
<td>CF</td>
<td>1-4-83</td>
<td>169</td>
<td>169N</td>
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<td>CF</td>
<td>11-1-83</td>
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<td>52</td>
<td>47R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>81R</td>
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</tbody>
</table>
Figure #3
Post Mortem Results of Exposed and Reactor Animals
Herd A & B

<table>
<thead>
<tr>
<th>#Slaughtered</th>
<th>Gross Lesion</th>
<th>No Gross Lesion</th>
<th>% Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Herd A
- Reactors: 81, 221, 303
- Gross Lesion: 49, 120, 194
- No Gross Lesion: 112, 102, 109
- % Lesion: 30%
- 47 calves
- no post mortem

Herd B
- Reactors: 74, 120, 194
- Gross Lesion: 7, 109
- No Gross Lesion: 47, 7
- % Lesion: 91%, 54%
- 67 calves
- no post mortem

Total of Both Herds
- Reactors: 464
- Gross Lesion: 243
- No Gross Lesion: 221
- % Lesion: 52%
- 114 calves
- no post mortem

Figure #4
Possible Sources of Infection
Herd A & B

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Commingle with known infected Elk—1981–82</td>
<td>Other Buffalo Purchases</td>
<td>1981 NE Purchase</td>
</tr>
</tbody>
</table>

Herd A & B
S. Dakota

1978 Elk Purchase

Llama Purchase 1981
Original Infected Herds

# Traced Herds Receiving Tuberculous bison

? Traced Herds Receiving Tuberculosis "Suggestive" Bison

F Tuberculosis Accredited-Free State

20 States
85 Herds
368 Exposed Bison
15 Traced Herds with Confirmed Bison
1 Traced Herd with TB Suggestive Bison
At the present time other infected herds have been found in the years 81, 83 and 84 that sales occurred. However, sales from the year 1984 were by far the greatest risk. A summary of infected herds by the year is listed in Figure 6.

It appears that sales occurring in the year 1984 have the greatest risk potential; however 1981-1983 herds are large (300-500 bison).

What happened in these herds from the possible exposure dates raises interesting questions. One explanation may be the infection was low at the beginning and did not reach its peak until 1984. It does illustrate that all answers are not known on the pathogenesis of tuberculosis.

CONCLUSIONS:

At the present this outbreak illustrates some of the problems associated with completion of the Bovine Tuberculosis Program. Some of the problem areas are as follows:

1. The extent of tuberculosis in bison as a whole is not positively known. It is hoped that the South Dakota problem was an exception but time only will answer that question. Slaughter surveillance is not adequate for bison in general to determine the exact status. Testing and testing procedures also have their limitations.

2. Research is needed to evaluate testing procedures and possible development of new testing methods. The experience in the South Dakota herds indicates there may be a sensitivity problem with the present caudal fold test procedures. There is also need to understand the pathogenesis of tuberculosis in bison.

3. This case of bovine tuberculosis in bison as well as the previous elk report illustrates one of the problems associated with final TB eradication. Captive bison herds and other captive wild animal herds are increasing in the United States. Consideration must be given to such herds for TB eradication. Most of this type of herd is maintained under conditions similar to domestic cattle. It would be a delusion to believe that tuberculosis eradication can be accomplished and still leave these reservoirs of infection.

4. This is the first known experience in the United States of tuberculosis in bison since 1954 when bovine tuberculosis decimated a herd of 60 bison established in 1910 on the Trexler-Lehigh Game Preserve, Schnecksville, Pennsylvania. Tuberculosis caused a rapidly progressive, fatal disease in these bison. This herd was depopulated in 1960.

Figure #6

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
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<tbody>
<tr>
<td>1981</td>
<td>1</td>
</tr>
<tr>
<td>1982</td>
<td>0</td>
</tr>
<tr>
<td>1983</td>
<td>1</td>
</tr>
<tr>
<td>1984</td>
<td>14</td>
</tr>
</tbody>
</table>
In Fiscal Year 1984, three new confirmed tuberculosis infected cattle herds were identified. This is the lowest number of newly detected infected herds reported since the Bovine Tuberculosis Eradication Programs began in 1917.

This has been a year of progress and of problems. Progress is evident by the decrease of new herd findings. Elation is modified by past experience and by the current investigations in progress that there are still more herds to be found. Each newly detected infected and exposed herd is unique. Each new herd appropriately receives a great deal of attention. The need is greater than ever in the eradication program to expend efforts to determine the source of each new foci of infection and to determine where infection may have gone. Specific examples can be cited where herds have been exposed 10 years or more prior to detection. Can the program permit this to continue? The epidemiology of slaughter tracebacks, movements of exposed animals, and investigations of adjacent and contact herds must be of the highest priority.

In Fiscal Year 1980, there was found a total of six tuberculous herds. This fiscal year there was a total of seven. One of the herds in FY 1980, a very large dairy in Arizona, had been carried over from FY 1979. This fiscal year two herds were carried over from FY 1983. The first herd was a dairy of approximately 300 head in El Paso, Texas. It was determined that infection entered the herd through cattle that had entered into the United States illegally. It was placed under "Constructive Seizure" by the U.S. Customs Service. This seizure did not prevent necessary program activities in the herd by state and federal animal health officials. This herd was owned by three individuals. The manager, who was also part owner of the herd, was responsible for the illegal animal movements. A disposal order was issued for 36 animals under the authority of the Secretary of Agriculture for animals that were identified as having been smuggled. This herd was depopulated. All gross lesions were in animals suspected of being smuggled. No federal indemnity has been authorized for reactor or exposed cattle that were owned by the person responsible for the illegal movements.

The second herd, also carried over and still under test, is a beef ranching operation of approximately 5,700 head on the western end of Molokai Island, Hawaii. A dispersal sale of Charolais cattle from a ranch on the eastern end of this island in 1973 resulted in the most extensive epidemiological traceout effort ever carried out in the program involving 1,650 head of cattle. Three herds have been depopulated from the eastern end of this island in 1976, 1980, and 1981. Following the depopulation of the most recent herd, two wildlife surveys were carried out that confirmed infection
by *M. bovis* in the feral swine population on the eastern end of the island. Trapping and hunting of the feral swine has led to a marked decrease of infection in this wildlife source. *M. bovis* has been isolated twice from wild deer on the island over a long period of time. The current infected herd was placed on a testing plan in January 1984 to evaluate the total herd situation. There have been problems in scheduling slaughter of reactor and exposed cattle in local packing plants. Reactor animals and lesioned animal carcasses must be cooked. Accelerated slaughtering of large numbers of exposed animals in Hawaii would upset a rather stable demand for locally slaughtered beef.

The five newly detected tuberculous herds for FY 1984 were located in Texas, Mississippi, and Idaho. One infected and two exposed beef herds were located in northeast Texas. The two small, exposed herds had mingled with the infected herd. In Mississippi, a small mixed beef operation purchased an infected calf originating from an extensively infected dairy herd that was depopulated in FY 1983. The fifth and most recent infected herd was a dairy in Idaho, where the last previously known infected herd was released from quarantine in April 1979. The current infected herd was purchased by the present owner in 1981 and was detected through a slaughter sample submission. Traceback success was the result of excellent cooperation between Federal meat inspection and local animal health officials and use of the Brucellosis Information System to establish animal movements. At the time of depopulation, it should be noted that all lesioned animals had been removed through use of tuberculin testing of the herd. The previous herd owner had purchased tuberculosis exposed cattle from a herd that was determined to be infected in 1977 and released from quarantine in 1979. The purchased exposed animals were negative to test at that time. This herd was located within a mile of the previously infected herd.

**Figure 1** — Two new States were added to those that are Tuberculosis Accredited-Free, Massachusetts (January 10, 1984) and Nevada (March 21, 1984). There are now 25 states, plus the Virgin Islands, that are Tuberculosis Accredited-Free, matching 25 states and the Commonwealth of Puerto Rico that are Modified Accredited.

There is a total of 9 states with freedom from *M. bovis* for 5 or more years. These are: Alaska, Oregon, Kansas, Oklahoma, Iowa, Indiana, Missouri, Tennessee, and Georgia. In FY 1984, three new states, Oklahoma, Indiana, and Iowa, were added to this list. Two were removed when they became Tuberculosis Accredited-Free and this year Idaho experienced an infected herd.

**Figure 2** — There was a national total of seven tuberculous herds for FY 1984. One newly detected herd was in Idaho, one in Mississippi, three in Texas. In addition, two herds; one in Hawaii and one in Texas, were carried over from FY 1983. Two of three new herds in Texas were exposed herds.

**Figure 3** — This figure illustrates methods for the location of five herds newly detected. Two infected herds were found from identification and traceback from routine slaughter samples. The three other herds, one
infected and two exposed, were found as a result of an investigation of animal movements and contacts.

Figure 4 — In FY 1983 and FY 1984, no new herds were found as a result of routine testing of herds.

Figure 5 — Six of seven herds were depopulated. The herd not depopulated is the large beef herd in Hawaii.

Figure 6 — This figure shows geographically the location of the herds in Figure 5 and the proportion of those depopulated.

Figure 7 — The importance of correct identification of slaughter samples is illustrated in these two bar graphs. They collectively represent 21 investigations closed, from a total of 1,881 suspicious sample submissions by state and federal slaughter plants. Many of these investigations were initiated in FY 1983. Tissues grossly indistinguishable from tuberculosis frequently are not supported by laboratory findings. The 21 cases illustrated were subjected to field investigations when tissues collected were compatible or suggestive on microscopic examination or if *M. bovis* was subsequently isolated. Selected cases are investigated when gross pathology, histopathological findings of granulomatous lesions, or preliminary source information is suspicious following case evaluation by the tuberculosis epidemiologists.

Seventeen unidentified cases were of feedlot origin. There was a 50 percent success rate in finding infected herds from four cases illustrated for identified adult samples. Two successful slaughter investigations led to the confirmation of one herd in Hawaii and one in Texas. Two cases were classified as unsuccessful when investigation procedures and herd testing did not identify the source.

Figure 8 — There were 1,717 (VS Form 6-35) slaughter investigations initiated from federal slaughter plants. The percent of adult animals was 46 percent compared to 60 percent in FY 1983. Both the number of identified adults and those with identification was somewhat less than FY 1983. Sixty-six versus 70 percent and 46 percent versus 49 percent. The adult cattle population sampled was less than last year in terms of total sample numbers and percentages. There were 942 adults sampled in FY 1983 and 795 sampled in FY 1984.

Figure 9 — These bar graphs illustrate numbers of annual sample submissions from federal slaughtering establishments. Of 1,717 submissions collected, in FY 1984, 82 have resulted in field investigations, most of which are ongoing. Eleven of these investigations are from adult animals and the remainder are from feedlot animals. The increased number of total slaughter submissions was a result of more submissions this year from young fed cattle. This follows a year of heavy importation of feeder cattle that are now being slaughtered. (Imported feeder steers: FY 1982 — 320,000, FY 1983 — 496,000).

This report indicating seven total known tuberculosis herds, five of which were newly detected, and the confirmation of bovine tuberculosis in
three of these herds is strong evidence of continued program progress. Historical data and epidemiological information would tell us that we cannot expect this low level of infection to be maintained. There will be expected resurgences in herd members as we continue to pursue total eradication.

Included with this status report is a brief summary of bovine tuberculosis in bison which occurred this fiscal year and which continues at this time. Limited experience with bovine tuberculosis in bison herds, the epidemiology of exposed animal movements, and findings in receiving herds points to the susceptibility and risk of transmission of infection in bison and to the potential for spread to cattle.
Tuberculosis Eradication

Bovine Tuberculosis Area Status

September 30, 1984

- Accredited Free States (25) plus Virgin Islands
- Modified Accredited Areas (25) plus Puerto Rico
- No M. Bovis for Over 5 Years (9)
Tuberculosis Eradication

Location of 7 Tuberculous Herds
FY 1984
Tuberculosis Eradication

Methods of Locating 5 Tuberculous Herds Initially Detected during FY-84

- Traceback of Regular Kill Slaughter Animals (2)
- Tracing Exposed Cattle from Affected Herds (3)
Tuberculosis Eradication

Detecting Herds with TB Infection: 1974 through 1984

1974 75 76 77 78 79 80 81 82 83 84

0 25 50 75 100

Epidemiologic Tracing

All Other Tuberculin Testing

Figure 4

STUMPF, ESSEY, PERSON, THORPE
Tuberculosis Eradication

Herds Found vs. Herds Depopulated
FY 1974-84

Herds Found

Herds Depopulated

1974 75 76 77 78 79 80 81 82 83 84

100 75 50 25 0
Tuberculosis Eradication

Proportion of Tuberculous Herds Depopulated

FY 1984
Tuberculosis Eradication

Traceback of 21 Tuberculous Cases Closed
(Regular Kill Animals) FY 1984

17 Unidentified

100% Unsuccessful

4 Identified

50% Successful

50% Unsuccessful
Tuberculosis Eradication

Number of 6-35's Submitted FY 84 (Federal Establishments)

Number of 6-35's Submitted

Number of Adult Animals

Number of Animals with Identification

Number of ID Devices Submitted


1717

795 (46%)

526 (66%)

238 (45%)
BOVINE TUBERCULOSIS IN BISON

Mitchell A. Essey — DVM

The bison herd of a South Dakota owner was confirmed tuberculous following the traceback from slaughter of a lesioned, 2-year-old bull. *Mycobacterium bovis* was isolated June 8, 1984. Eighty-one reactors were disclosed in 237 bison tested, of which 74 (91 percent) were lesioned.

It is believed that bovine tuberculous was introduced to this herd through association with tuberculous elk of this owner’s son. The son’s elk herd was depopulated for tuberculosis under state authority in 1982. Tuberculosis spread from the elk herd to a bison herd owned by the son but was limited to a single cow found infected in December 1982. The son’s herd underwent three negative caudal fold tuberculin tests during 1983, and the father’s herd underwent one negative test that year. The elk herd may have become infected by the introduction of two elk purchased in 1978 from a small zoo in another state. These were sick on arrival and died shortly thereafter. This incident was followed by a series of unexplained deaths in the elk herd.

Tuberculous lesions were reported in 194 (64 percent) of 303 bison that received a postmortem examination in the father’s herd. Lesions were seen in 49 (30 percent) of 161 bison upon the depopulation of the son’s herd.

Bison moved from the two South Dakota herds into 20 states, plus Canada. This includes eight states currently classified as Tuberculosis Accredited-Free; Montana, Colorado, Nebraska, North Dakota, South Dakota, Nevada, Minnesota, and Michigan. Approximately 368 bison have been traced to 85 herds. These herds include an additional 2,200 bison and about 3,600 cattle. Bovine tuberculosis has been confirmed by histopathology compatible for tuberculosis or by culture of *M. bovis* in bison traced from the South Dakota foci into 15 herds located in the following states: five in Colorado, two each in Minnesota, Kansas and Tennessee, and one each in North Dakota, South Dakota, Nebraska, and Ohio. Also, one herd in Alabama received a bison heifer with thoracic lesions “suggestive” of tuberculosis on histopathology.

The Code of Federal Regulations, Title 9, Part 50, Bovine Tuberculosis Indemnity, was amended July 6, 1984, so that “all provisions for cattle also apply to bison herds in South Dakota found on June 1984 to be foci of tuberculosis infection and to any other bison affected with or exposed to tuberculosis because of such South Dakota foci of tuberculosis infection.”

The preponderance of positive cases were seen in animals that were purchased in 1984. The earliest confirmed case was a cow purchased by a Kansas owner in 1981. A Colorado herd received a confirmed tuberculous cow in 1983. No instance of transmission of bovine tuberculosis from bison to cattle has been shown as yet. However, the potential is believed to be great. The number of cattle herds considered exposed or potentially exposed ranges from 10–15 herds in each of three infected bison herds found.
Tuberculosis Eradication

Tuberculosis Traceback Investigations Submitted (Regular Kill) FY 1984

Cases not tuberculosis
Cases of tuberculosis

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases not tuberculosis</td>
<td>1512</td>
<td>1512</td>
<td>1512</td>
<td>1512</td>
<td>1512</td>
<td>1512</td>
<td>1512</td>
<td>1512</td>
</tr>
<tr>
<td>Cases of tuberculosis</td>
<td>58</td>
<td>65</td>
<td>62</td>
<td>52</td>
<td>53</td>
<td>78</td>
<td>27</td>
<td>82</td>
</tr>
</tbody>
</table>
In some cases involving smaller numbers of animals, cattle have run directly with infected bison.

The current outbreak has provided evidence that bovine tuberculosis transmits efficiently among bison. In one case, an entire herd of 50 bison was condemned for tuberculosis upon federal postmortem inspection. This included 32 purchased bison plus 18 original herd members. In virtually all cases, tuberculous lesions involved the thoracic cavity. Problems with tuberculin testing have been recognized in bison. Bison appear to be less sensitive to tuberculin than are cattle. However, this has yet to be completely evaluated. This is the first known experience in the United States of tuberculosis in bison since 1954 when bovine tuberculosis decimated a herd of about 60 bison established in 1910 on a Pennsylvania game preserve. Tuberculosis caused a rapidly progressive, fatal disease in the Pennsylvania outbreak.
Bovine Tuberculosis in Bison—FY 84

- States receiving tuberculosis exposed bison (20)
- Tuberculosis accredited free states receiving tuberculosis exposed bison (8)
- Herds initially infected (2)

- Herds receiving bison with lesions compatible for tuberculosis on histopathology (14)
- Herds receiving bison with lesions suggestive of tuberculosis on histopathology (2)
THE JOHNE'S DISEASE RESEARCH PROJECT IN PENNSYLVANIA

R. H. Whitlock,* H. A. Acland,* C. E. Benson,* L. T. Glickman,*
J. L. Bruce,* J. Fetrow,** C. Rossiter,*** S. Harmon,***
L. T. Hutchinson,** J. Dick,† R. Merkel††

INTRODUCTION

The Commonwealth of Pennsylvania, Department of Agriculture has recognized Johne's disease as an important disease of livestock for many years and is a reportable disease in the state. The true prevalence of the disease is unknown as no comprehensive survey has been done in the state. The Pennsylvania State Diagnostic Laboratory at Summerdale is currently filled to capacity with requests for Johne's cultures, which indicates the level of concern the producer and veterinarian has for Johne's disease. Since indemnity may be paid for Johne's positive cows by the BAI, the direct cost of Johne's disease to the state is substantial, i.e., greater than $100,000/year. Johne's disease has become recognized as a major disease that is deserving of national attention. One manifestation of this interest was the International Symposium on Johne's Disease held at Ames, Iowa, June, 1983.

Johne's disease is not peculiar to Pennsylvania, but is generally considered an emerging disease of international proportions. Many countries have recognized the disease for years but only recently have they devoted modest resources to further document the prevalence and investigate new diagnostic tests.

Paratuberculosis (Johne's disease), a chronic infective disease of ruminants, has been recognized in the United States for about 75 years. The causative agent is Mycobacterium paratuberculosis, a facultative intracellular acid-fast bacterium. Infection with Mycobacterium paratuberculosis is difficult to control because of a long incubation time, the absence of clinical signs in the early stages, the lack of effect of antibiotics or other drugs, and the lack of quick reliable methods for diagnosis.

A battery of diagnostic tests have been introduced and evaluated which aim to identify the causative organisms directly via histological and cultural techniques or which utilize immunologic responses of the host.

