PROCEEDINGS

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ANNUAL MEETING

of the
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ANIMAL HEALTH
ASSOCIATION

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William W. Hawkins, Jr., Dillon, MT
A. L. Klingsporn, Bowie, MD
H. E. Metcalf, Lakewood, CO
C. E. Terrill, Silver Spring, MD
Donald W. Baker, Albuquerque, NM
W. A. Hickman, Pierre, SD
Howard Whitford, College Station, TX
H. A. Hancock, Laramie, WY

John Neimi, Buffalo, SD
Olin H. Timm, Dixon, CA
J. E. Pearson, Ames, IA
Richard F. Hall, Caldwell, IA
M. E. MacHeak, Ames, IA
R. E. Simmons, Boise, ID
Laird Noh, Kimberly, ID
John Morris, Denver, CO
Glenn Helm, Davis, CA
J. S. Hourrigan, Hyattsville, MD
T. L. Barber, Denver, CO
Guy E. Reynolds, Corvallis, OR

Committee on Evaluation and Development of State-Federal Programs—1976

Dr. J. L. O’Harra, Chairman, Reno, NV
Dr. Douglas R. Stauffer, Co-Chairman

J. F. Andrews, Atlanta, GA
L. E. Bartelt, Sacramento, CA
W. L. Bendix, Richmond, VA
J. R. Bishop, Tipton, IN
D. E. Flagg, Bismarck, ND
Tom Cook, Denver, CO
H. E. Goldstein, Reynoldsburg, OH
J. L. Hourrigan, Hyattsville, MD

T. A. Ladson, Annapolis, MD
Robert Larramore, Gillette, WY
E. A. Schilf, Hyattsville, MD
H. Q. Sibley, Austin, TX
O. H. Timm, Dixon, CA
W. C. Tobin, Denver, CO
C. D. VanHouweling, Washington, DC
Paul Zillman, Hinsdale, IL

A. E. Janawicz, Montpelier, VT

Committee on State-Federal Relations—1976

Dr. A. E. Janawicz, Chairman, Montpelier, VT

H. E. Goldstein, Columbus, OH
L. E. Bartelt, Sacramento, CA
W. L. Bendix, Richmond, VA
J. F. Andrews, Atlanta, GA

J. C. Shook, Frederick, MD
T. A. Ladson, Annapolis, MD
D. H. Spangler, Lacey, WA
T. F. Zweigart, Raleigh, NC

N. R. Swanson, Cheyenne, WY
Committee on Transmissible Diseases of Poultry—1976

Dr. R. A. Bankowski, Chairman, Davis, CA
Dr. W. Butterfield, Co-Chairman, Plum Island, NY

E. S. Bryant, Storrs, CN
Lloyd Jones, Hopkinsville, KY
A. H. Dardiri, Greenport, L.I., NY
F. Golan, College Station, TX
J. E. Hanley, Dade City, FL
A. E. Janawicz, Montpelier, VT
W. E. Merritt, Washington, DC
W. C. Patterson, Athens, GA
W. C. Schofield, St. Louis, MO
C. J. Pfo, Hyattsville, MD
James B. Roberts, Muldrow, OK
T. B. Ryan, Cary, NC
John A. Smiley, Augusta, ME
H. W. Towers, Dover, DE
F. G. Buzzell, Augusta, ME
A. E. Docteau, Waltham, MA
J. B. Thomas, Columbia, SC
L. C. Grumbles, College Station, TX
R. L. Hogue, Lafayette, IN
T. L. Landers, Hot Springs, AR
H. E. Nadler, Albany, NY
I. L. Peterson, Beltsville, MD
B. S. Pomeroy, St. Paul, MN
A. S. Rosenwald, Davis, CA
Porter Halbert, San Augustine, TX
R. McCapes, Davis, CA

Committee on Transmissible Diseases of Swine—1976

Dr. E. A. Butler, Chairman, Des Moines, IA
Dr. T. F. Zweigart, Co-Chairman, Raleigh, NC

E. H. Bohl, Wooster, OH
John R. Ragan, Nashville, TN
W. C. Stewart, Ames, IA
Norman Kruse, Lincoln, NE
D. P. Gustafson, Lafayette, IN
R. E. Hall, Madison, WI
R. E. Thompson, Hyattsville, MD
J. E. Fox, Ashland, OH
M. Ristic, Urbana, IL
Don Brothers, Paducah, TX
E. O. Haelterman, Lafayette, IN
Taylor Woods, Jefferson City, MO
Don L. Larson, Brookings, SD
Robert Glock, Ames, IA
John P. Kluge, Ames, IA
Lowell Hinchman, Indianapolis, IN
Tom Powell, Athens, GA
Fred Wertman, Des Moines, IA
Don L. Kruger, Olympia, WA
Howard Hill, Ames, IA