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††National Animal Disease Laboratory, Mycobacteriology Laboratory, Ames, Iowa.
with various in vivo or in vitro techniques. Most methods presently employed suffer from lack of accuracy in that they fail to detect many infected animals, may detect exposed animals which are not currently infected, and they may not distinguish between infections from other mycobacteria or other bacterial species which cross react antigenically with Mycobacterium paratuberculosis. The fecal culture test for M. paratuberculosis and histological examination of tissues obtained at surgery or slaughter are generally recognized as being the most reliable tests to which the ELISA and other tests for M. paratuberculosis will be compared.

This research project is intended to help define the economic impact of Johne's disease on the dairy industry. The following is a very conservative estimate of the present impact of the disease in Pennsylvania.

Dairy cows in Pennsylvania: 740,000
Percent infected with Johne's disease: 5% (studies to date have estimated this to be as high as 10-15%)
Percent of cows slaughtered per year: 33% (slaughter rate for Johne's cows is probably higher)
Loss due to decreased weight when slaughtered: $100.00

ESTIMATED COST OF JOHNE'S DISEASE PER YEAR IN PENNSYLVANIA:

$1,221,000/year

As noted above, this estimate is very conservative since it ignores both the cost of reduced milk production in infected cows and the premature loss of cows with fewer lactations due to premature culling. These two costs are very likely to be much greater than simply the cost of reduced carcass value at slaughter. One of the major objectives of this study will be to measure the actual value of these losses.

OBJECTIVES FOR THE PENNSYLVANIA JOHNE'S DISEASE RESEARCH PROJECT:

YEAR 1: 1. To determine frequency and geographic distribution of Johne's disease by examining adult dairy cows at slaughter.
2. To establish the economic loss of Johne's disease cows identified at slaughter.
3. To compare diagnostic modalities for Johne's disease in adult cows at slaughter from the aspect of specificity, sensitivity and cost of the test.

YEAR 2: 1. To determine the prevalence of Johne's disease within Pennsylvania dairy herds identified by positive and negative cows as in Year I.
2. To compare the sensitivity and specificity of diagnostic tests for Johne's disease in the herd survey.
3. To evaluate the economic loss associated with Johne's dis-
JOHNS DISEASE RESEARCH PROJECT

ease in dairy herds and to establish economic constraints for Johne's disease control programs.

4. To determine the frequency of isolation of Johne's bacillus (*Mycobacterium paratuberculosis*) from controlled inoculated environmental sites.

YEAR 3: 1. To evaluate alternative laboratory tests as a means of diagnosis for Johne's disease.

2. To determine the viability of Johne's organisms from environments naturally contaminated by cows infected with *M. paratuberculosis*.

3. To cooperate with the Pennsylvania Department of Agriculture, Bureau of Animal Industry in development of Johne's disease control program for the state.

PROCEDURES:

Year I — Objective 1: Blood samples, fecal samples and tissues from the ileum; ileocecal colic lymph node and rectum will be collected from about 1,500 adult Holstein dairy cows from a major slaughter house in Northeastern Pennsylvania (Taylor's Packing Company — Wyalusing, PA). This plant processes more than 200,000 animals per year and accounts for the processing of more than 10% of the culled dairy cows in the United States. Several days each month a research team will visit the slaughter house to collect the specimens from randomly killed cows (approximately every 12th animal in the processing line for that day). Each month for a year, approximately 120 animals will be sampled for a total of about 1500 animals.

The frequency of Johne's disease in slaughtered cows will be determined by a collective assessment of the diagnostic tests to detect Johne's disease in a specific animal. The tissues (3/cow) will be processed and examined histologically for acid-fast bacilli compatible with the Johne's bacilli. One fecal sample will be cultured for *M. paratuberculosis* from each cow. Serum samples will be analyzed for antibody to *M. paratuberculosis* by the complement fixation test, enzyme linked immunosorbant essay (ELISA) and the agar gel immunodiffusion test. Dr. Richard Merkel at Ames, Iowa, National Animal Disease Center, will make the reagents available for the agar gel immunodiffusion and ELISA tests. Thus an upper limit estimate of the prevalence of Johne's disease in the state will be determined.

Year I — Objective 2: An estimate of the economic impact of Johne's disease on the Commonwealth of Pennsylvania will be developed. The total cost of Johne's disease to Pennsylvania's dairy industry will be estimated from the prevalence data and the dollar loss from each infected cow with Johne's disease. The estimate will be derived in a way that will allow the figures to be kept current for any existing market conditions. In order to be useful a Johne's control program must cost less than the disease itself. These economic constraints will be established.

Only adult female Holsteins will be evaluated for Johne's disease and
the following data will be obtained from each animal sampled at the slaughter house:

1. Live weight
2. State ear tag number(s)
3. Backtag identification number to permit trace back to the auction the animal was purchased.

Using the Pennsylvania state ear tag number(s), we will then contact the Bureau of Animal Industry (BAI) to locate the herd of origin in the state. This step will provide the following information:

1. Owner's name
2. Address
3. Phone number
4. BAI number

The herd owner will then be mailed a detailed questionnaire to obtain the following information:

**HERD:**

1. Number of adult cows in herd
2. Housing for adult cows
3. Calving area and practices
4. Estimate of how long calves are left with cow after birth
5. Calf housing up to weaning
6. Calf feeding practices
7. Heifer housing and management
8. DHIA rolling herd average
9. Percent butterfat
10. Percent days in milk

**INDIVIDUAL:**

1. Birth date
2. Date of last calving
3. Age at last calving
4. Date culled
5. Duration of lactation at time of culling
6. Reason culled
7. Total lifetime DHIA milk production
8. Total lifetime DHIA fat production
9. Price received when culled from herd

We will then compare this data for the positive and negative cows to identify risk factors for Johne's infection.

Analysis of economic data between Johne's positive and negative cows will provide the following information:

1. Difference in total lifetime milk and fat production
2. Difference in value when culled
3. Difference in total loss for Johne's positive cow:

The economic loss in an individual cow due to Johne's can be esti-
mated to consist of the three following components:

a. Loss of milk production prior to culling:
   A distribution of lifetime milk production by age at culling will be created for positive and for negative cows. For each positive cow the her total difference between production and the production in age matched negative animals will be considered to be the milk lost due to infection with Johne's. The value of that milk will be adjusted for fat content if appropriate. We will not adjust for reduced feed consumption to produce less milk, presuming that the reduction in digestive and metabolic efficiency accounts for the reduced production rather than simply reduced intake.

b. Loss due to reduced value at culling:
   The difference in price at culling between Johne’s positive and negative cows will be calculated. The sample size should allow us to detect with 80% certainty a 5% reduction in body weight due to Johne’s, given a prevalence of 5% in slaughtered cattle.

c. The lost value of the remaining lifetime of a Johne’s positive cow had she not been infected:
   The best estimate of that value is the price for replacing the culled cow with a healthy cow matched for age and stage of lactation, and originating from a herd with the same DHIA rolling herd average as that of the Johne’s positive cow. In order to obtain estimates of those values data will be collected through dairy sales agents selling cattle for dairy purposes. For cows sold for dairy use, the following data will be collected:
   (1) Age
   (2) Stage of Lactation
   (3) Price paid
   (4) DHIA rolling herd average of herd of origin

   The total cost for a cow infected with Johne’s will be the sum of the above three costs. The estimate will be conservative since it will ignore the added expense of treatments and veterinary services associated with the disease. These expenses are presumed to be small.

Because the above calculated cost of Johne’s disease is independent of the status of other cows in the herd, the overall economic impact of the disease on a herd will then be a simple function of the prevalence of the disease in the herd, the value of the cows in the herd (as estimated by their average milk production), and price of milk and cull cows. The economic impact of the disease could thus be calculated for the specific situation in any herd. That calculated cost could be used to determine the economic desirability of working towards Johne’s eradication in the herd.

The overall impact on the total Pennsylvania herd (or the national herd) can thus be calculated given the total herd average and prevalence (determined by herd cultures in the second year of this trial) and the total
herd average level of milk production. As was true on an individual herd basis, the economic incentive for a statewide or national campaign to control Johne's could be determined.

The economic impact of Johne's will obviously change as the value of dairy cows and of milk changes. Given the basic model and biological data derived from the trial, calculating this new impact in the future will simply be a matter of using the then current prices prevailing in the market place.

**Year I — Objective 3:** Diagnostic tests for Johne's disease.

Currently the fecal culture is the "accepted" test for Johne's disease but it requires a 12–16 week incubation period and is relatively expensive ($5–10/sample). The accuracy, sensitivity and specificity of several existing and recently developed tests for Johne's disease detection will be compared and include:

1. Complement fixation test
2. Culture of fecal sample, ileum and ileocecal lymph node
3. Enzyme-linked-immuno absorbent assay (ELISA)
4. Agar gel immunodiffusion test
5. Histopathology of the ileum, ileocecal lymph node and rectum
6. Immunoelectrophoresis

The purpose of this objective is to develop the necessary data so one of the new tests may supplant the current fecal culture test if the new techniques are superior.

**Year II — Objective 1:** Approximately 25 herds will be randomly selected from the 1700 dairy herds in the state arranged by zip code and stratified according to herd size. Only Holstein dairy herds with milk production records kept by the Dairy Herd Improvement Association (DHIA) will be used. Initially every 50th herd from the BAI herd list was selected and then stratified according to herd size.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cows Range</th>
<th>DHIA Herd Count</th>
<th>DHIA Herd %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1–29 cows</td>
<td>750 herds on DHIA</td>
<td>NA</td>
</tr>
<tr>
<td>1</td>
<td>30–59 cows</td>
<td>3,170 herds on DHIA</td>
<td>66.7</td>
</tr>
<tr>
<td>2</td>
<td>60–89 cows</td>
<td>1,066 herds on DHIA</td>
<td>22.4</td>
</tr>
<tr>
<td>3</td>
<td>90–119 cows</td>
<td>304 herds on DHIA</td>
<td>6.3</td>
</tr>
<tr>
<td>4</td>
<td>120–149 cows</td>
<td>132 herds on DHIA</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>150–179 cows</td>
<td>59 herds on DHIA</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>180–209 cows</td>
<td>28 herds on DHIA</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>&gt;210 cows</td>
<td>24 herds on DHIA</td>
<td></td>
</tr>
</tbody>
</table>

4,758 100%

Only herds with more than 30 and less than 210 cows were included in the original pool of herds. This constraint resulted in a proportional sample of DHIA herds. Owners with herds in each group will be contacted about their willingness to participate in the study and two herds will be sampled each month from a random listing.
Year II — Objective 2: Following the identification of fecal culture positive animals in a herd, serum from each animal will be further evaluated to determine the sensitivity and specificity of the ELISA, the complement fixation test, and AGID test and the immunoelectrophoresis test.

Year II — Objective 3: The economic loss associated with Johne's disease will be further defined on an individual and a herd basis using milk production data; reproductive data; and information on the prevalence of other diseases.

Year II — Objective 4:

Experimental Protocol: Sample plots at New Bolton Center will be fenced apart from the rest of the field and seeded with *Mycobacterium paratuberculosis*. These sites will be chosen to access the impact of various soil types and housing conditions on the maintenance and viability of the Johne's bacillus. The proposed sites are: a) sandy soil, b) loam soil, c) clay soil, d) soil with high limestone, e) free stall, f) calving stall, g) alley way, h) dry lots, i) calf hutch site, and j) calf stall. These sites will be sampled once a month for up to two years or until three consecutive cultures are negative. The specimens will be processed according to the technique of Ellsworth et al (AJVR 41:1526–30).

Justification: Completion of this objective will define the limit of viability of the Johne's bacillus in a wide variety of environmental conditions. This data can be utilized to establish the protocol hazard of a contaminated site to uninfected animals. The long term survival of this microbe in specific sites has not been reported and such information would provide the foundation for the development and implementation of appropriate decontamination procedures. We anticipate that the changes in weather will affect the isolation rate and propose to continue this objective over a maximum period of two years to include a typically cold Pennsylvania winter.

Year III — Objective 1: The laboratory tests including fecal culture, ELISA, gel immunodiffusion, immunoelectrophoresis and complement fixation will be utilized in the herd study to assess the accuracy and sensitivity of these tests on a herd basis. The final goal is to determine which test will give the most accurate information for the least money.

Year III — Objective 2: (Continuation of Year 2 Objective: Controlled site survey)

Technique: The approach to this survey may be modified by the data during Year 2 — Objective 4. The current approach is to screen farms (5 positive for Johne's) for *Mycobacterium paratuberculosis* at monthly intervals (10 sites per farm). Comparable soil and stall sites will be selected on both positive and negative farms. The approximate number of microbes per 100 gram soil (or per sq. meter for stalls) will be determined by conventional environmental survey techniques (Soil microbiology, Alexander, 1961, Wiley).
Justification: Completion of this objective will provide information on the mycobacterial population in environments frequently contaminated by shedders. Comparison with the data collected from the contaminated controlled environments may provide valuable insights on the management approaches to controlling the disease within a herd as well as suggesting the impact of frequency of soil inoculation on the disease rate.

The Pennsylvania Johne’s Research project is truly a cooperative project involving the School of Veterinary Medicine, University of Pennsylvania; Penn State University; The Bureau of Animal Industry, Pennsylvania Department of Agriculture; and the National Animal Disease Laboratory. The initial project objectives were outlined for three years and was funded by the Commonwealth of Pennsylvania at the recommendation of the State Grange. As the data is evaluated and analyzed, it is anticipated that herds infected with *M. paratuberculosis* can be monitored and recommendations established to facilitate the eradication of Johne’s disease from the farms.
REPORT OF THE COMMITTEE ON TUBERCULOSIS AND JOHNE’S DISEASE

Chairman: S. B. Hurley, Madison, WI
Vice Chairman: V. P. LaBranche, Boston, MA

J. A. Acree, FL; Don Agresti, CA; L. R. Barnes, IN; C. E. Boyd, SC; J. M. Dick, PA; M. A. Essey, CA; J. G. Flint, MN; G. H. Frye, MD; S. K. Harris, IA; E. M. Himes, IA; Glen Hoffsis, OH; R. L. Hosker, MD; D. E. Hughes, SD; C. A. Lamb, CA; L. L. Larson, WI; A. R. McLaughlin, WI; R. S. Merkal, IA; M. E. Oetting, MO; W. J. Owen, IA; W. L. Searles, TX; M. S. Silberman, GA; D. H. Smith, WA; P. L. Smith, CA; G. R. Snyder, VA; P. L. Spencer, IL; C. D. Stumpff, KS; C. O. Thoen, IA; Bruce Widger, NY

The committee on Tuberculosis and Johne’ Disease met at 1:30 p.m. on October 23, 1984, at the Hyatt Regency-Fort Worth Hotel with thirty-six members and guests in attendance under chairmanship of Dr. S. Hurley of Madison, Wisconsin.

I. Dr. Ralph Hosker of the USDA staff, Hyattsville, Maryland, opened the meeting with a status report of the State-Federal Tuberculosis Eradication Program. Recent outbreaks in the United States were discussed. Four infected herds were located in Texas, one in Mississippi, one in Idaho, and one in Hawaii. All herds excepting the Hawaiian herd have been depopulated. There are now 25 TB accredited free states and 25 modified accredited free states. It has been demonstrated that most of the detection was done by meat inspection and not routine testing. There were 1,717 cases submitted, from which 82 investigations were initiated. Seventy-one of these were of feedlot origin, fifty-one of which involved Mexican cattle.

A report on the Tuberculosis Program Evaluation meeting was given by Dr. Ralph Hosker of the USDA staff. A copy will be included in the proceedings book.

II. Dr. William B. Searles of the USDA staff of Austin, Texas, spoke on methods of reintroduction of tuberculosis into the United States. Wildlife including bison, elk, exotic animals, zoos, Mexican Feeders, etc. The use of tuberculin and the evaluation of responses in other species was discussed. A subcommittee is to be appointed to evaluate these concerns.

III. An epidemiological study of M. bovis in American Bison was presented by Dr. Charles Stumpff, USDA staff, DeSoto, Kansas. A report will be included in the proceedings book.

IV. Dr. D. E. Hughes, South Dakota state veterinarian, submitted the following resolution which was accepted by the committee:

Whereas, because of recent widespread outbreaks of tuberculosis in buffalo in the United States and the lack of information on 1) the pathogenesis of disease; 2) the apparent lack of efficacy of tuberculin skin tests for use in the diagnosis of tuberculous animals
REPORT OF THE COMMITTEE

and 3) the threat of introducing tuberculosis in cattle, in which the incidence is very low, the following resolution is presented.

Be it resolved, the United States Department of Agriculture and the buffalo associations should provide support for research on tuberculosis (*Mycobacterium bovis* infection) in buffalo with particular emphasis on improving diagnostic tests for field use in identifying infected animals.

VI. A preliminary report was given on progress of nationwide paratuberculosis survey by A. M. Hintz of Ames, Iowa.

VII. Dr. Norman W. Kruse, state veterinarian of Nebraska, presented a resolution, signed by twelve state veterinarians, expressing concern over the *M. bovis* problems in American bison. It was urged that this be declared an emergency disease problem. The resolution was tabled until the next day.

The meeting was adjourned at 5:30 p.m.

The second session of the Tuberculosis and Johne’s Disease committee was held at 1:30 p.m. on October 24, 1984.

I. Over forty members and guests were in attendance. Dr. J. K. Atwell of Hyattsville discussed the resolution at length. This resolution was defeated.

A second resolution was submitted by Dr. D. E. Hughes. It was voted to accept this resolution which is as follows:

*Whereas, American bison are raised under the same conditions and for the same purposes as cattle; and,*

*Whereas, *M. bovis* infection has been shown to exist in several herds of bison in many different states; and,*

*Whereas, the current regulations pertaining to the handling of tuberculosis in bison shall pertain to only those animals infected from two South Dakota ranches.*

*Therefore, be it resolved that the USAHA goes on record as strongly recommending that all bison raised under agricultural conditions shall be handled under the same tuberculosis testing, indemnity and depopulation conditions as cattle except that prior to January 1, 1987, state classification will not be affected by *M. bovis* infection in bison herds.*

II. A report of the Johne’s disease program in Wisconsin was given by Dr. Joan Arnoldi. A field manual can be obtained by writing to the Animal Health Division, Wisconsin Department of Agriculture, 801 W. Badger Rd., P.O. Box 8911, Madison, Wisconsin 53708.

III. Subcommittee reports

A. Johne’s Disease by Dr. S. Hurley

Several new approaches for diagnosis were discussed. A brief
Recommendations were approved for presentation to the full committee. The following were approved:

1. The code of Federal Regulations, Part 71, should be amended to remove restrictions on the movement of Johne's vaccinates in interstate commerce. THE MOTION WAS APPROVED UNANIMOUSLY.

2. It is recommended that a staff officer position should be created within APHIS, Cattle Diseases, to deal specifically with Johne's disease. THE MOTION WAS APPROVED UNANIMOUSLY.

The intention of this recommendation is not to embark upon a grandiose control plan but simply to provide an individual to work on regulatory change and public education.

3. The Code of Federal Regulations, Part 71, should be amended to allow cattle exposed to Johne's disease to move interstate after the herd of origin has been tested by an approved protocol and found negative. THE MOTION WAS APPROVED UNANIMOUSLY.

4. Procedures and funding should be established to approve laboratories to conduct Johne's testing and to standardize antigens, other reagents, and protocols for use in conducting these tests. THE MOTION WAS APPROVED UNANIMOUSLY.

5. A letter should be sent to Dr. Earl Splitter, USDA, CSRS, to request that Johne's disease continue to be listed as a specific priority item for funding by the Special Competitive Grants. THE MOTION WAS CARRIED UNANIMOUSLY.

B. Embryo Transplant report by Dr. L. L. Larson and A. R. McLaughlin. The committee moved to accept the report, a copy of which will be included in the proceedings book.

C. Tuberculosis in Imported Mexican Feeder Steers, a report presented by Dr. Ralph Hosker, USDA, Hyattsville, Maryland. The report was accepted, a copy of which will be included in the proceedings book. Dr. Michael Bedoya, Asesor Del Director, de Sanidad Animal, Mexico, briefly addressed the committee. He indicated that his country and the livestock producers would not strongly oppose the use of the hot brand for identification of imported Mexican feeder steers. He proposed the cheek as the brand site to be applied to the animals prior to border crossing. If the United States wishes this means of permanent identification, it will have to press for agreement in negotiations and require it as a condition for U.S. importation of feeder steers.
REPORT OF THE COMMITTEE

D. UM&R changes by Dr. Ralph Hosker

Part II Recommended Procedures (Minimum requirement)

J. Procedures in Affected Herds

Disclosure of tuberculosis in any herd shall be followed by a complete epidemiologic investigation. All cattle in herds from which tuberculosis cattle originate and all cattle that are known to have associated with affected cattle shall be tested promptly. These procedures shall apply to adjacent and contact herds as well as to the evaluation and testing of possible source herds for the affected herd. Herds that have received exposed animals shall be tested following the slaughter or testing of exposed animals. Every effort shall be made to assure the immediate elimination of the disease from all species of domestic livestock on the premises. The first consideration in affected herds is the depopulation of the entire herd. If depopulation cannot be accomplished, the herd shall be handled as outlined under Section N, Quarantines.

A motion was made and passed for its adoption.

J-A Procedures in Tuberculosis Infected Feedlot

A tuberculosis infected feedlot shall be handled in the same manner as an affected herd in regard to epidemiologic investigation and the development of epidemiologic tracings for animal movements into and out of the feedlot. Emphasis on investigation and testing shall be to detect possible spread from the feedlot. Cattle in feedlots known to be exposed to tuberculosis cattle shall be quarantined and shipped under permit directly to slaughter. Feedlot or portions of feedlots that have contained affected and exposed cattle shall be vacated, cleaned, and disinfected following the removal of such cattle to slaughter.

A motion was made and passed for its adoption.

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

The committee met at 9:00 a.m., Thursday, October 25, 1984, to conclude unfinished business.

Dr. Hosker opened the meeting explaining proposed changes in the indemnity program.

It was proposed that in order to gain greater program support through more participation by the industry and State governments and to extend present appropriations for indemnity payments to more herd owners, the Federal indemnity payments should be reduced. Four proposals were presented for a one-third sharing of present indemnity costs by participation of the individual States and the individual herd owners.

After considerable discussion it was moved to let the indemnity remain as it is presently, because industry people had no input into discussions and greater cooperation among herd owners would result if it was left as is.

The following amendment is proposed as a new item for addition to quarantine procedures when herds suspicious of tuberculosis are detected
as a result of slaughter traceback procedures. The finding of newly detected herds has continued to decrease in the last 10 years. As the end point of eradication approaches, more decisive action will hasten that date. It was stated at the recent tuberculosis conference that accelerated closing of traceback investigations would be counterproductive. However, the expeditious initial quarantining of suspicious herds would be productive in stopping the further dissemination of disease. The proposed amendment is underlined.

Part II, Recommended Procedures (Minimum Requirements)

N. Quarantine

6. Herds identified as the source(s) of slaughter traceback case investigations shall be placed under quarantine within 30 days of notification and a herd test scheduled.

This was adopted by the committee after much discussion.

After several proposals concerning infected Bison herds, it was decided to adopt the following amendment:

Include Bison throughout the UM&R with all the same criteria as for cattle except that state status would not be affected by the presence of *M. bovis* infected Bison herds until January 1, 1987.

Dr. J. G. Flint of Minnesota was commended for twenty-five years of faithful dedication.

The meeting was adjourned at 11:00 a.m.

REPORT OF SUBCOMMITTEE ON TUBERCULOSIS AND PARATUBERCULOSIS REGARDING EMBRYO TRANSPLANT (ET)

Sub-Committee on Embryo Transplant (SCET) is in beginnings of establishing uniform hygiene methods. Logically, TB-Paratuberculosis Committee is the group from which advice will be sought by SCET on TB and Para TB in their effort to establish uniform hygiene methods for ET.

We are here, of course, talking about the EMBRYO itself. Once an EMBRYO is placed into a recipient for transport, any hygiene question relates to the recipient, i.e., a live animal.

There are several biological principles which need to be taken into consideration when contemplating the attainment of the goal of SPF embryos for transfer/transport.

1) The nature of ovum development is such that as long as *Zona pel-lucida* is intact, ovum/blastocyst cannot become infected, especially by bacteria.

   — The source of any ZP surface contamination by *M. bovis* (or any other agent) is the internal genital tract via the flushing fluid.

2) State-of-the-art ET flushes ZP intact embryos from donor 6–7 days post estrus.

3) Washing of ZP intact embryos with sterile flushing fluid can remove all micro organisms especially bacteria, which might be contaminating ZP surface.
4) A unique opportunity also exists for meaningful culture of an embryo's environment (flushing fluid) for pathogens.

Summary

ET, itself, can provide a screen against genital transfer of pathogens.

- It would, seemingly, be a travesty of sorts to NOT avail ourselves of these biological security factors in any recommendations the TB/Johne's might make towards ET hygiene methods regarding TB and Para TB.

SEPARATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FATTY ACIDS FROM M. PARATUBERCULOSIS

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School of Veterinary Medicine, Oregon State University
Veterinary Services, Oregon Department of Agriculture

Summary

Johne's disease or paratuberculosis is a chronic debilitating bacterial infection of cattle and a major disease concern of the dairy industry. There have been multiple approaches to diagnosis of the disease, but present tests are either insensitive, unreliable or take too long, i.e., months. A new approach utilizing high performance liquid chromatography to separate and identify the unique pathognomonic long chain fatty acids of the causative agent, Mycobacterium paratuberculosis is being developed. The long term objective is to be able to extract and identify the presence of the organisms direct from fecal samples. An extraction and fluorescent tagging protocol has been developed. Preliminary trials utilizing several strains of Mycobacterium sps. have verified the feasibility of the procedure.

Introduction

Paratuberculosis or Johne's disease has become increasingly widespread in recent years, especially in dairy cattle and goat herds. Paratuberculosis is a very costly disease and once it is established in a herd, eradication is a lengthy process. Oregon Department of Agriculture estimated a 29 million dollar loss within the state last year. Two other states have reported losses between 37 and 52 million dollars per year. Moreover, because no effective diagnostic test exists the true incidence of the disease is speculative and hard to quantitate. If corrective measures are not instituted to check the spread of the infection within a herd, eventually almost all of the animals will be affected (Withers, 1959).

The diagnosis of clinical Johne's disease in a herd is difficult and with present knowledge, identification of carrier animals is almost impossible. Historically, the methods of diagnosis either involved clinical examination, skin testing, serological tests, and/or histopathological examination of intestinal samples obtained through a pinch biopsy or necropsy. Clinical examination and skin tests can suggest but not definitively diagnose the disease. Serologi-
cal complement-fixation tests have problems with false positives, especially in animals infected with the common bacterium *Corynebacterium renale*. Most of the serological and hypersensitivity tests devised for similar bacteria have proved unsatisfactory as a diagnosis test for paratuberculosis. Culture of *M. paratuberculosis* from feces was the most sensitive and reliable test, but weeks and sometimes months were required before definitive results can be reported.

Recently, other researchers have used gas chromatography for differentiation of microorganisms growing in vitro. This test was found to be extremely sensitive for detection of several mycobacterial metabolites. More recently liquid chromatography has been used for the separation of components of the bacterial cell wall. Mycobacterium sps. have unique long chain fatty acids in their cell walls that are effectively separated on HPLC. The cell wall structure has been demonstrated by freeze etching (Fig. 1). The mycolic acids in the middle layer are comprised of the long chain fatty acids. These long chain fatty acids comprise approximately 55% of the total amount of fatty acids in the Mycobacterium. The remaining forty-five percent are short chain fatty acids common to many bacteria. Recently the alpha, beta and methoxy mycolic fatty acids from *M. tuberculosis* H37Ra were able to be separated by a HPLC protocol. Figure 2 demonstrates the separation of these three classes of compounds. Note that there is between three and five peaks representative of each class.

In this paper we report on the current developmental stage of a new rapid diagnostic test that could determine both clinical and subclinical infected animals. This new test takes advantage of the unique long chain fatty acids found within the cell wall of the paratuberculosis bacterium. These fatty acids have been extracted from organisms present in culture medium and separated by high performance liquid chromatography (HPLC). The long chain fatty acids from *M. tuberculosis* and *M. paratuberculosis* have been tagged with a new fluorescence compound. The chromatograph fingerprint created by the unique fatty acids of the cell wall not only identify the genus but also the species of mycobacterium. This test, though in its infancy, could also prove to be a new method for identifying the bacteria grown in cultures.

**Materials and Methods**

Several types of Mycobacterium species were used for this study. They include *M. tuberculosis*, strain H37Ra, *M. paratuberculosis*, strain 21, and a wild strain of *M. paratuberculosis* isolated from Eastern Oregon. These latter two strains were obtained from Dr. Merkel (National Animal Disease Laboratory) and Oregon State Department of Agriculture. All cultures were grown in Hanson's media with Micobactin J added. Cells were harvested by centrifugation and due to the slow growth of these organisms, population densities were not adjusted.

A 4 ml sample of the culture was saponified for 20 minutes using a 5% KOH solution. Saponification was necessary in order to separate the hydrogen bonding between adjacent pairs of mycolates. This basic solution of Mycobacteria was acidified to pH 1 using HCl. By changing the solution to an acid pH, the carboxylic acid groups were protonized. The fatty acids
were then extracted three times with diethyl ether. The 10 ml of pooled extract was then dried over nitrogen.

The second step was to derivatize the carboxylic group with 4-bromo-7-methyl-2-methoxy coumarin. The coumarin moiety was used as a fluorescent tag in the separation of the long chain fatty acids by HPLC. The procedure consisted of reconstituting the dried extract in 5 ml of benzene. Then this benzene solution was gently heated and allowed to react for one hour. One ml of that solution was then applied to a silica SEPPAK; washed with 5 ml distilled water and extracted with 2 ml of methanol. The effluent was collected and run on reverse phase HPLC. The reverse phase consisted of a gradient elution of the fatty acids by a mobile phase of 45 to 60% chloroform with the remaining percentage of solvent being acetyl nitrile. This gradient was run over a time interval of 60 minutes.

The fluorescence of the coumarin was excited at 285 nm and the emission measured a 340 nm. The use of fluorescence eliminated a number of UV interfering contaminants; moreover, fluorescence emission is specific for coumarin and thus for the fatty acids.

The instrumentation used for this separation was as follows: HPLC consisted essentially of two Beckman 100A pumps for gradient elution, a model 300 microprocessor, a model 401 autoinjector and CR1A integrator. Fluorescence was measured by a Perkin-Elmer LH-4 spectrofluorometer. A summary flow diagram of the extraction technique is seen in Figure 3.

Results and Discussion

Figure 4 illustrates the gradient elution of *M. tuberculosis*, H37Ra. The major components of mycolic acids are eluted between 40 and 60 minutes. The five major peaks found in this time period represent long chain fatty acids with chain lengths that range from C48 to C76. These peaks were consistent in elution time and in the relative proportion to each other with multiple cell type sampling. This separation compares with that found by Takayama21 in the same bacterium species. However, some eluting peaks in these previous separations have been further resolved, resulting in a larger number of separated mycolic acids.

The solvent gradient used did not separate other fatty acid peaks still coeluting with similar carbon chains. The peaks eluted at 43 minutes and at 46 minutes have at least 2 subspecies of mycolic fatty acids contained within. Further identification of these peaks using gas chromatography-mass spectrometry (CG-MS) has not been done.

Figures 5 and 6 illustrate the multiple peaks of *M. paratuberculosis*, strain 21 and wild strain respectively. In Fig. 5 the three major peaks are found with averaged elution times tending to be longer than those seen in Fig. 4 with the *M. tuberculosis* strain H37Ra. Although exact molecular structures were not determined, longer mycolic acids are indicated. Total number of peaks including minor components are more numerous when compared to strain H37Ra.

In Figure 6, elution patterns of the wild isolate seen also indicate a more complex structure and longer chain length of mycolic acids of this bacterium compared to that of *M. tuberculosis*. In this separation a large
number of peaks with short retentions can be identified. These represent short chain fatty acids (C₈–C₂₂) and other molecules that were derivatized by the procedure. A clean up procedure utilizing a SEPPAK C₁₈ column was used to eliminate these nonessential compounds in Figures 4 and 5. The gradient elution program can separate most of the fatty acids; however, some overlap of different peaks does exist and improvement of the separation gradient is still developing.

A major improvement with this new technique is the derivatizing of the carboxylic moiety with the fluorescent (and absorbant) coumarin rather than the phenylacetyl ester previously used. Figure 7 is a plot of both the linearity and the improved sensitivity of the coumarin tag as compared to the phenylacetyl ester tag. Coumarin has a linearity range that includes the higher concentrations as well as the more sensitive lower limits. The improved sensitivity is essential if HPLC separation of fatty acids is to be used as a diagnostic test for Johne's. Assuming that 40% of the fatty acids in the cell wall are of the long chain form, calculations have shown HPLC can determine quantities as low as $2 \times 10^2$ cells/gram. This sensitivity is more than adequate to isolate the bacteria from positive fecal samples.

At present, with our limited number of separations completed, it is evident that different Mycobacterium species will give unique separation patterns. The next objective is to identify and characterize the components of the various peaks separated by HPLC utilizing GC-MS. This information derived from known pure cultures will be catalogued for each species of Mycobacterium paratuberculosis and would comprise a library of fingerprints for these organisms.

The final step is to use this procedure to isolate *M. paratuberculosis* from fecal samples. Known positive samples will be tested initially. The possible presence of saprophilic Mycobacterium species in feces will be investigated. If present, they will have to be quantitated and their role in peak formation further defined.

This assay for rapid diagnosis of Johne's disease holds great promise. It is rapid, taking only hours to complete. It is economically feasible, costing less than a dollar per sample in an equipped laboratory. It is highly selective for the bacteria in question, thus false positives would be minimal. And finally, it shows promise to be sensitive enough to be able to detect subclinical carriers shedding the organism.

**REFERENCES**

REPORT OF THE COMMITTEE


Superficial lipids (Mycosides, cord factor, sulpholipids)

Mycolic acid

Arabinogalactan

Peptidoglycan

Cell membrane

Figure 1: The cell wall structure of Mycobacterium sp.
C18 Column  
p-dioxane: ACN  
(11:9, V/V)  

Takayama  

Figure 2: Three classes of long chain fatty acids separated on TLC solid by HPLC.
Figure 3: Flow diagram of extraction technique of M. paratuberculosis.
Figure 4: Gradient elution of *M. tuberculosis*, strain H37Ra, C$_{18}$ reverse phase 45–60% CHC$_3$ (in ACN).
Figure 5: Gradient elution of *M. paratuberculosis*, strain 21, C$_{18}$ reverse phase 45–60% CHCl$_3$ (in ACN).
Figure 6: Gradient elution of *M. paratuberculosis*, wild strain, C18 reverse phase.

HPLC Separation of
*Mycobacterium Paratuberculosis*
*Mycolic Acids*

C18 Column
45-60% CHCl₃ (in ACN)
over 50 min
Figure 7: Linearity and sensitivity comparison of fluorescence versus absorbance.

M. tuberculosis long chain fatty acids

○ modified by 4-bromomethyl-7-methoxycoumarin, C36:0
△ modified by phenacyl ester

Excitation $\lambda = 322$ nm
Emission $\lambda = 390$ nm
## REPORT OF THE COMMITTEE
### SLAUGHTER INVESTIGATIONS
#### FY 1979 - 1984

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JOHNE'S SUBCOMMITTEE MEETING

The Johne's subcommittee meeting was convened at 1:35 p.m. Members present were Drs. J. G. Flint, Glen Hoffsis, A. R. McLaughlin, and S. S. Hurley.

Dr. Bob Jones from Pat Brennan's lab at Colorado State University discussed the possibility of chemically synthesizing antigens specific for *Mycobacterium paratuberculosis* as has been accomplished with *M. leprae*. They have purified what they feel is an oligosaccharide sidechain unique to *M. paratuberculosis* strain 18 and are further investigating the possibility of using this antigen for ELISA testing.

Dr. Maurie Craig at Oregon State University reported on the approach that his research is taking to identify *M. paratuberculosis*. Mycolic fatty acids are separated by lipid chromatography and the resulting "fingerprint" may be useful in separating *M. paratuberculosis* from other mycobacterial species.

Dr. Sarah Hurley of the University of Wisconsin gave a brief report on her work to develop a DNA hybridization probe which would identify *M. paratuberculosis*.

Dr. Robert Whitlock gave a report on the Pennsylvania Johne's program. They have just completed the first phase of a two part prevalence and economic impact assessment. In the first phase 1500 cows were sampled from a slaughter plant killing 1100 cows/day. Serum, fecal samples, ileocaecal nodes and valves and rectal ileal, and jejunal biopsies were collected from each of the animals. The serum will be used to run AGID, CF, ELISA, CIE and EF. It appears that 4–5% of the animals samples are culture positive on one or more tissues. Tracebacks have been made to the herds from which culture positives have come and management information is being collected.

The second phase of the program will involve the random selection of Pennsylvania herds, evaluation of their Johne's disease status and then comparative evaluations of infected and uninfected herds.

Ada Mae Hintz from NADC presented a preliminary report on the nationwide Johne's survey being conducted by ARS and Meat Inspection. These results indicate a positive culture on approximately 2% of the ileocaecal lymph nodes cultured. A more detailed report will be given to the full Committee on Tuesday.

Several recommendations were approved for presentation to the full committee. These are as follows:

1. The code of Federal Regulations, part 71, should be amended to remove restrictions on the movement of Johne's vaccinates in interstate commerce. THE MOTION WAS APPROVED UNANIMOUSLY.

2. It is recommended that a staff officer position should be created within APHIS. Cattle Diseases to deal specifically with Johne's disease. THE MOTION WAS APPROVED UNANIMOUSLY.
The intention of this recommendation is not to embark upon a grandiose control plan but simply to provide an individual to work on regulatory change and public education.

3. The Code of Federal Regulations, Part 71, should be amended to allow cattle exposed to Johne's disease to move interstate after the herd of origin has been tested by an approved protocol and found negative. THE MOTION WAS APPROVED UNANIMOUSLY.

4. Procedures and funding should be established to approve laboratories to conduct Johne's testing and to standardize antigens, other reagents, and protocols for use in conducting these tests. The motion was approved unanimously.

5. A letter should be sent to Dr. Earl Splitter, USDA, ARS, to request that Johne's disease continue to be listed as a specific priority item for funding by the Special Competitive Grants. THE MOTION WAS CARRIED UNANIMOUSLY.

TUBERCULOSIS IN IMPORTED MEXICAN FEEDER STEERS

The concern of the Bovine Tuberculosis Eradication Program is two-fold in regard to Mexican feeder steer importations. Most of these steers do not directly enter a feedlot, and secondly, fed cattle are not identified in the same manner as adults. A problem that the investigator of slaughter traceback of feedlot cases must always address is, "Did this steer originate from a United State breeding herd?"

These steers are grazed in many States other than those that border on Mexico. When on pasture, there is a risk by association with native cattle herds. Some steers are selected out of feeder lots and used on the rodeo circuit as roping steers. These steers travel extensively and contact other livestock before eventually entering feedlots. Another risk is the sale of "poor doers" that may have tuberculosis from feedlots that may enter family type feeding operations.