Committee on Tuberculosis and Paratuberculosis—1976

Dr. P. L. Smith, Chairman, Sacramento, CA
Dr. A. R. McLaughlin, Co-Chairman, Madison, WI

R. W. Bennett, Hyattsville, MD
Neal Black, St. Paul, MN
Joseph L. Blair, Washington, DC
Carl E. Boyd, Columbia, SC
Albert M. Carey, Beltsville, MD
Charles S. Duncan, Albany, NY
J. G. Flint, St. Paul, MN
G. H. Frye, Hyattsville, MD
D. W. Johnson, Roseville, MN
A. F. Kaufmann, Atlanta, GA
Victor LaBranche, Boston, MA
A. B. Larsen, Ames, IA
W. L. Mallmann, East Lansing, MI
A. P. Schneider, Boise, ID
G. R. Snyder, Washington, D.C.
G. W. Spangler, Des Moines, IA
R. J. Stadler, Hartford, CT
R. M. S. Temple, Bristolville, OH
Charles Thoen, Ames, IA
K. M. Weinland, Lafayette, IN
Lindsey Horn, Chicago, IL
H. Q. Sibley, Austin, TX
Paul L. Spencer, Springfield, IL
J. B. Young, Austin, TX
Joseph L. McMillan, Wheatland, CA
H. E. Nadler, Albany, NY
Committee on Wild and Marine Life Diseases—1976

Dr. Frank A. Hayes, Chairman, Athens, GA

Don E. Cooperrider, Kissimmee, FL
Ahmed H. Dardiri, Greenport, L.I., NY
Joe T. Finley, Jr., Encinal, TX
Harry G. Geyer, Washington, DC
Lynn A. Griner, San Diego, CA
Andrew H. Hulsey, Little Rock, AR
Theodore P. Kistner, Corvallis, OR
Stewart H. Madin, Berkeley, CA

Benjamin S. Pomeroy, St. Paul, MN
Robert E. Putz, Washington, DC
John R. Ragan, Nashville, TN
James S. Smith, Hyattsville, MD
Gilberto S. Trevino, Hyattsville, MD
James B. White, Cheyenne, WY
Lonnie L. Williamson, Washington, DC
Gilliam G. Winkler, Lawrenceville, GA

Committee on Zoological Gardens—1976

Dr. R. M. S. Temple, Chairman, Aurora, OH

Dr. Dale Swindaman, Co-Chairman

George Pearson, Washington, DC
Gordon Hubbell, Miami, FL
Don D. Farst, Brownsville, TX
Jim Banks, Lorena, TX

George Becker, Jr., Orlando, FL
Gordon Hubbell, Miami, FL
Don D. Farst, Brownsville, TX
Jim Banks, Lorena, TX

Committee on Food Animal Hygiene and Inspection—1976

Dr. Walt Fechner, Chairman, Little Rock, AR *

Dr. E. Baker, Chairman, Madison, WI

Dr. James K. Payne, Co-Chairman, Washington, DC

David Bedell, Athens, GA
Vernon Coiner, Boise, ID
W. H. Dubbert, Washington, DC
Jim Fowler, San Francisco, CA
M. R. Humphrey, Washington, DC
D. C. Kelley, Manhattan, KS
M. R. Levy, Philadelphia, PA
Mrs. Joan Donnelly, Elizabethtown, PA
Miss Sarah Brite, Washington, DC
L. J. Raforth, Alameda, CA
James A. Bell, Raleigh, NC

D. D. Breeden, Lincoln, NE
C. T. Domingues, Lansing, MI
J. W. Holcombe, Oklahoma City, OK
E. E. Kerr, San Francisco, CA
R. L. Parker, Atlanta, GA
M. A. Simmons, Denton, TX
R. J. Lee, College Park, MD
J. A. Libby, St. Paul, MN
Gary Smith, College Station, TX
Henry Woodard, Atlanta, GA
George Estes, Richmond, VA