The collection of suspicious lesions from fed cattle is necessary and in one recent case led to the identification of an infected herd in West Virginia. These feedlot cases each generate costly laboratory procedures for histopathology and bacteriology.

The enclosed chart indicates the findings from slaughter cases for the last 6 years. The number of cases of Mexican origin for FY 1984 is estimated since most of these are still under investigations. A total of 149 slaughter traceback cases of feedlot origin were investigated in the period FY 1979–1983, of which 106 or 71 percent were of Mexican origin. In summary, Mexican steers appear to be responsible for 40 percent of slaughter investigations and 60–75 percent of feedlot investigations.

The Committee on Tuberculosis and Johne's Disease in 1983 went on record as supporting a cooperative agreement between Mexico and the United States to enter into a joint effort that would eventually lead to the eradication of bovine tuberculosis from the country of Mexico.

The risk of tuberculosis from Mexico is not only from imported feeders but from the accidental and deliberate introduction of infected breeding
animals. *M. bovis* has been confirmed in stray cattle and in those smuggled into the United States.

A meeting held with Mexican animal health officials should consider:

1. Exchange of technical information by laboratory and field personnel. That is, laboratory training in culture techniques and training in field testing procedures and guidelines.

2. An import requirement that steers be exported only from herds that are accredited free. These herds might be jointly tested by United States and Mexican teams. (This would be more than the present system with Mexican accredited herds).

3. A cooperative effort (such as a joint commission) to reduce the level of infection in Mexican border States and in the chief exporting Mexican States such as Chihuahua.

Alternative for the United States to unilaterally consider may be as follows:

1. Prohibit entry of Mexican steers.

2. Obtain the authority for postentry quarantine and postentry re-testing. (Such as permit to quarantined feedlots only).

3. Retest of steers at the port of entry by United States personnel and rejection of reactor lots of steers.

4. Identification of imported steers:
   a. **External** – Hot branding. This would assist in identification of the live animals.
   b. **Internal** – Dye marking. This would assist the inspector on the kill floor.

The anticipated meeting of animal health personnel of both countries at this year's USAHA should be fruitful even if it is only the first step in dealing with the long time problem of infected feeder steers.
BLUETONGUE IN CALIFORNIA'S WILD RUMINANTS: DISTRIBUTION AND PATHOLOGY

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B. I. Osburn, DVM, PhD**
W. P. Heuschele, DVM, PhD***

ABSTRACT

Between 1978 and 1984 more than 2,000 serum samples were collected from California's resident black-tailed and mule deer (Odocoileus hemionus sp.), tule elk (Cervus elaphus nannodes), Roosevelt elk (C.e. roosevelti), pronghorn antelope (Antelocapra americana), and three races of bighorn sheep (Ovis canadensis sp.) and analyzed for agar gel precipitating (AGP) antibodies to bluetongue (BT) virus. AGP and serum neutralization (SN) tests for antibodies to epizootic hemorrhagic disease (EHD), a closely-related orbivirus of deer, were also conducted on many samples. Parallel virus isolation procedures yielded all four international serotypes of BT10,11,13,17 and one serotype of EHD.1 The herds sampled were representative of those found in various habitats under various management schemes in California. An attempt was made to clarify the influence of temperature, weather, location, season, adjacency to livestock and other factors potentially influencing rates of infection. Several disease syndromes associated with isolation of bluetongue virus and/or bluetongue antibodies were discovered. This is the first attempt to quantitate the levels of orbivirus infection and estimate their impact on California's native wildlife.

INTRODUCTION

Bluetongue (BT) and Epizootic Hemorrhagic Disease (EHD) are closely-related, frequently fatal or debilitating diseases of North American domestic and wild ruminants. Both orbiviruses are transmitted within and between species by blood feeding culicoid gnats. In South Africa, BT was reported as early as the 1870s in European breeds of sheep.1,3,7,19,20 Wild ruminants were suspected to be resevoir hosts, as no clinical disease was recognized in them.3 In the 1930s atypical BT lesions in South African sheep and cattle were distinguished from foot and mouth disease.3 Names such as “sore muzzle”, “necrotic” or “mycotic stomatitis”, “blacktongue”, “hemorrhagic septicemia” and “pseudo-foot-and-mouth disease” have been used to describe the disease in domestic livestock. The term “hemorrhagic disease” has been used to describe peracute and acute BT and EHD in wildlife.7,11

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In the United States, BT was first described in domestic sheep in the 1940s and the virus was isolated in California in 1953. The susceptibility of white-tailed deer was noted in 1955. That same year EHD virus was isolated from white-tailed deer in Michigan and New Jersey. Several times since then, most recently in the early 1970s, BT and EHD epizootics in the western United States resulted in major losses of white-tailed deer, mule deer, antelope, and bighorn sheep. Both viruses are believed to be endemic now in white-tailed deer in the southern states. BT antibodies have been found in native buffalo (Bison bison), moose (Alces alces), and caribou (Rangifer tarandus), but no association with disease was noted.

Bluetongue virus has been isolated from 10 species of exotic ruminants and was believed to have caused fatalities in 8 of those. A recent survey for BT agar gel precipitating antibodies in North American zoo animals reported positive results in 22% of 1,900 animals and increased to 85 the number of seropositive species of 102 tested.

Acute fatal BT or EHD infection ("hemorrhagic disease") in adult white-tailed deer is manifested as fever, rapid difficult breathing, swollen cyanotic tongue, excessive salivation, serous to catarrhal nasal exudate, and occasionally, bloody diarrhea. Animals that do not die acutely show lameness and coronary band lesions for a week or more following beginning of convalescence. Heavy overgrowth of the hoof follows, and from 1 to 7 months elapse before deer regain their original condition. Secondary bacterial infection of the coronary band, soft tissues of the leg, hooves, tongue, palate, gums and rumen often follow nonfatal viremia. These sequelae are similar to "footrot" or "Modoc mud disease" reported in mule deer in California.

White-tailed deer fawns have been shown to be susceptible to fatal BT infection following decline of colostral antibodies. Abortion and neonatal weakness following persistent maternal infection is reported in Rocky Mt. elk. Persistent subclinical infection has been reported in cattle, elk, pronghorn, and captive exotic animals. Primary viral pneumonia and secondary bacterial bronchopneumonia have been reported to result from BT infection of bighorn sheep and pronghorn antelope. The pathogenesis of these lesions has not been fully explained.

Bekker first pointed out the consistency of the locations of lesions in sheep, being areas of mechanical stress; lower lips opposite incisors, dorso-lateral aspect of the tongue opposite molar teeth and the muscular pillars of the rumen, esophageal groove and pylorus. Immunofluorescence and histopathological techniques revealed that BT virus had an affinity for endothelium, periendothelial cells, pericytes of capillaries, precapillary arterioles and venules in sheep and white-tailed deer. Maximum virus specific fluorescence was observed in deer in small vessels underlying stratified squamous epithelium, and in reticuloendothelial cells of lymph nodes draining the head, and was not observed in epithelial cells. Endothelial cell necrosis, fluid leakage, and regenerative hyper-
plasia resulted in vascular occlusion causing hypoxia in the overlying epithelium. Severity of secondary lesions were influenced by mechanical factors and secondary infection. Although not mentioned in reviews of BT pathogenesis, exposure of endothelial collagen could result in acutely fatal coagulation defects and explain lesions of “hemorrhagic disease”.

MATERIALS AND METHODS

From 1978 until 1981 both bluetongue agar gel precipitin antibody testing and virus isolation attempts were conducted at University of California, Davis, by techniques described elsewhere. Prior to 1978, and from 1981 through 1983, this same work was conducted at the California Department of Food and Agriculture (CDFA) laboratory at Fresno which also conducted serum neutralization and agar gel precipitin testing for EHD antibodies. In 1984 AGP testing for both BT and EHD antibodies were conducted at the San Diego Zoological Park by methods described by Jochim. All locations used similar, but not identical techniques for agar gel immunodiffusion tests. All initial virus isolation attempts were from sonicated blood, inoculated intravenously into 10–11 day old chicken eggs. CDFA laboratories preferred clotted blood to washed cells for virus isolation.

In this study, some deer from which BT virus was isolated had only a short-lived and weak AGP antibody response. Although various authors have noted that the group specific BT agar gel precipitating (AGP) antibodies do not always appear following documented bluetongue infection the AGP remains the most practical antibody test for mass screening procedures. There is some cross-reactivity between BT and EHD in the AGP test. It is insensitive, thus incidence of infection derived must be considered as conservative.

With the exception of California mule deer collected in Santa Barbara County for a case control study of association between bluetongue exposure and lesions of ‘footrot’, all samples collected were samples of convenience. Most antelope and deer samples were from hunter-kills and thus were older males. All bighorn and elk and some deer and antelope were sampled subsequent to capture for relocation, and thus were primarily females and young animals. Carcasses routinely submitted to the Wildlife Investigations Laboratory were necropsied. When post-mortem lesions suggestive of BT or EHD were noted, blood and/or spleen were submitted for virus isolation in buffered physiological saline.

RESULTS

Prevalence of BT and EHD AGP antibodies in pronghorn antelope can be found in Table 1. Because sample size was smaller and less consistent for countries other than Modoc, only the results by year from Modoc County are presented on Graph 1.

Prevalence of BT and EHD AGP antibodies in bighorn sheep are presented by subspecies in Table 2. The only population of bighorn consistently showing both BT and EHD antibodies were those in the Santa
Prevalence of BT AGP, EHD AGP antibodies in tule elk and Roosevelt elk are presented in Table 3. The seasonal percentages of BT antibodies in tule elk in the Owens Valley are presented in Graph 3.

Prevalence of BT AGP, EHD serum neutralizing and EHD AGP antibodies in black-tailed deer are presented in Table 4, and those for the races of mule deer in Tables 5, 6, and 7.

The percent of BT AGP positives for each species are shown on a rough species distribution map of California in Figure 5.

DISCUSSION

The prevalence of bluetongue antibodies in wild ruminants in California shows a distinct southern and eastern distribution (Figure 5). Coastal, high mountain and very arid habitats show lowest BT exposure rates. These areas correspond with absence of gnat breeding habitats.

Antelope: Antelope in Modoc County appear to be consistently and frequently exposed to bluetongue. Over 200 samples were collected in six years during the last week in August, the height of seasonal bluetongue occurrence in northeastern California. Just over 40% were positive. The antelope blood samples collected yearly in Modoc County represent between 1% and 2% of the population in that county. Yearly fluctuations of BT AGP antibodies are apparent in Figure 1; BT prevalence was highest during the drought years of 1978 and 1979.

Bluetongue viruses have been isolated from apparently normal hunter-killed antelope (Table 1). The significance of this is unknown. Clinical bluetongue was not recognized in California antelope.

The most likely cause of yearly fluctuations in BT exposure are factors influencing gnat populations, such as altitude, rainfall and ambient temperature, and factors influencing rate of transmission, such as combined livestock and antelope numbers, and proximity between species. It should be noted that the highest BT antibody prevalence rates occurred during the driest years 1978 and 1979.

Of all wildlife species sampled in this study, antelope in Modoc County have the closest contact with range cattle, particularly at water sources in late summer. These water sources are warm, stagnant, or fecal-contaminated, and are ideal for reproduction of the culicoid gnats which transmit BT and EHD. Antelope have limited contact with deer which are at higher elevations during summer. The deer adjacent to antelope ranges consistently show lower incidence of BT AGP antibodies (Table 6).

Bighorn Sheep: Bluetongue does not appear to be active in bighorn sheep in the Sierra Nevada mountains or in the Mojave Desert, the incidence of AGP antibodies being nil. In 1980 and 1982, approximately 10% of the entire Sierra Nevada population was sampled. The significance of EHD antibodies in these bighorn in 1980 is unknown. These bighorn winter at approximately 6,000 feet, depending on snow line and summer at
Table 1. Prevalence of Bluetongue and Epizootic Hemorrhagic Disease AGP Antibodies in Pronghorn Antelope in California

<table>
<thead>
<tr>
<th>COUNTY</th>
<th>DATE</th>
<th>BT AGP+</th>
<th>PERCENT</th>
<th>EHD AGP+</th>
<th>PERCENT</th>
<th>VIRUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modoc</td>
<td>8/78</td>
<td>25/30</td>
<td>83</td>
<td>ND</td>
<td>ND</td>
<td>(1)BT-10</td>
</tr>
<tr>
<td>Lassen</td>
<td>&quot;</td>
<td>6/6</td>
<td>100</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Modoc</td>
<td>8/79</td>
<td>14/16</td>
<td>88</td>
<td>&quot;</td>
<td>&quot;</td>
<td>(1)BT-17</td>
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<tr>
<td>Lassen</td>
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<td>1/4</td>
<td>25</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Modoc</td>
<td>8/80</td>
<td>4/23</td>
<td>17</td>
<td>4/5</td>
<td>80</td>
<td>--</td>
</tr>
<tr>
<td>Lassen</td>
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<td>0/1</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>Siskiyou</td>
<td>&quot;</td>
<td>0/7</td>
<td>0</td>
<td>0/2</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Modoc</td>
<td>8/81</td>
<td>5/20</td>
<td>25</td>
<td>1/21</td>
<td>5</td>
<td>--</td>
</tr>
<tr>
<td>Siskiyou</td>
<td>3/82</td>
<td>1/23</td>
<td>4</td>
<td>0/24</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Modoc</td>
<td>8/82</td>
<td>18/31</td>
<td>58</td>
<td>3/33</td>
<td>9</td>
<td>--</td>
</tr>
<tr>
<td>Modoc</td>
<td>8/83</td>
<td>10/43</td>
<td>23</td>
<td>9/43</td>
<td>21</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>6 yrs</td>
<td>84/204</td>
<td>41</td>
<td>17/133</td>
<td>2</td>
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</tr>
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</table>

Figure 1. AGP Antibodies to Bluetongue and EHD in Pronghorn Antelope in Modoc County, California

%AGP +

75%
50%
25%
Table 2. Prevalence of Bluetongue and Epizootic Hemorrhagic Disease AGP Antibodies in Bighorn Sheep in California

<table>
<thead>
<tr>
<th>HERD</th>
<th>COUNTY</th>
<th>DATE</th>
<th>BT+</th>
<th>EHD+</th>
<th>ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. Baxter</td>
<td>Inyo</td>
<td>3/79</td>
<td>0/9</td>
<td>0/9</td>
<td>0</td>
</tr>
<tr>
<td>Lava Beds</td>
<td>Modoc</td>
<td>2/80</td>
<td>0/5</td>
<td>1/2</td>
<td>50</td>
</tr>
<tr>
<td>Mt. Baxter</td>
<td>Inyo</td>
<td>3/80</td>
<td>0/30</td>
<td>12/26</td>
<td>46</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>3/82</td>
<td>0/21</td>
<td>0/21</td>
<td>0</td>
</tr>
<tr>
<td>(Ovis canidensis californica)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joshua Tree</td>
<td>Riverside</td>
<td>7/78</td>
<td>0/2</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Death Valley</td>
<td>Inyo</td>
<td>8/80</td>
<td>0/4</td>
<td>0/4</td>
<td>ND</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>8/81</td>
<td>0/3</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Kofa</td>
<td>(Arizona)</td>
<td>11/81</td>
<td>0/10</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Old Dad</td>
<td>San Bernardino</td>
<td>8/83</td>
<td>0/11</td>
<td>0/11</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>12/83</td>
<td>1/16</td>
<td>0/16</td>
<td>0</td>
</tr>
<tr>
<td>Marble Mts.</td>
<td>&quot;</td>
<td>8/83</td>
<td>0/10</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>San Gabriel</td>
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<td>12/83</td>
<td>0/9</td>
<td>0/9</td>
<td>0</td>
</tr>
<tr>
<td>Mts.</td>
<td>&quot;</td>
<td>0/23</td>
<td>0</td>
<td>0/23</td>
<td>0</td>
</tr>
<tr>
<td>(Ovis canidensis nelsoni)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Santa Rosas</td>
<td>Riverside</td>
<td>1-5/77</td>
<td>7/13</td>
<td>54</td>
<td>4/8</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>4/81</td>
<td>5/13</td>
<td>38</td>
<td>9/13</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>11/81</td>
<td>7/14</td>
<td>50</td>
<td>11/13</td>
</tr>
<tr>
<td>Living Desert</td>
<td>&quot;</td>
<td>2/83</td>
<td>0/6</td>
<td>0</td>
<td>4/6</td>
</tr>
<tr>
<td>Santa Rosas</td>
<td>&quot;</td>
<td>5/83</td>
<td>11/20</td>
<td>55</td>
<td>0/20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30/66</td>
<td>45</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 2. AGP Antibodies to Bluetongue and EHD in Peninsular Bighorn Sheep in Riverside County, California

%AGP +

75%

50%

25%

<table>
<thead>
<tr>
<th>BT</th>
<th>EHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>APRIL - NOVEMBER 1980</td>
</tr>
<tr>
<td>1983</td>
<td>1983</td>
</tr>
</tbody>
</table>
Table 3. Prevalence of Bluetongue and EHD AGP Antibodies in California Elk

<table>
<thead>
<tr>
<th>HERD</th>
<th>COUNTY</th>
<th>DATE</th>
<th>BT AGP</th>
<th>% AGP</th>
<th>% EHD</th>
<th>VIRUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owens Valley</td>
<td>Inyo</td>
<td>11/77</td>
<td>25/26</td>
<td>96</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/78</td>
<td>17/23</td>
<td>74</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10/78</td>
<td>22/26</td>
<td>85</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td></td>
<td>11/78</td>
<td>20/25</td>
<td>80</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/78</td>
<td>10/11</td>
<td>91</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>San Luis Is1</td>
<td>Merced</td>
<td>3/78</td>
<td>7/8</td>
<td>83</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tupman</td>
<td>Kern</td>
<td>12/78</td>
<td>8/8</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>San Luis Is1</td>
<td>Merced</td>
<td>11/79</td>
<td>18/20</td>
<td>90</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pt. Reyes</td>
<td>Merced</td>
<td>5/12/59</td>
<td>6/15</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>Concord</td>
<td>Contra</td>
<td>11/79</td>
<td>0/4</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Owens Valley</td>
<td>Inyo</td>
<td>10/79</td>
<td>14/38</td>
<td>37</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/81</td>
<td>15/33</td>
<td>45</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total Inyo County</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total All Tule Elk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Roosevelt Elk (Cervus elaphus roosevelti)

<table>
<thead>
<tr>
<th>HERD</th>
<th>COUNTY</th>
<th>DATE</th>
<th>BT AGP</th>
<th>% AGP</th>
<th>% EHD</th>
<th>VIRUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold Bluffs</td>
<td>Humboldt</td>
<td>3/82</td>
<td>0/16</td>
<td>0</td>
<td>0/16</td>
<td>0</td>
</tr>
<tr>
<td>Prairie Crk</td>
<td></td>
<td>11/83</td>
<td>0/4</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0/20</td>
<td>0</td>
<td>0/16</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3. Seasonal Fluctuations in Bluetongue AGP Antibodies in Tule Elk in Owens Valley of Inyo County, California

%AGP +

75%
50%
25%

Table 4. Prevalence of Bluetongue and EHD Antibodies in Black-Tailed Deer

<table>
<thead>
<tr>
<th>COUNTY</th>
<th>DATE</th>
<th>BT AGP</th>
<th>%+</th>
<th>EHD AGP</th>
<th>%+</th>
<th>EHD SN&gt;1:32 %+</th>
<th>ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monterey</td>
<td>9/79</td>
<td>11/29</td>
<td>38</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>9/80</td>
<td>22/48</td>
<td>46</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>9/81</td>
<td>28/42</td>
<td>66</td>
<td>ND</td>
<td>0/40</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>9/82</td>
<td>Pending</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>9/83</td>
<td>26/77</td>
<td>33</td>
<td>10/77</td>
<td>ND</td>
<td>13</td>
<td>--</td>
</tr>
<tr>
<td>Trinity</td>
<td>1-4/80</td>
<td>0/39</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>12/80</td>
<td>0/10</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>12/81</td>
<td>0/17</td>
<td>0</td>
<td>ND</td>
<td>0/17</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>12/82</td>
<td>0/17</td>
<td>0</td>
<td>0/7</td>
<td>ND</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Humboldt</td>
<td>9/81</td>
<td>0/10</td>
<td>0</td>
<td>ND</td>
<td>0/10</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Siskiyou</td>
<td>12/81</td>
<td>0/7</td>
<td>0</td>
<td>ND</td>
<td>0/7</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Shasta</td>
<td>9-10/83</td>
<td>0/17</td>
<td>0</td>
<td>2/17</td>
<td>ND</td>
<td>12</td>
<td>--</td>
</tr>
<tr>
<td>W. Tehama</td>
<td>1-4/80</td>
<td>1/5</td>
<td>20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>&quot;</td>
<td>1981</td>
<td>2/9</td>
<td>22</td>
<td>ND</td>
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</tr>
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<td>&quot;</td>
<td>4/82</td>
<td>2/17</td>
<td>12</td>
<td>ND</td>
<td>0/17</td>
<td>0</td>
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</tr>
<tr>
<td>&quot;</td>
<td>4/83</td>
<td>3/15</td>
<td>20</td>
<td>1/15</td>
<td>ND</td>
<td>6</td>
<td>--</td>
</tr>
<tr>
<td>Lake</td>
<td>3/81</td>
<td>1/5</td>
<td>20</td>
<td>3/5</td>
<td>ND</td>
<td>60 (2) EHD-1</td>
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</tr>
<tr>
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<td>Pending</td>
<td>ND</td>
<td>0/22</td>
<td>8/22</td>
<td>18</td>
<td>--</td>
</tr>
<tr>
<td>Santa Clara</td>
<td>1981</td>
<td>0/69</td>
<td>0</td>
<td>0/52</td>
<td>0/52</td>
<td>0</td>
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<tr>
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<td>1982</td>
<td>2/48</td>
<td>4</td>
<td>1/72</td>
<td>1/72</td>
<td>2</td>
<td>--</td>
</tr>
<tr>
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<td>1983</td>
<td>2/32</td>
<td>6</td>
<td>0/12</td>
<td>0/12</td>
<td>0</td>
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</tr>
<tr>
<td>Marin</td>
<td>9/81</td>
<td>0/134</td>
<td>0</td>
<td>0/30</td>
<td>0/30</td>
<td>0</td>
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</table>

Total 100/647 15

Figure 4. Case Control Study Design for Association Between Bluetongue AGP Antibody and Lesions of ‘Footrot’ in Deer

<table>
<thead>
<tr>
<th>Disease ('Footrot')</th>
<th>Exposure (Bluetongue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>A 14</td>
</tr>
<tr>
<td>-</td>
<td>C 1</td>
</tr>
<tr>
<td>+</td>
<td>B 8</td>
</tr>
<tr>
<td>-</td>
<td>D 5</td>
</tr>
</tbody>
</table>

Odds Radio $\frac{AD}{BC} = \frac{70}{8} = 8.75$
Table 5. Prevalence of Bluetongue and EHD Antibodies in Mule Deer in California (Odocoileus hemionus Californicus)

<table>
<thead>
<tr>
<th>COUNTY</th>
<th>DATE</th>
<th>BT</th>
<th>AGP+</th>
<th>% AGP+</th>
<th>SN&gt;1:32</th>
<th>% ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tulare</td>
<td>1980</td>
<td>6/10</td>
<td>60</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kern</td>
<td>4/81</td>
<td>0/10</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>6/81</td>
<td>0/6</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>9/81</td>
<td>11/109</td>
<td>10</td>
<td>ND</td>
<td>0/77</td>
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</tr>
<tr>
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<td>11/81</td>
<td>6/9</td>
<td>66</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>9/20</td>
<td>45</td>
<td>ND</td>
<td>1/20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>8/82</td>
<td>1/10</td>
<td>10</td>
<td>ND</td>
<td>1/11</td>
<td>8</td>
</tr>
<tr>
<td>Tulare</td>
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<td>0/11</td>
<td>0</td>
<td>ND</td>
<td>0/11</td>
<td>0</td>
</tr>
<tr>
<td>Monterey</td>
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<td>5/12</td>
<td>42</td>
<td>ND</td>
<td>5/12</td>
<td>42</td>
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<td>38/188</td>
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<td>ND</td>
<td>ND</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 6. Prevalence of Bluetongue and EHD AGP Antibodies in Rocky Mt. Mule Deer (Odocoileus hemionus hemionus)

<table>
<thead>
<tr>
<th>COUNTY</th>
<th>DATE</th>
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<th>AGP+</th>
<th>% AGP+</th>
<th>SN&gt;1:32</th>
<th>% ISOLATION</th>
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Table 7. Prevalence of Bluetongue and EHD Antibodies in Inyo and Burro Mule Deer

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<td></td>
<td>3/81</td>
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<td>75</td>
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<tr>
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Inyo Mule Deer

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Figure 5. Percent BT AGP Positive by Subspecies

Antelope 41%
California Bighorn 0%
Nelsons Bighorn 0%
Peninsular Bighorn 45%
Tule Elk 63%
Roosevelt Elk 0%
Black-tailed Deer 15%
Rocky Mountain Mule Deer 12%
California Mule Deer 20%
Burro Mule Deer 87%
Inyo Mule Deer 9%
elevations of approximately 11,000 feet. These altitudes would seem to preclude the possibility of gnat vectoring of orbiviruses. Most desert bighorn exist at lower elevations, but in extremely remote locations, separated by as much as 100 miles of desert from human habitation, livestock, and other susceptible wildlife.

The Peninsular bighorn of the Santa Rosa mountains appear to be consistently and frequently exposed to bluetongue with antibody prevalence rates averaging 45% of sampled animals. Two serotypes of bluetongue virus (11, 17) have been isolated from free-ranging pneumonic bighorn lambs (Table 2), and three (11, 13, 17) from captive adult ewes. One EHD virus isolation has also been made from a sick lamb. Pneumonia appears to affect lambs at an average age of two months during late winter and spring. Primary pneumonia resulting from bluetongue infection has been reported previously in bighorn sheep from Texas.12

Development of water for homes and golf courses may be influencing gnat vector populations near the Santa Rosa mountains. Studies of gnat populations in the adjacent Coachella Valley have shown an inverted seasonality. Gnats are present during winter, fall, and the spring lambing season but absent in the hottest summer months. The range of the Peninsular bighorn is less arid than that of the desert bighorn. Also seasonal cattle grazing occurs more frequently.

Elk: Tule elk in the Owens Valley appear to be consistently and frequently infected with bluetongue, 68% showing BT AGP antibodies. Antibody levels peak in December (Figure 3). All four serotypes of bluetongue have been isolated from these elk (Table 3), but only during October and November. It has been reported that like cattle, elk can maintain circulating bluetongue virus for long periods of time.16 The Owens Valley tule elk population fluctuates between 400–500 animals. Samples taken in 1977, '78, '79, and ‘81 are from 5%–7% of the total yearly population. The elk range is 3,800 to 4,000 feet in the floor of the valley. The Owens River and stock ponds offer ideal gnat breeding sites. Contact with beef cattle is often quite close. No clinical disease resembling bluetongue has been reported from tule elk.

It is interesting to note that tule elk in Kern, Marin and Merced counties show high incidence of BT AGP antibodies. The animals at the former two locations are also maintained at low elevations in historic river bottoms. All three populations are close to cattle. No evidence of BT or EHD activity was found in Roosevelt elk in Humboldt County.

Deer: Black-tailed deer from the northern forested and brushy mountains of California (Humboldt, Shasta, Trinity and western Siskiyou counties) have shown no incidence of bluetongue antibodies and little evidence of EHD antibodies (Table 4). Some cattle grazing and limited sheep grazing occur in this area. This can be contrasted to data from scrub pine and chaparral areas of the coastal ranges of Lake, Mendocino, and western Tehama counties where BT AGP antibodies occur in approximately 20% of black-tailed deer. Livestock grazing is comparatively heav-
ier in this area. Evidence of EHD activity was apparent in some locations. EHD Type 1 virus was isolated from the spleens of two healthy deer in Lake County in 1981. It should be noted that most black-tailed deer are not migratory with home ranges as small as one quarter mile; thus their antibody prevalence rates are probably a better indicator of local exposure than are those of migratory deer.

In 1982 and 1983 a number of deer carcasses with lesions suggestive of "hemorrhagic disease" were found in adjacent areas of Marin and Sonoma counties. In 1984 similar lesions were seen in black-tailed deer in eastern Tehama County. Dozens of decomposing deer carcasses were later found. Pulmonary edema was consistently found, and areas of necrosis on the soft palate, gums, dental pads or tongues were seen in all animals. Petechial and ecchymotic hemorrhages on serosal and mucosal surfaces and massive effusions of fluid into the pleural and, to a lesser extent, the peritoneal cavities were striking. The pleural effusion is quite similar to that seen in sheep with South African strains of bluetongue and with African horse sickness, another gnat transmitted orbivirus. Type 11 bluetongue viruses were isolated from blood and/or spleen of one deer from Sonoma and two deer from Tehama counties.

The prevalence of BT AGP antibodies in hunter-killed black-tailed deer from Camp Roberts and Fort Hunter Liggett in Monterey County is consistently in the range of 40% (Table 4). Some evidence of EHD activity was also apparent in 1983. Both sheep and cattle are grazed on these military bases. Deer numbers in adjacent areas of Monterey, San Benito, San Luis Obispo, and Santa Clara counties appear to be declining. These areas have traditionally suffered fall dieoffs of deer often ascribed to "footrot".

In summary, bluetongue and EHD exposure as measured by antibodies in black-tailed deer increased from north to south. Average ambient temperature generally increases and rainfall generally decreases from north to south. Interior coastal mountain ranges have more evidence of orbivirus activity than do ranges closer to the ocean. Some correlation with livestock use and incidence of BT and EHD antibody may exist, but this cannot be statistically confirmed.

Rocky Mountain mule deer in Modoc County have shown relatively low (12% mean) prevalence rates for BT and EHD antibodies (range 0%–22%) (Table 6). This stands in contrast with results for antelope in the lower grasslands sampled 30 days before the deer (Table 1). This may be the result of altitude, the dispersed nature of deer or the association of antelope with livestock watering ponds.

Migratory herds in Alpine County showed no evidence of exposure to either virus in 1983. An adjacent migratory herd from Mono County the previous year had a 9% prevalence of antibodies to BT. The higher elevation mountain pastures used by Rocky Mountain mule deer in Modoc, Alpine, and Mono counties in the summer are often heavily grazed by domestic sheep.
Inyo mule deer which migrate up the eastern slope of the Sierra Nevada in spring and winter in the Owens Valley, occupy an altitudinal gradient (4,000'—5,500') between tule elk and bighorn sheep about five months of the year. Prevalence of BT AGP positive serum samples was 9%, far less than tule elk, but greater than bighorn sheep. This may indicate that some exposure occurs at the lower altitudes near the Owens Valley floor, perhaps even in winter.

Exposure to bluetongue was very high in burro mule deer (87% AGP+) captured along the Colorado River in 1980 and 1981, despite being extremely isolated, unexposed to livestock and the lowest density deer population in the state. Apparently, deer density was less important than other factors such as low altitude, long warm season, and extensive gnat habitat along the Colorado River.

California mule deer captured in the Tehachapi mountains above Tejon Ranch in the spring and summer of 1981 showed no evidence of BT exposure (Table 5). Six of these animals were placed in captivity on the ranch adjacent to livestock. Gnat breeding habitat was created between the pens. During August and September, cattle, sheep, and goats in adjacent pens seroconverted, some sheep showed oral lesions of bluetongue and Types 11 and 17 virus were isolated. Eventually all deer became BT AGP positive, although two showed only transient seroconversion. Type 11 bluetongue virus was isolated from two of the deer. Concentration of susceptible host animals and creation of gnat breeding habitat appear to have facilitated transmission of BT.

That fall a sample of over 100 hunter-killed deer from higher elevations above the ranch showed only a 10% incidence of BT AGP antibodies. A Type 10 bluetongue virus was isolated from one hunter-killed deer. Antibodies and virus were generally in deer near, and believed to be using, stock ponds. EHD antibodies were not found in any deer in this study area.

In the fall of 1981–82, 'footrot' affecting approximately half of 200 resident California mule deer on a cattle ranch in Santa Barbara County was investigated. This was an overpopulated non-hunted herd which depended on nightly foraging in irrigated alfalfa fields. A special deer collection was instituted. Deer with swollen feet or sloughed hooves, lesions typical of 'footrot', and a small control group without lesions were shot. Serum was tested for AGP antibodies to bluetongue and blood and spleen were collected for virus isolation. Results of this study (Graph 4) revealed a much higher rate of exposure to bluetongue in cases than in controls (odds ratio 8.75). No bluetongue virus was isolated.

The deer population was reduced by 50% and all cattle were sold off. The following year (1982), 'footrot' did not occur and incidence of BT AGP antibodies in deer was greatly reduced. Cattle brought onto the ranch from a coastal area in the spring of 1982, following deer population reduction did not seroconvert for BT AGP antibody. This study seems to indicate: (1) an association between bluetongue infection and the disease called 'footrot' in California mule deer, and (2) an association between overpopulation
and concentration of deer and ‘footrot’.

It is interesting to note that in the 1950s ‘footrot’, which was believed to be bacterial in origin, was blamed for the deaths of thousands of mule deer. It was felt to be the most important disease of California deer. During approximately the same time period BT Type 10 was causing major losses of sheep. The lesions of ‘footrot’ cannot be reproduced in deer by the bacteria isolated from cases. Foot lesions in deer begin at the coronet, not in the hoof. These lesions and those of the oral mucosa, tongue and rumen are identical to those of nonfatal ‘hemorrhagic disease’ of white-tailed deer. ‘Footrot’ dieoffs have historically occurred around water sources in late summer and fall, killing does, fawns, and bucks in apparent good flesh in approximately equal ratios.

**SUMMARY**

This study seems to indicate a strong association between residence at lower altitudes, concentration about limited water sources, contact with livestock, and exposure to bluetongue. The picture for EHD is less clear. Migratory ruminants at high altitudes, those residing in cold, moist, or marine habitats, or isolated by deserts from gnat breeding sources and/or livestock appear to be less at risk of exposure.

Acute fatal bluetongue characterized by lesions of ‘hemorrhagic disease’ and chronic oral and hoof lesions of ‘footrot’ appear to result from some bluetongue infections of deer in California. Recent studies on captive mule deer indicate that abortion and neonatal deaths may result from bluetongue infection during the last trimester of pregnancy (Jessup, unpublished data). It is also apparent that quite frequently bluetongue infection of deer, as well as elk and antelope, may not result in recognizable disease signs.

Pneumonia in Peninsular bighorn lambs appears to be associated with bluetongue infection. Bluetongue virus has been isolated early in the course of disease from lambs. This pneumonia problem appears to be occurring only in a mountain range where evidence of bluetongue infection exists and not in ranges where it does not.

Bluetongue is an extremely complex and highly variable disease in wildlife, as it is in domestic livestock. It has the potential to adversely impact wildlife populations through adult mortalities, through neonatal mortalities and decreased recruitment rates. Considered together in all their diversity, the gnat-borne orbiviruses may be the most important infectious disease of California’s wild ruminants.

**REFERENCES**


ACKNOWLEDGEMENTS

This work is to be submitted by the lead author in completion of a Masters Degree in Epidemiology and Preventive Medicine. It was supported by Pittman Robertson Project W-52-R, 'A Wildlife Investigations Laboratory', Mzuri Safari Foundation, and Bighorn Research Institute. The authors thank Kathy Else, Mike Oliver, Maureen Dorsey, Russ Mohr, Henry Colletto, Jim DeForge and numerous DFG employees for technical assistance, especially Sue Records for her secretarial expertise; Karen Jones and Bill Clark, who took blood samples and kept the faith. Much of this paper was previously at the First International Symposium of Bluetongue held at Asilomar, California in February 1984.
The Committee on Wildlife Diseases convened at 1:30 p.m., October 25, 1984. Twelve committee members and 16 visitors were present. The first order of business was to review the Committee Report of 1983 and consider the status of each item previously recommended for further consideration. Items with apparent need for carry-over from Old and New Business of the 1983 Report herein are considered Old Business. Summary statements with a synopsis of action taken to date are cited as follows:

**OLD BUSINESS**

1. *Compensation for Relocation of Wildlife in the Event of Depopulation as an Essential Measure for Preventing Spread of a Dangerous Contagious Disease*

   This item has been on the agenda of the Wildlife Diseases Committee since 1975. Current authority held by the USDA will not allow payment for wildlife relocation costs if wildlife are killed to control livestock or poultry diseases, and additional Congressional action may be the only way to move forward on this issue.

   Currently both the USAHA and the International Association of Fish and Wildlife Agencies (IAFWA) are on record supporting this approach to mitigation of wildlife losses due to a necessary depopulation. At their Annual Meeting in September 1984, IAFWA decided to send a letter to the Administrator of APHIS encouraging the agency to continue their efforts toward getting authority to pay relocation costs. The IAFWA expressed a willingness to assist APHIS in the legislative process.

   **RECOMMENDED ACTION:** That the President of USAHA write a letter to the Administrator of APHIS similar to the one recently sent by IAFWA in which support and assistance were offered in getting any necessary legislation enacted to resolve the problem of restocking wildlife.

2. *Tuberculosis in Captive Bison*

   A report was presented by Dr. C. D. Stumpff on bovine tuberculosis disclosed in two American bison herds in South Dakota in 1984. Post-mortem examination revealed a gross lesion rate of 52 percent in animals slaughtered. Sales occurred to 20 other states and involved 87 herds. Infected herds have been found in 10 states with 17 confirmed bovine TB
infected herds. A preliminary investigation of native deer in the immediate vicinity of the South Dakota herds did not reveal evidence of infection.

**RECOMMENDED ACTION:** That the Committee on Wildlife Diseases endorse resolutions previously prepared by the Zoological Animals Committee as follows: 1) that the interstate movement of American bison be subject to the same testing and controls as required for cattle, and 2) that the USDA be the lead agency for collecting information and data concerning similar testing requirements for all other wild ruminants and carry out investigative studies as to the efficacy of such tests in the various species of wild ruminants.

3. **African Swine Fever (ASF) Surveillance in Feral Pigs in the Republic of Haiti**

The depopulation of domestic swine from the Republic of Haiti was declared completed in February 1984. As domestic swine were removed, it became apparent that residual foci of true feral pigs were present in several locations. Approximately 4 months prior to the end of the depopulation brigade activities, reports of feral pigs were received by the program. Surveillance teams from the Southeastern Cooperative Wildlife Disease Study (SCWDS) were dispatched to investigate reports of feral swine and obtain samples where possible.