* Deceased
## RECORD OF PREVIOUS MEETINGS

<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<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. 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>Dec. 2-4, 1918</td>
<td>Chicago, Ill.</td>
<td>*Dr. M. Jacob, Knoxville, Tenn.</td>
<td>*Mr. S. H. Ward, St. Paul, Minn.</td>
</tr>
<tr>
<td>Nov. 28-30, 1921</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. F. Crewe, Bismarck, N.D.</td>
<td>*Dr. D. M. Campbell, Chicago, Ill.</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. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Name</td>
<td>City</td>
</tr>
<tr>
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<td>-----------------------------</td>
</tr>
<tr>
<td>33. Dec. 4-6, 1929</td>
<td>Chicago, Ill.</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
<td></td>
</tr>
<tr>
<td>34. Dec. 3-5, 1930</td>
<td>Chicago, Ill.</td>
<td>*Dr. A. E. Wright, Wash., D.C.</td>
<td></td>
</tr>
<tr>
<td>36. Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
<td></td>
</tr>
<tr>
<td>37. Dec. 6-8, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N.Y.</td>
<td></td>
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<tr>
<td>38. Dec. 5-7, 1933</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Robinson, Providence, R.I.</td>
<td></td>
</tr>
<tr>
<td>43. Dec. 6-8, 1938</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
<td></td>
</tr>
<tr>
<td>44. Dec. 4-6, 1939</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wy.</td>
<td></td>
</tr>
<tr>
<td>45. Dec. 3-5, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. A. Crossman, Boston, Mass</td>
<td></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></td>
</tr>
<tr>
<td>49. Dec. 5-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
<td></td>
</tr>
<tr>
<td>50. Dec. 4-6, 1946</td>
<td>Chicago, Ill.</td>
<td>*Dr. William Moore, Raleigh, N.C.</td>
<td></td>
</tr>
<tr>
<td>53. Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. O. Brandenburg, Bismarck, N.D.</td>
<td></td>
</tr>
<tr>
<td>57. Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>*Dr. T. Childs, Ottawa, Canada.</td>
<td></td>
</tr>
<tr>
<td>60. Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------</td>
<td>-----------------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, Fla</td>
<td>Dr. John G. Milligan, Montgomery, Ala</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>63. Dec. 15-18, 1959</td>
<td>San Francisco, Calif</td>
<td>Mr. F. G. Buzzell, Augusta, Me</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>64. Oct. 17-21, 1960</td>
<td>Charleston, W.Va</td>
<td>Dr. J. R. Hay, Chicago, Ill</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>65. Oct. 3-Nov. 1-3, 1961</td>
<td>Minneapolis, Minn</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, N.M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R.I.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>70. Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>71. Oct. 16-20, 1967</td>
<td>Phoenix, Ariz.</td>
<td>Dr. Grant S. Kaley, Albany, N.Y.</td>
<td>Dr. W. L. Bendix, Richmond, Va</td>
</tr>
<tr>
<td>72. Oct. 6-11, 1968</td>
<td>New Orleans, La.</td>
<td>Dr. John F. Quinn, Lansing, Mich</td>
<td>Dr. W. L. Bendix, Richmond, Va</td>
</tr>
<tr>
<td>76. Nov. 5-10, 1972</td>
<td>Miami Beach, Fla</td>
<td>J. C. Shook, Mechanicsburg, Pa</td>
<td>Dr. W. L. Bendix, Richmond, Va</td>
</tr>
<tr>
<td>77. Oct. 14-19, 1973</td>
<td>St. Louis, Mo</td>
<td>W. C. Tobin, Denver, Colo</td>
<td>Dr. W. L. Bendix, Richmond, Va</td>
</tr>
</tbody>
</table>

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

A. A. Erdmann, D.V.M.

Heavenly Father, we thank Thee for the privilege of being together this evening at the 79th Annual Meeting of the United States Animal Health Association.

We pray that you will continue to guide and aid us in the decisions we must make concerning ourselves and those with whom we live and work.

Teach us to understand that man's ultimate happiness depends on his concern and desire to seek wisdom and comprehension for living and sharing with all mankind.

May we always handle our responsibilities at work, in our home, in our church, in our tasks and in our community in a manner that will be pleasing to Thee.

We pray for our nation and its leaders and ask that you grant to them the knowledge, wisdom and leadership to make these decisions that will be beneficial to this nation.

Amen
MEMORIAL SERVICE

A. A. Erdmann, D.V.M.
Madison, WI

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

At this time, as is our custom, we pause for a moment to pay tribute to those esteemed colleagues who are now in our book of memories and another greater and better world. They are:

Dr. Chester Manthei (Michigan State University)
Died August 17, 1975

Dr. Guy W. Eberhardt (Georgia)
Died September 14, 1975

Dr. James R. Porteus (New York State)
Died December 20, 1974

Dr. John Poole
Died Spring, 1975

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

Amen
REPORT OF THE SECRETARY

W. L. Bendix, D.V.M., Richmond, Virginia

During the 1975 fiscal year just past it is a pleasure to report that 710 individual members paid dues as compared to 610 for the previous fiscal year. This is of course, an even one hundred gain. This is due largely to the efforts of our Treasurer, Dr. John Shook. His diligence at our meetings and throughout the year is to be highly commended.