Feral swine surveillance was continuous from April 1983 through September 1984. Rumors of feral swine were numerous and many reports only required a brief investigation; however, feral pig activity was confirmed on seven areas. In a 17 months period there were 106 feral pig captures, and ASF testing was done on 85 of these animals. Only one area had pigs that tested positive for ASF antibodies, an area called LaPierre near the village of Gonaives. There, 8 of 25 pigs tested positive serologically. Extensive hunting pressure was put on the LaPierre population and no pigs have been killed since March 3, 1984. Forty-nine pigs tested from other areas since March 3 have been negative, and the current feral pig population estimate for the entire Republic of Haiti is only 30 animals. The results of this surveillance indicate that ASF is not entrenched in the extremely small numbers of feral swine in Haiti and that repopulation of the Republic should proceed without hazard.

**RECOMMENDED ACTION:** None required except to commend the countries and agencies involved for a successful eradication program.

4. **Distribution Maps for Cloven-hooved Wildlife**

Last October, it was reported that color-coded animal density maps had been made for white-tailed deer, feral swine, and collared peccary populations for the entire United States. These maps had been assembled by the Southeastern Cooperative Wildlife Disease Study through a Cooperative Agreement with USDA,APHIS, Veterinary Services, for the purpose of providing a nationwide inventory of foot-and-mouth disease- and rinderpest-susceptible wild animals. Dr. Victor Nettles announced the remaining indigenous cloven-hooved wild species, i.e., moose, pronghorn
antelope, elk, mountain goat, bighorn sheep, and Dall’s sheep have also been completed.

**RECOMMENDED ACTION:** None required.

**NEW BUSINESS**

1. **Pullorum in Chukar Partridge Intended for Release**

Dr. Tom Thorne reported on information provided by Dr. Michael Marshall, Utah Assistant State Veterinarian, regarding an outbreak of pullorum in captive chukar. The birds were imported as 3,500 eggs from a private supplier in British Columbia, Canada, hatched by a private hatchery in Utah, and transferred to a game bird farm near Salt Lake City. Three thousand of the young birds were sold to an individual in California where they were to be released for hunting.

In September 1984, the Utah owner submitted dead birds for necropsy and pullorum was diagnosed. Pheasants and bobwhite quail were also present on the Utah bird farm. With the cooperation of the Utah Department of Wildlife Resources, the Utah State Veterinary Office quarantined the bird farm and notified the California State Veterinarian.

Blood tests revealed 17.5 percent of the chukar were pullorum reactors while there were no positive bobwhite or pheasant. Many of the chukar sent to California died and the rest were voluntarily killed once the owner was informed of the presence of pullorum and its ramifications. The remaining chukar in Utah will be killed.

**RECOMMENDED ACTION:** The Utah Departments of Agriculture and Wildlife Resources should be commended for their actions which prevented release of pullorum infected chukar. The USAHA Wildlife Diseases Committee will take action with the Fish and Wildlife Health Committee of IAFWA to inform state wildlife management agencies of the importance of this consideration of disease prevention when game birds are to be introduced into their respective states.

2. **Disease Testing in Wild Turkeys Intended for Translocation**

In this Committee’s report to the Association last year, we alerted the membership that there were several accounts of mycoplasmosis in wild turkeys. This disease is of major significance to domestic turkeys and possibly could be important in the wild. Whether true wild turkeys harbor the organism is still speculative; however, the Committee favored cooperative efforts to investigate the mycoplasmosis status of wild turkey flocks, particularly birds intended for interstate translocation.

During the past year, the Wildlife Disease Association (WDA) has formed a Wild Turkey Disease Monitoring Committee. The WDA’s Committee has completed an in-depth evaluation of all known diseases and parasites of wild turkeys. An Advisory Statement on Disease Monitoring in Wild Turkeys has been prepared which suggests a protocol for testing wild turkeys for diseases of major significance. It was emphasized by the
WDA's Committee that their recommendations not be interpreted as requirements because the Committee was fully aware that there may be instances where disease monitoring may impede translocation of birds. Still, it was stressed that the many successful wild turkey restoration programs should be protected from unwarranted criticism because of inadequate consideration of disease potentials.

The Advisory Statement was reviewed and endorsed by the Fish and Wildlife Health Committee of the IAFWA in September, and they recommended that copies be sent to all state and Federal wildlife agencies.

RECOMMENDED ACTION: The Wildlife Diseases Committee of USAHA endorsed the concept of pre-release disease monitoring of wild turkeys intended for transaction. The Committee recommended that each State Veterinarian also receive a copy of the WDA's material on wild turkeys.

3. Experimental Leptospira Interrogans Serovar Hardjo in Pronghorn Antelope

Dr. Tom Thorne reported upon a cooperative research project conducted at the Wyoming Game and Fish Department's Sybille Wildlife Research Unit. Six pregnant pronghorn antelope were artificially inoculated by intravenous injection of *Leptospira interrogans* serovar *hardjo*, an important cause of bovine leptospirosis. Antelope were monitored for clinical signs of illness. Culture of urine demonstrated leptospirosis for at least 381 days postinoculation. Blood serum tested for leptospiral antibodies demonstrated titer development and duration using microscopic agglutination. Clinical features of acute and chronic serovar *hardjo* infection and pathology were minimal.

RECOMMENDED ACTION: None required.

4. Biological Terrorism

During February of this year, a new form of extortion surfaced in Australia. An anonymous threat was made to introduce foot-and-mouth disease into that country unless certain concessions were made regarding prisons in Queensland. Of particular concern to the USAHA Wildlife Diseases Committee was the statement in the threat notice that wild animals would be infected to ensure an “ineradicable reservoir for continued infection of livestock.”

The temporary problem in Australia has been resolved; the threat letter was traced to a typewriter in a Queensland prison. Nevertheless, the real harm of this situation was the notoriety this threat generated. Public attention has been drawn to our vulnerability in this regard, and it is likely that such extortion will be tried again.

Protection of our wildlife resources from this form of biological terrorism is a nearly impossible task, but there are steps that can be taken. Since the crucial factor in stopping exotic diseases will be the speed with which infected animals are recognized and eliminated, renewed effort should be made with state and Federal wildlife agencies to be prepared for disease
emergencies. The Memorandum of Understanding between the USDA and all state wildlife agencies and the U.S. Fish and Wildlife Service serves as an excellent document to express the mutual concern by these parties for the health of our nation's wild and domestic animals. However, to be fully prepared for a foreign animal disease emergency, a workable Cooperative Agreement must be drafted, and the matter of wildlife repopulation (restocking) costs must be resolved.

Increased disease awareness and foreign animal disease training also would be valuable. In this regard, three wildlife veterinarians who work directly with state or Federal wildlife agencies recently have been included in USDA's Foreign Animal Disease Training Course. Additional wildlife veterinarians should be trained as time and space permit. Dr. William Taylor of NVSL, Ames, Iowa, also suggested that more of APHIS's foreign animal disease diagnosticians receive training in basic wildlife management and diseases. At the suggestion of Dr. A. M. Dardiri, it was decided that the Wildlife Disease Association and the American Association of Wildlife Veterinarians be notified of new publications that are available on foreign animal diseases.

In recent years, task forces to combat foreign disease outbreaks have been manned by members of the Regional Emergency Animal Disease Eradication Organization (READEO). READEO members include personnel from USDA, state agricultural agencies, universities, etc., and in every READEO there is a slot for a wildlife officer. Originally, three alternate wildlife officers were designated for each of five regions in the Nation, and these persons were committed to move quickly to any disease emergency within or outside of their home state.

If this contingency plan for wildlife activities is to remain in effect, the current list will need a major updating and the new designees will require training. An alternate system would be to have one person per state wildlife agency designated as State Wildlife Liaison Officer. These persons would require less extensive training and would not need to travel to other states. Instead, they would be used very effectively to assist the Task Force Wildlife Officer designated by Emergency Programs, APHIS, USDA. Wildlife activities in the task force will be the responsibility of the Wildlife Officer, and disease surveillance would be handled jointly by wildlife disease biologists available through a current cooperative agreement of Emergency Programs, APHIS, USDA and state or federal wildlife agency personnel who would be reimbursed through a cooperative agreement signed at the time of need.

The Cooperative Agreement between APHIS, USDA, and the South-eastern Cooperative Wildlife Disease Study (SCWDS), The University of Georgia, has proved to be an effective way for APHIS to maintain quick wildlife response capabilities in foreign or domestic animal disease outbreaks. Incorporation of SCWDS activities into Veterinary Services' ongoing Animal Health Programs has provided invaluable data on relationships between wildlife and livestock or poultry while simultaneously
affording USDA with closer ties to state and Federal fish and wildlife agencies. The continued relationship among the multiple cooperators through SCWDS will help maintain security from diseases in all animal resources, wild or domestic.

RECOMMENDED ACTION: The aforementioned items should be considered as the bare essentials in plans to protect our wildlife resources against biological terrorism. The members of the Wildlife Diseases Committee of USAHA should deliberate on any additional steps that would be possible to decrease our vulnerability and encourage greater cooperation between wildlife and agricultural interests at all levels. Time and effort spent in preparation will prove the best investment for handling a foreign animal disease introduction.

5. **Duck Virus Enteritis Discussion Panel Sponsored by the U.S. Fish and Wildlife Service**

In July 1982, the U.S. Fish and Wildlife Service (FWS) convened a panel of avian disease and wildlife management specialists comprised of representation from The Wildlife Society (Dr. George Burger), International Association of Fish and Wildlife Agencies (Dr. Forest Kellogg), the National Academy of Sciences (Dr. Charles Beard), the North American Game Breeders and Shooting Preserve Operators (Dr. J. Richard Cain), and the Wildlife Disease Association (Dr. Louis Leibowitz). The purpose of the Panel was to examine FWS policies, guidelines, and operating procedures related to the following specific issues: (1) the status of duck virus enteritis (DVE) in North American waterfowl, (2) the use of vaccines for the prevention and control of DVE, and (3) the DVE status of captive waterfowl released into the wild.

The Panel met on three occasions and received input from the following consultants: Dr. J. E. Pearson, Dr. Christopher Brand, Dr. Wallace Hanson, Dr. Milton Friend, Dr. Sajjad Haider, Dr. David Long, Mr. Jack Frost, Mr. William Stark, Mr. Leo Whalen, Mr. C. D. Murphy, Mr. Paul Spencer, and Dr. Robert Lange. The final recommendations of the DVE Discussion Panel were as follows:

**Status of DVE in North American Waterfowl**

1. USDI and USDA should resolve any jurisdictional problems that may exist in the management of waterfowl disease. USDI has legislatively mandated responsibility for wild migratory waterfowl. USDA provides services for disease prevention and control in support of the domestic duck industry. Federal and state governments must clarify and/or establish their appropriate responsibilities to effectively manage the critical interface between captive and wild waterfowl.

2. The FWS should recognize that DVE can no longer be considered an "exotic" disease among North American waterfowl.

3. The FWS should support research efforts:
   a. to develop more reliable, sensitive, and economical means of determining the DVE status of waterfowl; i.e., the ELISA test; and
b. to verify if vertical transmission of DVE virus occurs.

Use of Vaccines in Prevention and Control of DVE

1. Use of vaccine should be allowed in captive waterfowl as a DVE preventative measure even though concerns exist regarding safety and efficacy of the vaccine. Some of these concerns could be eliminated by the development and use of an inactivated (killed) virus vaccine.

2. Use of vaccine should be allowed in the face of an outbreak to reduce mortality and the quantity of virulent virus shed into the environment. Waterfowl vaccinated in the face of an outbreak should not be released or transported except for slaughter.

DVE Status in Captive Waterfowl Released on FWS Lands

1. DVE-free Status: — It is virtually impossible to assure that waterfowl removed from an open aquatic rearing area are free of DVE. They could appear healthy, test negative, and yet be in the incubation stages of the disease. Therefore, we believe that requirements outlined in the FWS Refuge Manual (7 RM 17.5) dealing with the pathogen-free status of birds destined to be released on National Wildlife refuges are unrealistic. If FWS continues this policy, captively reared birds cannot be released on National Wildlife Refuges.

   If FWS wishes to revise this policy, then the following procedures should reduce the likelihood of introducing DVE virus to wild birds on National Wildlife Refuges by released birds. A monitoring program could be initiated with state and private participation that would include the following actions by captive waterfowl propagators:
   a. Maintain a prescribed system of production and mortality records.
   b. Report unusual mortality or illness to FWS.
   c. Open facilities to FWS inspection.
   d. Cooperate with FWS in obtaining statistically valid serological and virus isolation samples from the premises.
   e. Allow use of susceptible sentinel birds to detect the presence of DVE virus.

   Once the above conditions were met and no evidence of DVE existed on the premises, then FWS could allow use of birds from this facility. Because USDA has experience in eradication and control of DVE in domestic ducks, they should be consulted with regard to statistically valid numbers to be sampled and on the scheduling of such sampling.

2. Other Diseases: Health certificate standards for birds released on FWS refuge lands should be clearly defined for each disease of concern. Requirements stated in FWS Refuge Manual are too vague and broad to allow precise and realistic implementation.

RECOMMENDED ACTION: The FWS should be commended for using this cooperative approach in obtaining input from numerous parties out-
side their Agency. The USDA and USDI should move forward on resolving questions of jurisdiction over pen-reared ducks intended for release into the wild.

6. Wildlife Studies Associated with the Lethal Avian Influenza Outbreak in Pennsylvania, Virginia, New Jersey, and Maryland

Dr. Robert G. Webster of St. Jude Children’s Research Hospital gave the Committee a brief review of the epizootiology of avian influenza (AI) virus. He emphasized that numerous virus subtypes and strains have been recovered from waterfowl and that the intensity of infection is greatest in the summer when there is a large number of susceptible fledglings. A variety of birds have been experimentally infected with the lethal AI virus by Dr. Webster, and it appears that the lethal virus is well adapted to gallinaceous birds and replicates poorly in other avian species.

An overview of the wildlife surveillance activities associated with the AI Task Forces in Pennsylvania and Virginia was presented. Virus isolation attempts were made on 4,133 birds and small rodents in Pennsylvania and only 2 H5N2 AI viruses were recovered. One was from a hunter-killed pheasant and the other from a pen-reared chukar. The lethal AI virus was not found in 334 birds collected in the Virginia AI Quarantine Zone. Over 1,500 waterfowl were screened for AI virus in the Chesapeake Bay region of Maryland with negative results. From these data, it was concluded that wild birds were not responsible for dissemination of lethal H5N2 AI virus among poultry.

The possibility that wild aquatic birds could be a silent reservoir for future lethal H5N2 AI virus outbreaks was discussed. A variety of aquatic birds in the Task Force surveys had antibodies against hemagglutinin type 5 and neuraminidase type 2, however, it could not be said with certainty that these antibodies were acquired from infection with H5N2 virus. An alternate hypothesis is that the birds received exposure to a combination of other influenza subtypes each having only an H5 or N2 component. Dr. Nettles said that the SCWDS is planning to monitor wild waterfowl for avian influenza viruses throughout the year in the Pennsylvania Quarantine Zone.

Dr. Victor Nettles presented the Committee with a resolution from the Avian Influenza Technical Collaborating Committee which recommended that State and Federal Fish and Wildlife Agencies use caution in shipping and/or receiving migratory waterfowl or pen-reared game birds. The resolution encouraged state and Federal fish and wildlife agencies to obtain laboratory testing of the birds in question prior to shipment. Dr. Nettles stressed that testing should be done not only to benefit the poultry industry but also to protect wildlife agencies from being accused of introducing the disease. It was pointed out that the resolution recently was endorsed by the Fish and Wildlife Health Committee of IAFWA.

RECOMMENDED ACTION: The Committee was pleased to hear that a thorough investigation of the epidemiology of avian influenza had been conducted in concurrence with other AI Task Force activities, and it
commended the Task Force and APHIS for their foresight on this aspect of the disease eradication program. Furthermore, the Wildlife Disease Committee of USAHA also wishes to go on record as supporting the resolution on testing translocated migratory waterfowl and pen-reared game birds. The Committee unanimously passed a resolution commending the four states and FWS for their assistance in dealing with this dangerous disease outbreak.
INTRODUCTION

Malignant catarrhal fever (MCF) is a generalized viral disease of cattle and many species of wild ruminants characterized by high fever, profuse nasal discharge, corneal opacity, panophthalmitis, leukopenia, generalized lymphadenopathy and severe inflammation of the upper respiratory and digestive tracts. Diarrhea and hemorrhagic enteritis, exanthematous dermatitis, CNS signs and non-suppurative arthritis may also occur in occasional cases. Histologically, a generalized necrotizing vasculitis, lymphoreticular proliferation and infiltration in many tissues are characteristic features (4,12,16,21).

In Africa, MCF in cattle has been recognized by Masai herdsmen for centuries as being associated with their cattle having had contact with areas grazed by wildebeest, particularly during the wildebeest calving season. In masailands of East Africa MCF is responsible for an annual mortality of about 7% of domestic cattle (12).

The virus of wildebeest-associated MCF was first isolated in 1960 by Plowright from a blue wildebeest, also known as a brindled gnu (Connochaetes taurinus taurinus) (14), and was identified as a herpesvirus (15).

A closely related herpesvirus was later isolated from a Coke’s hartebeest (Alcelaphus buselaphus cokei) by Reid and Rowe (18). Subsequently, a similar virus was isolated by Mushi et al. from a topi (Damaliscus korrigum) (10). Reid et al. proposed that MCF viruses of antelope origin be named alcelaphine herpesvirus rather than bovine herpesvirus since antelope of the subfamily Alcelaphinae (wildebeest, hartebeest, etc.) were the primary reservoir hosts of African MCF viruses (12,19).

In Europe, MCF was described in cattle and associated with contact with sheep in the 1700s (17). Since then sporadic MCF in cattle associated with sheep, especially during the lambing season, has been recognized in most areas of the world. The virus of sheep-associated MCF has not yet been identified, although several other incidental viruses have been isolated from cattle, bison and deer with clinical MCF in the United States (25). Workers in Scotland and Australia have succeeded in transmitting sheep-associated MCF to domestic rabbits by inoculation of blood or tissues from...
cattle and captive deer with clinical MCF (20,21). Transmission was subsequently maintained continuously by rabbit-to-rabbit transfer for four years and over 100 passages. Cultured infected rabbit lymphoid cells characterized as T-lymphocytes have been shown to be infective (20). The virus involved has not, however, been identified or visualized by electron microscopy.

Clinical signs and pathologic lesions of African or alcelaphine MCF and sheep-associated MCF are virtually identical in cattle, wild ruminants, and experimentally infected rabbits. Transmission of MCF virus from wildebeest most likely occurs by inhalation or ingestion as neonatal wildebeest shed cell-free infectious virus in their nasal and ocular secretions and feces for up to three months (5,6,12).

For many years, MCF in North America and other areas of the world, has generally occurred as sporadic individual cases in cattle, and in a few instances in bison and deer. It is probable that MCF is underdiagnosed or misdiagnosed because of the similarity of signs and lesions in MCF to those seen in the more commonly occurring bovine viral diarrhea-mucosal disease complex, and in some cases of bluetongue and other diseases causing oral erosions, ocular lesions, diarrhea or profuse nasal discharge. Maré (9) in 1977 described recent outbreaks of MCF in the US in which herd morbidity was relatively high. Some outbreaks were strongly associated with proximity or contact of involved cattle with sheep or areas grazed by sheep. A few outbreaks in cattle were linked to the proximity of a wild animal park in which alcelaphine antelope (wildebeest, hartebeest and topi) were kept and in which MCF had occurred in susceptible Asian exotic ruminants. Dealing only with reported episodes of MCF in cattle, Maré concluded that there was an alarming increase of MCF in the USA (9).

Pierson et al. (13) described an epizootic of MCF occurring in 1971–72 in a Colorado feedlot in which 87 (37%) of one group of 231 cattle died within 68 days. Although the disease was successfully transmitted by inoculation of infectious blood to experimental cattle, the virus involved was not isolated or identified. Cattle involved in this outbreak were typically gathered from various sources, sale barns, etc., and their origins could not be traced. Sheep were present in adjacent pens and were presumed to be the source of the MCF agent involved.

Ranching of exotic game animals has become an increasingly popular and growing enterprise in North America. Often such ranching is in conjunction with or adjacent to cattle ranching operations. It has been estimated, conservatively, that over 300,000 exotic ruminants are on ranches in Texas alone. There have been several reports of MCF occurring in exotic ruminants on Texas ranches (5); and anecdotal unpublished accounts of many episodes of MCF in cattle and exotic hoofed stock on such ranches have been related to these authors. This leads one to the conclusion that the hazard of MCF for domestic livestock, indigenous wildlife, and captive exotic and endangered ruminant species is indeed increasing. A need for greater surveillance for MCF, possible regulation of movement
of potential carriers, and mandatory reporting of MCF cases throughout the USA appear to be appropriate considerations.

A virus of MCF, identified as alcelaphine herpesvirus was first isolated in the United States by Castro et al. (1) from whole blood from an Indian gaur (Bos gaurus) and a greater kudu (Tragelaphus strepsiceros) with clinical MCF during an outbreak occurring at the Oklahoma City Zoo in late summer, 1979. Isolations of MCF virus were subsequently also made from white-tailed wildebeest at the same zoo (2,3).

The San Diego Zoo (SDZ) and San Diego Wild Animal Park (SDWAP) experienced episodes of MCF in Javan banteng (Bos javanicus) and Indian gaur at the Zoo in 1974, and again at the WAP in 1976 and 1979 (4). In 1980, nine Pere David's deer (Elaphurus davidianus) at the SDWAP succumbed to peracute MCF. Other species affected by MCF at the SDWAP during this period were reported previously (5).

RECENT EPIDEMIOLOGIC FINDINGS

The first isolations of alcelaphine herpesviruses at the SDWAP from a sick nilgai (Boselaphus tragocamelus) in 1981; and subsequent isolations in 1982 from clinically ill axis deer (Cervus axis), barasingha deer (Cervus duvaucelli) and Formosan sika deer (Cervus nippon taionanus) and an asymptomatic white-tailed gnu (Connochaetes gnou) calf and adult white-bearded gnu (Connochaetes taurinus albojubatus) have been previously reported (5,6).

Because of the serious losses of valuable captive exotic ruminants from MCF, and the hazard it poses for domestic livestock, we have continued a study initiated in 1981 of epidemiologic aspects of MCF.

PREVALENCE OF MCF ANTIBODIES IN CAPTIVE EXOTIC AND DOMESTIC RUMINANTS

To date we have tested sera from over 2,300 exotic ruminants from zoos throughout the USA, and a small sampling of domestic ruminants for MCF antibodies by indirect immunofluorescence (IIF) and by virus neutralization (VN) using standard beta microtitration methods (6). In the course of these studies we found that the IIF test detected crossreaction of antibodies to other bovine herpesviruses with alcelaphine herpesviral antigens, which was previously reported (5,6). We therefore decided that, although the IIF test was useful for rapid serologic screening, the VN test provided a more reliable test basis for prevalence statistics, diagnostic confirmation, and identification of possible MCF virus carriers. The results of our MCF antibody prevalence studies are summarized in Tables 1, 2 and 3. As anticipated, the subfamily Alcelaphinae of the family Bovidae (comprised of wildebeest, hartebeest, topi, blesbok and bontebok species) had a high (48.3%) prevalence of VN antibodies to MCF. Interestingly, we also found a remarkably high VN antibody prevalence (45.6%) among members of the subfamily Hippotraginae (addax, and various oryx species). Mushi and Karstad reported finding MCF virus neutralizing antibodies in sera of 50
MALIGNANT CATARRHAL

fringe-eared oryx (Oryx beisa callotis) in captive herds in Kenya (11). They were not successful in isolating MCF virus from blood or nasal secretions from any of these animals but felt they should be further studied.

In our investigation we found that all species of oryx and addax had relatively high MCF VN antibody prevalence (Table 3). In both the Alcelaphinae and Hippotraginae the VN antibody titers, when present, tended to be relatively high in a majority of seropositive individuals (Table 4). In the subfamily Caprinae the VN antibody prevalence was moderately high (29.5%), but titers as a rule were found to be low in most individuals (Table 3).

Usually, positive sera from wild sheep and goats and from domestic sheep, goats and cattle associated with episodes of sheep-associated MCF had strongly positive IIF serum antibody reactivity with alcelaphine herpesvirus antigens, but low titer or negative VN activity. These data and similar findings reported by other investigators lead us to the speculation that the agent of sheep-associated MCF is a herpesvirus related but not identical to alcelaphine herpesviruses (22,23). This view would also be supported by the close similarity of the complex pathologic manifestations of both alcelaphine and sheep-related MCF. Lymphoproliferative response, even extending to neoplasia is a characteristic of the Gammaherpesvirinae subfamily of herpesviruses which includes Marek's disease virus, Epstein-Barr virus, herpesvirus ateles, Herpesvirus saimiri, Herpesvirus sylvilagi, and alcelaphine herpesviruses (7).

VIRUS ISOLATION FROM SPECIES WITH HIGH MCF ANTIBODY PREVALENCE

Having identified a number of ruminant species besides wildebeest with high MCF antibody prevalence, we then sought to isolate virus from many of these species by methods previously reported. Isolations of viruses confirmed as alcelaphine herpesvirus have been made in our laboratory from, in addition to wildebeest, clinically healthy, seropositive Cape hartebeest (Alcelaphus buselaphus caama), Jimela topi (Damaliscus lunatus jimela) and scimitar-horned oryx (Oryx gazella dammah). Each of these isolations were made by co-cultivation of buffy coat leukocytes from heparinized blood with fetal aoudad kidney cells in culture at 33°C as previously described.

We similarly attempted virus isolations from several fringe-eared oryx, Himalayan tahr goats, Cretan goats, African pygmy goats and domestic sheep and goats, and a few domestic cattle with clinical sheep-associated MCF without success.

The sheep-associated MCF virus continues to be elusive and will no doubt require application of the modern techniques of molecular virology before it will be revealed.

We found additional evidence that the virus of sheep-associated MCF is a herpesvirus related to alcelaphine herpesvirus by application of a direct fluorescent antibody test for alcelaphine herpesvirus to blood leukocyte
smears of seropositive African pygmy goats and Cretan goats. In scattered leukocytes in these preparations there was bright specific nuclear and cytoplasmic fluorescence similar to that encountered in smears from a nilgai and sika deer with acute clinical MCF which had been examined by this technique. Blood and buffy coat smears from several seronegative wild ruminants treated by this method had no fluorescing cells with the MCF conjugate used. A detailed description of this direct FA procedure is reported elsewhere. A summary of species from which alcelaphine herpesviruses have been isolated in the USA is listed in Table 4.

THE ROLE OF WILD RABBITS AND GROUND SQUIRRELS IN MCF EPIDEMIOLOGY

Because rabbits are susceptible to both alcelaphine and sheep-associated MCF experimental infection,17,20,21 we have begun serologic examinations of wild brush rabbits and ground squirrels which inhabit the SDWAP in large numbers, many in close cohabitation with wildebeest or hartebeest. To date we have found low titer MCF VN antibody (1:4) in 2 of 16 brush rabbits examined.

DISCUSSION

Episodes of clinical MCF in zoos and on ranches in the US have been circumstantially linked with the presence of domestic sheep, domestic goats, wild sheep or goats, or alcelaphine antelope on the same premises or neighboring premises. Some zoos have had cases of MCF in valuable endangered wild ruminant species and domestic cattle while there were no alcelaphine antelope in the zoo collection. In these cases wild sheep and/or domestic sheep and goats were suspected as the source of infection, a conclusion supported in most cases by finding MCF antibodies in these suspect carriers on the premises.

It is a recognized fact that herpesviruses in general are notorious for establishing latent persistent infections which, under appropriate circumstances may recrudesce to active productive infections with virus shedding. One must therefore assume that any animal with serum VN antibodies to MCF virus is likely to be a carrier of the viral genome in some form. Whether every seropositive animal is capable of shedding readily infectious cell-free MCF virus, as do wildebeest calves, is at present unknown. It would seem prudent to consider any animal seropositive for MCF by VN testing as a potential source for MCF virus. The testing of exotic and domestic ruminants with documented high prevalence of MCF antibodies prior to movement or entry, i.e., species highly suspect as MCF carriers, therefore may be an appropriate preventive strategy against the introduction of MCF to a premise which has not experienced or had evidence of MCF infection. Increasing the population of MCF carriers on a premise potentially creates an increased risk of clinical cases of MCF and

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possible evolution of the causative virus to a state of higher cell-free transmissibility. Such a situation could develop with serious, if not disastrous consequences for both valuable exotic animal collections and domestic cattle, and possibly also domestic sheep and goats. Clinical MCF has been produced experimentally in domestic sheep and natural clinical cases of MCF in domestic sheep have also been reported.

The increase in private collections of exotic ruminants, often in association with domestic livestock operations may well contribute to an increased hazard of MCF for both enterprises if such continue without some surveillance of the MCF status of animals involved and imposition of mandatory reporting of MCF cases. Requirements for serotyping potential MCF carriers before entry or movement would seem to be warranted also.

REFERENCES


ACKNOWLEDGEMENTS

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Table 1: Prevalence of Malignant Catarrhal Fever Neutralizing Antibodies Among Captive Wild Ruminants By U.S. Region

<table>
<thead>
<tr>
<th>Region</th>
<th>VN Antibody Prevalence (%)</th>
<th>Number Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast</td>
<td>31.5</td>
<td>148</td>
</tr>
<tr>
<td>Southeast</td>
<td>12.9</td>
<td>155</td>
</tr>
<tr>
<td>North Central</td>
<td>30.3</td>
<td>185</td>
</tr>
<tr>
<td>South Central</td>
<td>24.3</td>
<td>383</td>
</tr>
<tr>
<td>West</td>
<td>27.2</td>
<td>1,437</td>
</tr>
<tr>
<td>Totals</td>
<td>26.3</td>
<td>2,308</td>
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</table>
Table 2: Prevalence of Malignant Catarrhal Fever Neutralizing Antibodies Among Captive Wild Ruminants By Family/Subfamily

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>Subfamily</th>
<th>VN Antibody Prevalence (%)</th>
<th>Number Tested</th>
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</thead>
<tbody>
<tr>
<td>ANTILOCAPRIDAE</td>
<td></td>
<td>0.0</td>
<td>11</td>
</tr>
<tr>
<td>BOVIDAE</td>
<td>Alcelaphinae</td>
<td>48.3</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Antilopinae</td>
<td>15.6</td>
<td>460</td>
</tr>
<tr>
<td></td>
<td>Bovinae</td>
<td>21.7</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>Caprinae</td>
<td>29.5</td>
<td>518</td>
</tr>
<tr>
<td></td>
<td>Cephalophinae</td>
<td>4.2</td>
<td>24</td>
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<tr>
<td></td>
<td>Hippotraginae</td>
<td>45.6</td>
<td>364</td>
</tr>
<tr>
<td></td>
<td>Neotraginae</td>
<td>23.5</td>
<td>17</td>
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<tr>
<td></td>
<td>Saiginae</td>
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</tr>
<tr>
<td></td>
<td>Tragelaphinae</td>
<td>14.4</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>27.9</td>
<td>1,906</td>
</tr>
<tr>
<td>CAMELIDAE</td>
<td></td>
<td>7.9</td>
<td>63</td>
</tr>
<tr>
<td>CERVIDAE</td>
<td></td>
<td>20.8</td>
<td>308</td>
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<tr>
<td>GIRAFFIDAE</td>
<td></td>
<td>7.7</td>
<td>13</td>
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<tr>
<td>TRAGULIDAE</td>
<td></td>
<td>50.0</td>
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### Table 3: Captive U.S. Exotic and Domestic Ruminants With High\(^a\) MCF Neutralizing Antibody Prevalence

<table>
<thead>
<tr>
<th>BOVIDAE</th>
<th>Antibody Prevalence</th>
<th>Antibody Mean Titer ± SD</th>
<th>Titer Range ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subfamily Alcelaphinae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-bearded gnu (Connochaetes taurinus albojubatus)</td>
<td>29/43(^b)(67)(^c)</td>
<td>2-256</td>
<td>115 ± 104</td>
</tr>
<tr>
<td>White-tailed gnu (Connochaetes gnou)</td>
<td>16/27 (59)</td>
<td>4-256</td>
<td>69 ± 89</td>
</tr>
<tr>
<td>Brindled gnu (Connochaetes taurinus taurinus)</td>
<td>4/11 (36)</td>
<td>12-256</td>
<td>137 ± 137</td>
</tr>
<tr>
<td>Cape hartebeest (Alcelaphus buselaphus caama)</td>
<td>9/20 (45)</td>
<td>4-256</td>
<td>72 ± 105</td>
</tr>
<tr>
<td>Jimela topi (Damaliscus lunatus jimela)</td>
<td>7/14 (50)</td>
<td>2-128</td>
<td>25 ± 46</td>
</tr>
<tr>
<td>Blesbok (Damaliscus dorcas phillipsi)</td>
<td>4/16 (25)</td>
<td>2-6</td>
<td>5 ± 2</td>
</tr>
<tr>
<td><strong>Subfamily Antilopinae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kenya impala (Aepyceros melampus rendilis)</td>
<td>14/53 (26)</td>
<td>2-48</td>
<td>12 ± 15</td>
</tr>
<tr>
<td>Angolan springbok (Antidorcas marsupialis angolensis)</td>
<td>12/51 (24)</td>
<td>2-20</td>
<td>7 ± 7</td>
</tr>
<tr>
<td>Dorcas gazelle (Gazella dorcas dorcas)</td>
<td>2/7 (29)</td>
<td>4-8</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>Roosevelt's gazelle (Gazella granti Roosevelti)</td>
<td>4/16 (25)</td>
<td>2-4</td>
<td>3 ± 1</td>
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<tr>
<td><strong>Subfamily Bovinae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic cattle (Bos taurus)*</td>
<td>16/53 (30)</td>
<td>2-256</td>
<td>20 ± 62</td>
</tr>
<tr>
<td>Water buffalo (Bubalus bubalis)</td>
<td>6/16 (38)</td>
<td>2-3</td>
<td>2 ± 0.5</td>
</tr>
<tr>
<td>African buffalo (Syncerus caffer)</td>
<td>4/19 (21)</td>
<td>2-8</td>
<td>5 ± 3</td>
</tr>
<tr>
<td><strong>Subfamily Caprinae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aoudad, Barbary sheep (Ammotragus lervia)</td>
<td>14/34 (41)</td>
<td>16-256</td>
<td>23 ± 67</td>
</tr>
<tr>
<td>Cretan goat (Capra aegagrus creti)</td>
<td>5/22 (23)</td>
<td>2-8</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>Turkomen markhor (Capra falconeri heptneri)</td>
<td>18/56 (32)</td>
<td>3-32</td>
<td>7 ± 7</td>
</tr>
<tr>
<td>Domestic goat (Capra hircus)</td>
<td>9/40 (23)</td>
<td>3-16</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>West Caucasian tur (Capra ibex caucasica)</td>
<td>10/17 (59)</td>
<td>2-8</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Alpine ibex (Capra ibex ibex)</td>
<td>4/4 (100)</td>
<td>2-8</td>
<td>4 ± 3</td>
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<tr>
<td>Nubian ibex (Capra ibex nubiana)</td>
<td>27/56 (48)</td>
<td>2-24</td>
<td>7 ± 6</td>
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<tr>
<td>Himalayan tahr (Hemitragus jemlahicus)</td>
<td>14/27 (52)</td>
<td>2-40</td>
<td>9 ± 10</td>
</tr>
<tr>
<td>Bighorn sheep (Ovis canadensis)</td>
<td>7/23 (30)</td>
<td>2-4</td>
<td>2 ± 0.8</td>
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<tr>
<td>Dall sheep (Ovis dalli dalli)</td>
<td>3/9 (33)</td>
<td>2-8</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>Mouflon sheep (Ovis orientalis musimon)</td>
<td>15/77 (20)</td>
<td>2-16</td>
<td>6 ± 5</td>
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Table 3 - continued

<table>
<thead>
<tr>
<th>Subfamily Rupicaprinae</th>
<th>Antibody Prevalence</th>
<th>Titer Range $^d$</th>
<th>Mean Titer $^d$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamois (Rupicapra rupicapra)</td>
<td>9/26 (35)</td>
<td>2-8</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subfamily Hippotraginae</th>
<th>Antibody Prevalence</th>
<th>Titer Range $^d$</th>
<th>Mean Titer $^d$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addax (Addax nasomaculatus)</td>
<td>33/73 (48)</td>
<td>2-160</td>
<td>25 ± 35</td>
</tr>
<tr>
<td>Roan antelope (Hippotragus equinus)</td>
<td>3/14 (21)</td>
<td>2-6</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Beisa oryx (Oryx gazella beisa)</td>
<td>1/5 (20)</td>
<td>0-2</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>Gemsbok (Oryx gazella gazella)</td>
<td>30/46 (65)</td>
<td>2-256</td>
<td>45 ± 54</td>
</tr>
<tr>
<td>Fringe-eared oryx (Oryx gazella callotis)</td>
<td>22/30 (73)</td>
<td>2-256</td>
<td>45 ± 54</td>
</tr>
<tr>
<td>Scimitar-horned oryx (Oryx gazella dammah)</td>
<td>55/102 (54)</td>
<td>2-256</td>
<td>60 ± 79</td>
</tr>
<tr>
<td>Arabian oryx (Oryx leucoryx)</td>
<td>12/19 (65)</td>
<td>2-48</td>
<td>14 ± 17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subfamily Reduncinae</th>
<th>Antibody Prevalence</th>
<th>Titer Range $^d$</th>
<th>Mean Titer $^d$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defassa waterbuck (Kobus ellipsiprymnus adolfi-friderici)</td>
<td>5/22 (23)</td>
<td>8-16</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Uganda kob (Kobus kob thomasi)</td>
<td>6/19 (32)</td>
<td>2-12</td>
<td>8 ± 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subfamily Tragelaphinae</th>
<th>Antibody Prevalence</th>
<th>Titer Range $^d$</th>
<th>Mean Titer $^d$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitatunga (Tragelaphus spekei)</td>
<td>8/34 (24)</td>
<td>2-8</td>
<td>3 ± 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cervidae</th>
<th>Antibody Prevalence</th>
<th>Titer Range $^d$</th>
<th>Mean Titer $^d$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sika deer (Cervus nippon)</td>
<td>9/35 (26)</td>
<td>2-512</td>
<td>115 ± 165</td>
</tr>
<tr>
<td>Eld's deer (Cervus eldi thamin)</td>
<td>3/14 (21)</td>
<td>2-64</td>
<td>33 ± 31</td>
</tr>
<tr>
<td>Reeve's muntjac (Muntiacus reevesi reevesi)</td>
<td>21/62 (34)</td>
<td>2-64</td>
<td>8 ± 13</td>
</tr>
</tbody>
</table>

$^a$ 20%
$^b$ No. positive/No. tested
$^c$ % positive
$^d$ Reciprocal of median endpoint
Table 4: Species From Which Alcelaphine Herpesviruses Have Been Isolated In the U.S.

<table>
<thead>
<tr>
<th>Clinical MCF Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian gaur (Bos gaurus gaurus)</td>
</tr>
<tr>
<td>Greater kudu (Tragelaphus strepsiceros strepsiceros)</td>
</tr>
<tr>
<td>Nilgai (Boselaphus tragocamelus)</td>
</tr>
<tr>
<td>Formosan sika deer (Cervus nippon taiouanus)</td>
</tr>
<tr>
<td>Indian axis deer (Cervus axis axis)</td>
</tr>
<tr>
<td>Watusi (Ankole) cattle (Bos taurus)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinically Healthy MCF Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>White-tailed gnu (Connochaetes gnou)</td>
</tr>
<tr>
<td>White-bearded gnu (Connochaetes taurinus albojubatus)</td>
</tr>
<tr>
<td>Jimela topi (Damaliscus lunatus jimela)</td>
</tr>
<tr>
<td>Cape hartebeest (Alcelaphus buselaphus caama)</td>
</tr>
<tr>
<td>Scimitar-horned oryx (Oryx gazella dammah)</td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE ON ZOOLOGICAL ANIMALS

Chairman: M. S. Silberman, Atlanta, Georgia

Vice Chairman: R. L. Crawford, Hyattsville, Maryland

R. A. Bowen, CO; P. M. Eppele, MD; Gene Erickson, IA; Milton Friend, WI; E. E. Grass, CA; D. E. Herrick, MD; Werner Heuschele, CA; C. J. Mikel, OK; G. P. Pierson, MD; Jeanne Roush, D.C.; D. F. Schwindaman, MD; R. M. Scott, MI; K. C. Sherman, KS; O. J. Williams, GA; R. J. Yedloutschnig, NY.

The meeting was called to order at 1:30 p.m. by Morton Silberman, Chairman. The meeting was attended by 10 committee members and 28 guests. An agenda previously sent to committee members was followed.

Dr. John Acree presented a proposed APHIS policy on the importation of zoo animals into the United States with suggested new disease testing and methods for eliminating the introduction of exotic ectoparasites. It was stressed that this was not a policy statement or impending regulation changes but was presented in order to receive input and comments from concerned groups. Dr. Acree advised that another meeting concerning these suggestions would be held in the Washington, D.C. area before any definite policy was developed.

Representatives from The American Association of Zoological Parks and Aquariums, The American Association of Zoo Veterinarians, Animal Importers, various zoos and universities, research investigators and wildlife disease experts were present. A lively discussion followed which pointed up possible disease hazards to the United States Animal Industry and to the native wildlife population. The discussion also pointed out the considerable economic impact upon the zoo animal industry by such requirements, and the fact that such requirements may well be detrimental to species survival plans for various endangered species. The fact was also pointed out that many tests are nonspecific and could well eliminate animals from consideration for import when in fact they would pose no threat to the United States animal industry. General consensus was that there was some danger for the introduction of exotic diseases but that more information is needed and more specific and definitive tests are needed before such restrictions are considered. Representatives of the zoo animal field requested that they be consulted and invited to attend meetings before any such policy or regulations were considered.

Dr. Larry Cornell presented a workshop report concerning "Animals on Display: Educational and Scientific Impact," and also presented a resolution "Acknowledging the Contributions of Zoological Parks and Aquariums," for committee consideration. No action was taken on the request as the committee did not have sufficient time to review and discuss the presented material.

Dr. R. L. Crawford presented information on the growing threat of disease dissemination, specifically, tuberculosis, by American bison. Bi-
son are becoming common in many areas, often in association with livestock, and move interstate without restrictions. It is now apparent that there is a tuberculosis problem in bison herds. Discussion followed and two resolutions were passed by unanimous vote: 1) That the interstate movement of American bison be subject to the same testing and controls that the UM & R requires for cattle movements, and 2) That the USDA be the lead agency for collecting information and data concerning similar testing requirements for all other exotic ruminants, and carry out investigative studies as to the efficacy of the such tests in the various species of exotic ruminants.

The growing problem of exotic animal auctions was discussed, which allow dangerous animals and exotic ruminants to move interstate freely and to be placed into situations which may be harmful to the livestock industry and to the public. A subcommittee was appointed to study this problem and to report back next year with their recommendations.

A brief discussion of zoos and the enforcement of the Animal Welfare Act was given and the need for more specific regulations for zoo animals was indicated. It was voted that considerable improvement has been made over the past 14 years and that continued strong enforcement is needed.

A brief update on MCF and testing capabilities was given by Dr. Werner Heuschele and it was indicated that diagnostic testing capabilities have been greatly improved and are now available.

A very brief report was given on the VVND problems associated with the illegal importation of yellowheaded parrots from Mexico. It was pointed out that consideration has been given to bringing such birds under regulation of the Animal Welfare Act, but has been dropped as being impractical at this time.

The meeting was adjourned at 4:30 p.m.
ARTICLE I—NAME

The name of this Association shall be "The United States Animal Health Association," a non-profit association.

ARTICLE II—PURPOSE

The purpose of this Association shall be the study of animal health science, milk and meat hygiene, and the dissemination of information relating thereto, the unification so far as possible of laws, regulations, policies, and methods pertaining to milk and meat hygiene, and to the prevention, control, and eradication of transmissible animal diseases; to maintain coordination among the various animal health regulatory organizations, and to serve as the animal health science clearing house between this Association and the following: The livestock owner, the animal health scientist, the milk and meat hygienist, the veterinary practitioner, the transportation and stockyard companies, the milk and meat producing and distributing companies, and various other interested agencies. The word "animal" as herein used shall be understood to include poultry.

ARTICLE III—MEMBERSHIP

There shall be five kinds of members: Official, allied organization, official membership.

OFFICIAL MEMBERSHIP

The animal health departments of each state, also the United States, and the Canadian, and Mexican governments, Puerto Rico, the Virgin Islands, and Los Angeles County, California, and of such other governmental units as the Executive Committee may be a two thirds vote approve, shall be eligible to official membership in this Association and be represented on the Executive Committee by the animal health executive official.

ALLIED ORGANIZATION MEMBERSHIP

Any nonprofit organization approved by the Executive Committee that is national in scope and activity and directly concerned with the interests and objectives of this Association as outlined in Article II—Purpose, may be elected to allied organization membership and be represented on the Executive Committee by a duly authorized member of the organization. Such organizations applying for membership shall have and shall continue to maintain no less than 50 (fifty) individual members of the U.S. Animal Health Association to qualify.

INDIVIDUAL MEMBERSHIP

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

Any individual member who has maintained membership in this Association for 35 years, or if such member is at the point of retirement, for 25 years, may be elected to life membership by the Executive Committee. Such life membership shall carry with it all the rights and privileges of regular individual membership, including receipt of the Annual Proceedings of this Association. Such life membership shall be exempt from the payment of dues or any other assessments. All past presidents shall automatically become life members. Members of the Executive Committee will be eligible for such life membership; but for such member, the requirements for maintaining
individual membership will be waived. But the period of time for such membership will be as herein provided.

The Executive Committee may, at its discretion, confer honorary individual memberships. Such memberships shall be exempt from the payment of dues or other assessments and may be withdrawn at the discretion of the Executive Committee.

ELECTED REGIONAL DELEGATE MEMBERSHIP

Such elected regional delegates as provided for in Article V — Executive Committee shall by virtue of such election automatically become members of this organization for such term or terms as may be decided by the Executive Committee and shall pay such dues as the Executive Committee may decide.

NONVOTING JUNIOR MEMBERSHIP

Students in agriculture, medicine, veterinary medicine, vocational agriculture, or any 4-H Club member, as well as future farmers under 21 years of age are eligible to election as nonvoting junior members.

ARTICLE IV — MEETINGS

The meetings of this Association shall be annual and special.

ARTICLE V — OFFICERS

The officers of this Association shall be: President, President-Elect, First Vice-President, Second Vice-President, Third Vice-President, Secretary, Treasurer, Board of Directors, and an Executive Committee.

BOARD OF DIRECTORS

The Board of Directors shall consist of the officers, including the immediate Past President with the exception of the Executive Committee. It shall handle the financial, administrative, and internal affairs of the Association during such time as the Association and/or the Executive Committee is not in session. It shall handle all other duties and responsibilities as may be assigned to it by the Executive Committee or as may be provided in the Constitution. The Board of Directors shall meet immediately after the adjournment of each annual meeting of this Association and at the same place. The purpose of such meeting is to review plans for the administrative functions of the Secretary for the coming year, to give administrative guidance to the Secretary, and to approve the operations of the office of the Secretary. The Board of Directors may meet at such other times and places as it, by a majority vote, deems necessary. The Secretary shall keep minutes of all meetings of the Board of Directors, and after approval of such minutes by the president, they shall be presented to the Executive Committee at the next annual meeting of this Association.

EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the executive officer representing the animal health departments of the various states, the principal animal health officer of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive animal health officer of Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, and of such other governmental units as may be approved for official membership by the Executive Committee, the elective officers of this Association, not more than eight (8) delegates at large representing the livestock industry, including poultry, and allied organization members. All past presidents in attendance not included in any other section shall be ex-officio members.*

* For the purpose of having proper credentials, the name of the Executive Committee representative or substitute, if applicable, shall be provided to the Association Secretary by the executive officer of those entities named herein.
There shall be five districts. Said districts shall be known as (1) the Northeast: consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont; (2) The North Central: consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; (3) The Southern: comprising the States of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico, and the Virgin Islands; (4) The Western district: consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming; (5) the District-at-Large: consisting of Allied Organization Members and all Elected Regional Delegate Members.

Each district, as provided above, shall, on a rotating basis, annually submit to the Nominating Committee, nominees for vacancies that shall occur in the following offices: President; President-Elect; First Vice-President; Second Vice-President; Third Vice-President. The order of rotation shall be as follows: Northeastern; Western; Southern; Region-at-Large; North Central. In the event that an elected officer is unable to complete an elected term, the District that originally submitted the nominee shall have the opportunity to resubmit a nominee to fill the vacancy; or, the provisions of Article VII — Duties of Officers shall apply.

The elected officers shall have the authority to place before the Executive Committee applications for allied organization membership. Not more than five (5) such applications shall be presented to the Executive Committee for consideration at any annual meeting of The United States Animal Health Association.

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies. All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee.

The President-Elect shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary for the Association. The Secretary shall receive such salary and allowance as may be fixed by the Executive Committee.

The Executive Committee shall cause to be audited annually, or oftener if deemed necessary, the receipts and disbursements of the Secretary and of the Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall accept or reject applications for individual and for allied organization membership properly placed before it. Three negative votes shall disqualify for either such membership.

That, with the exception of a change in the name of this Association, upon the dissolution of this corporation or the termination of activities thereof, all remaining assets thereof shall be contributed for utilization in the advancement of research of diseases of animals, and no part of the net assets shall insure to any person or group of persons for private gain.

ARTICLE VI — PROGRAM COMMITTEE

The President, the Chairman of the Executive Committee, the Secretary, the Treasurer, and the Chairmen of the respective committees shall constitute the Program Committee. It shall be the duty of the members of the Program Committee to make the necessary arrangements and provide the program for the annual and special meetings.

ARTICLE VII — DUTIES OF OFFICERS

1. President: It shall be the duty of the President to preside at all meetings of this Association and of the Board of Directors; to appoint all committees excepting the Executive and officer faction of the Program Committee; to call special meetings of the Association whenever he considers the holding of such meetings necessary for the good of the livestock industry or upon written request of five members of the Executive Committee. The President shall be an ex-officio member of all committees.
The President shall officially represent this Association in such places and
at such meetings as he, with the concurrence of a majority of the Board of
Directors, deems desirable or necessary in the best interests of this
Association. He may at his discretion designate a member of the Executive
Committee to substitute for him. A report of such attendance shall be made
annually to the membership, and all actual expenses incidental thereon shall
be paid by this Association.

2. President-Elect: The President-Elect shall be chairman of the Execu-
tive Committee. In the absence of the President, he shall preside at the
meetings of the Association. In the event of the absence, disability, or
resignation of the President, he shall perform all duties of the President. He
shall be an ex-officio member of the Executive and Program Committees and
of the Board of Directors.

3. First Vice-President: The First Vice-President shall assume the duties
of the President in the event of the absence, disability, or resignation of the
President and President-Elect. He shall assume the chairmanship of the
Executive Committee in the event of the absence, disability, or resignation of
President-Elect. He shall be an ex-officio member of the Executive
Committee and the Board of Directors.

4. Second Vice-President: The Second Vice-President shall assume the
duties of the President in the event of the absence, disability, or resignation
of the President, President-Elect, and First Vice-President. He shall assume
the chairmanship of the Executive Committee in the event of the absence,
disability, or resignation of the President-Elect and First Vice-President.
He shall be an ex-officio member of the Executive Committee and of the
Board of Directors.

5. Third Vice-President: The Third Vice-President shall assume the duties
of the President in the event of the absence, disability, or resignation of the
President, President-Elect, First Vice-President, and Second Vice-President.
He shall assume the chairmanship of the Executive Committee in the event of the
absence, disability, or resignation of the President-Elect, First Vice-President,
and Second Vice-President. He shall be an ex-officio member of the Executive
Committee and of the Board of Directors.

6. Secretary: The Secretary shall keep an accurate record of the proceed-
ings of the Association. Whenever authorized so to do by the Executive Committee,
he shall publish said proceedings and distribute them to the members of the
Association. The Secretary shall also keep an accurate record of the proceedings
of the Executive Committee. He shall forward to each Executive Committee member
a copy of each regulation approved by the Association.

He shall keep an accurate account of all Association moneys received and
disbursed. All moneys due this Association received by the Secretary shall be
promptly turned over to the Treasurer, accompanied by transmittal infor-
mation identifying the amount, the source, and such other information as the
Treasurer and the Board of Directors may require. He shall draw on the
Treasurer, on proper warrants, over his signature and that of the President,
such sums as may be necessary to discharge the financial obligations of this
Association, provided however that for the payment of incidental expenses of
his office, the Secretary may draw on the Treasurer from time to time sums
not to exceed twenty-five dollars ($25) at any one time on his own authority.
He shall also present to the chairman of the Executive Committee a list giving
the name, occupation, and address of each applicant for individual member-
ship for the approval of the Executive Committee. He shall prepare forms for
applicants for allied organization membership and shall notify each of the
elected officers upon receipt of such completed application. He shall perform
such other duties as may be authorized and prescribed by the Executive
Committee. He shall be ex-officio secretary of the Executive Committee,
ex-officio secretary of the Board of Directors, and an ex-officio member and
secretary of the Program Committee. He shall be bonded for not less than ten
thousand dollars ($10,000).

6. Treasurer: The Treasurer shall keep an accurate account of all
Association moneys received and disbursed. He shall receive from the
Secretary all moneys of the Association paid directly to the Secretary along
with proper identification of such moneys. By and with the approval of the
Board of Directors, he shall deposit the funds of this Association in such
types of accounts as may be approved by the Board of Directors, and he shall
invest the funds of the Association or liquidate Association investments in
such manner as may be approved by the Executive Committee upon recommendation of the Board of Directors. He shall honor warrants for the proper expenditure of Association funds furnished him by the Secretary over his signature and that of the President. He shall honor warrants from the Secretary on the Secretary's own authority for incidental expenses of the Secretary's office in sums not to exceed twenty-five dollars ($25) for any given expenditure. He shall be given guidance and general administrative supervision by the Board of Directors, and he shall furnish the Executive Committee with a financial statement of the Association's funds annually. He shall be bonded for not less than ten thousand dollars ($10,000), and he shall receive such salary as the Executive Committee may from time to time determine.

ARTICLE VII – AMENDMENTS

The Constitution and Bylaws of this Association may be amended by a two-thirds vote of the members of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting, printed in the annual proceedings, and further provided that the amendment has received the approval of a majority of the Executive Committee members present and voting.

ARTICLE IX – ELECTION OF OFFICERS AND ELECTED REGIONAL DELEGATES

The Nominating Committee shall annually report to the membership of this Association at the first morning general session. Their recommendations for the offices of President, President-Elect, First Vice-President, Second Vice-President, Third Vice-President, and Treasurer, and Elected Regional Delegates shall constitute their report. Nominations shall not originate within this committee but shall be submitted by the appropriate district. Said recommendations shall be posted on the registration bulletin board immediately following their presentation. Any member may propose amendments to the slate presented by the Nominating Committee prior to, or at the second morning general session.

The report of the Nominating Committee, and proposed amendments of the report shall be presented to the Executive Committee for consideration. The acceptance of the report or amendment shall constitute election.

BYLAWS

ARTICLE I – ORDER OF BUSINESS

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

A suspension of the Bylaws may be made by a two-thirds majority for the purpose of changing the order of business or to facilitate important business.

** The phrase "at the second morning general session" shall be deemed to mean at a time certain specified in the program the Report of the Action of the Nominating Committee during that session; provided that if a paper is being presented at that specified time, its presentation will be completed, immediately after which the Nominating Committee Report will be read. Provided further, if the program is ahead of schedule for that session, a recess will be taken until the time certain established in the program for the Report of the Action of the Nominating Committee.
ARTICLE II – APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary. The application shall give the name, occupation, and address of the applicant and shall be accompanied by a fee of thirty dollars ($30) which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary, who shall in turn submit them to the Executive Committee.

Applications for allied organization membership shall be made in writing to the Secretary on an appropriate form prepared by him. In turn, notice of receipt of such application shall be provided each of the elected officers.

An individual or allied organization member may be expelled for cause by the Executive Committee. A majority vote by the members of the Executive Committee present and voting shall be required in order to expel any such member.

ARTICLE III – MEETINGS

The annual meetings shall be held in a location selected at a previous annual meeting by a majority of the members of the Executive Committee. The annual meetings shall be held in a location selected at a meeting of the geographical districts as outlined in Article V, Executive Committee, on a rotating basis as follows: North Central, Northeast, Western, Southern, and in concurrence with the executive officer of the animal health department of the state in which the meeting is proposed.

Each meeting site in the selected location shall be determined by the secretary with the approval of the Board of Directors, and in consultation with the executive officer representing the animal health department of the state in which the meeting is to be held. The Executive Committee shall be advised of said selecting at least five (5) years in advance of any annual meeting.

The annual meetings shall begin in the month of October. The Board of Directors is authorized to select an alternate location and a site in the event that the previous selections, because of any unforeseen circumstance, become unavailable and/or unacceptable. The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

ARTICLE IV – QUORUM

Twenty-five members of the Association shall constitute a quorum. Thirty members of the Executive Committee shall constitute a quorum, providing at least two-thirds of this number are executive officers representing the animal health departments of their respective states.

ARTICLE V – DUES

The dues for individual membership in this Association shall be thirty dollars ($30) per annum, payable in advance (on or before January 1st of each year) to the Secretary of the Association.

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

The dues for official and allied organization memberships shall be two hundred dollars ($200) each per annum, payable in advance (on or before January 1st each year) to the Secretary of this Association.
This Constitution and Bylaws was considered by the Executive Committee and unanimously approved on October 13-15, 1981 — St. Louis, Missouri, and approved by the membership at the 86th Annual Meeting — Nashville, Tennessee, November 9, 1982.

The interpretation of the constitution as reflected in the explanatory footnotes was concurred in the Executive Committee on October 26, 1984 at the 88th Annual Meeting in Fort Worth, Texas.
89th ANNUAL MEETING
October 27 – November 1, 1985
THE MARC PLAZA HOTEL
Milwaukee, Wisconsin

90th ANNUAL MEETING
October 19-24, 1986
EXECUTIVE WEST HOTEL
Louisville, Kentucky

91st ANNUAL MEETING
October 25-30, 1987
HOTEL UTAH
Salt Lake City, Utah