We currently have 36 applications for individual members which will be presented for action at this meeting.

Since amending our Constitution to admit industry organizations to, “Allied Organization Membership,” each with a seat on our governing body, The Executive Committee, we have approved the admission of ten such members. One more will be presented this year for approval and we are expecting one additional shortly. This clearly indicates wide industry support of our policies and active participation of industry in making policy. Our Association is much the better for it.

Treasurer Shook will bring the membership up to date on our finances in just a few moments. Inflation continues as do our problems related directly to it. Ten new active members from each state would, however solve most of our dilemma and permit us to provide some additional services which are needed.

It has been nearly ten years since our Constitution and Bylaws were brought up to date. An ad hoc committee has been appointed to suggest whatever revisions may be necessary to again up date the Constitution. A preliminary meeting was held yesterday. We solicit your comments and suggestions (in writing, please) what should be added, deleted, changed? Contact Dr. T. J. Grennan, Chairman, or the Secretary. Nothing will be presented in all probability until next year so you have all of the 1976 fiscal year to think about it and act. Remember, the Executive Committee wants your association to reflect your wishes, so speak or better still, write your mind.

We have 32 regular standing committees and generally one or two ad hoc groups working. New committees are added as need arises. The Secretary is well aware of the importance of the work of each committee. It is vital to our work that all segments of industry and any others who may be affected by our activities and programs be given opportunity to state their views and make suggestions and offer criticisms. Our committees are the forum for this, and must continue to function as such. The Secretary is also aware that there are other reasons that a direct interest or concern relating to the subject matter of a committee’s principal area of debate that prompts committee membership. There is however a limit to committee size which is related directly to committee effectiveness. It is hoped that
each chairman will carefully review his committee membership with this in mind. This subject is also respectfully suggested to the ad hoc committee (sic!) on the Constitution and Bylaws for their consideration.

Virginia was delighted to be your host for the 1974 meeting held at Roanoke. We are still getting favorable comments about that session. Hotel Roanoke wants you back. The Secretary hopes this week in Portland will prove both pleasant and instructive. Anything we at the desk can do to make your stay more satisfactory is yours for the askings. The local group and Dr. Rea have worked hard to insure a successful week.

Next year we go back to the Americana of Bal Harbour at Miami Beach, Florida. After much trading back and forth we have secured the week, November 7-12, 1976. This is the week after election day and is just about the right time for our gathering. As a part time resident of Florida, the Secretary wants to extend a cordial invitation to one and all to come down to the Sunshine State in 1976 and enjoy life.

Our 81st Annual meeting in 1977 is set for October 16-21 in Minneapolis, Minnesota at the Radisson Hotel, Downtown. The Executive Committee had tentatively selected Rapid City, South Dakota as the 1977 meeting site provided this site was satisfactory as to accommodations and all the other requirements that we must have for a successful meeting. Minneapolis was suggested as the alternate city.

When Mrs. Blanton scheduled a trip this spring to Portland to begin laying out the format for this meeting the Secretary (in the interest of economy) asked her to drop off at both of these places. Dr. M. D. Mitchell of South Dakota was most considerate and helpful, but circumstances suggested that Rapid City would not be the location of choice. Transportation, accommodations, meeting room costs, all created some problem. We have, and we hope with your approval, have selected Minneapolis. The site for our 1978 meeting will be selected at this meeting. The priority for recommendation falls to the Northeast region. The priority for selection for 1979 will fall again to the Western region and should be presented at Miami Beach next year.

Thank you and have a good week.

Respectfully submitted,
W. L. Bendix, D.V.M.
Secretary
UNITED STATES ANIMAL HEALTH ASSOCIATION  
1910 BYRD AVENUE, ROOM 118  
RICHMOND, VIRGINIA 23230

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR  
PERIOD OCTOBER 1, 1974 THROUGH SEPTEMBER 30, 1975

CASH BALANCE—OCTOBER 1, 1974:

<table>
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<tr>
<th>Account</th>
<th>Amount</th>
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<tr>
<td>Southern Bank and Trust Company</td>
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<tr>
<td>Checking Account</td>
<td>$328.42</td>
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<tr>
<td>Savings Account</td>
<td>2,811.64</td>
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<tr>
<td>Local Arrangements</td>
<td>151.95</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>$3,292.01</strong></td>
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<td>Trevose Savings and Loan Association</td>
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<tr>
<td>Morrisville, Pennsylvania</td>
<td>1.00</td>
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<tr>
<td>Sandia Savings and Loan Association</td>
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<tr>
<td>Albuquerque, New Mexico</td>
<td>1.00</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>$3,294.01</strong></td>
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INCREASED BY CASH RECEIPTS:

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<tr>
<th>Item</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Individual Dues</td>
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<tr>
<td>Official Dues</td>
<td>9,150.00</td>
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<tr>
<td>Proceedings</td>
<td>5,003.83</td>
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<tr>
<td>Reprints</td>
<td>2,783.98</td>
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<tr>
<td>Foreign Animal Books</td>
<td>75.00</td>
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<tr>
<td>Junior Membership Dues</td>
<td>6.00</td>
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<tr>
<td>Registration Fees</td>
<td>16,223.30</td>
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<tr>
<td>Tours</td>
<td>712.50</td>
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<td>Interest Income</td>
<td>762.24</td>
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<td>Sale of one U. S. Treasury Bond</td>
<td>8,504.17</td>
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<tr>
<td>Transferred from</td>
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<tr>
<td>Local Arrangements Account</td>
<td>151.95</td>
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<tr>
<td>Miscellaneous—Postage</td>
<td>2.70</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>57,095.67</strong></td>
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TOTAL BEGINNING BALANCE AND RECEIPTS   **$60,389.68**
UNITED STATES ANIMAL HEALTH ASSOCIATION
1910 BYRD AVENUE, ROOM 118
RICHMOND, VIRGINIA 23230

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR PERIOD OCTOBER 1, 1974 THROUGH SEPTEMBER 30, 1975

DECREASED BY EXPENDITURES:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
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<tr>
<td>Annual Meeting</td>
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<td>Printing</td>
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<td>Office Supplies</td>
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<td>Salaries</td>
<td>11,800.08</td>
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<tr>
<td>Social Security Tax</td>
<td>494.00</td>
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<tr>
<td>Communication</td>
<td>2,727.99</td>
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<tr>
<td>Travel:</td>
<td></td>
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<tr>
<td>Dr. Harry E. Goldstein</td>
<td>339.48</td>
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<td>Dr. J. C. Shook</td>
<td>96.32</td>
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<tr>
<td>O. H. Timm</td>
<td>810.00</td>
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<tr>
<td>Bob Laramore</td>
<td>219.11</td>
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<tr>
<td>Dr. W. L. Bendix</td>
<td>1,250.74</td>
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<tr>
<td>Ella R. Blanton</td>
<td>377.99</td>
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<tr>
<td>Rent—Office Space</td>
<td>1,810.79</td>
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<tr>
<td>Other Meetings</td>
<td>85.00</td>
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<tr>
<td>American Association of Veterinary</td>
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<tr>
<td>Livestock Diagnosticians</td>
<td>3,750.00</td>
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<tr>
<td>Virginia Unemployment Insurance</td>
<td>5.92</td>
</tr>
<tr>
<td>Surety Bond—Treasurer</td>
<td>50.00</td>
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<tr>
<td>Miscellaneous Expense</td>
<td>374.48</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>26.43</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>$52,282.16</strong></td>
</tr>
</tbody>
</table>

CASH BALANCE—SEPTEMBER 30, 1975:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash on Hand—September 30, 1975</td>
<td>$137.46</td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
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<tr>
<td>Richmond, Virginia</td>
<td></td>
</tr>
<tr>
<td>Checking Account</td>
<td>187.73</td>
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<tr>
<td>Savings Account</td>
<td>7,780.33</td>
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<tr>
<td></td>
<td><strong>$8,105.52</strong></td>
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<tr>
<td>Trevose Savings and Loan Association</td>
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</tr>
<tr>
<td>Morrisville, Pennsylvania</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td></td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td><strong>8,107.52</strong></td>
</tr>
</tbody>
</table>
UNITED STATES ANIMAL HEALTH ASSOCIATION
1910 BYRD AVENUE, ROOM 118
RICHMOND, VIRGINIA 23230

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1974 THROUGH SEPTEMBER 30, 1976

REVENUE:

Total Cash Receipts $ 57,095.67
LESS—Expenditures 52,282.16
Excess of Receipts over Expenditures $ 4,813.51

NET WORTH—SEPTEMBER 30, 1975

Cash on Hand—September 30, 1975 $ 137.46
Accounts Receivable 1,110.90

Balance:
Southern Bank and Trust Company, Richmond, Virginia
Checking Account 187.73
Savings Account 7,780.33

Balance:
Trevose Savings and Loan Association
Morrisville, Pennsylvania 1.00

Balance:
Sandia Savings and Loan Association
Albuquerque, New Mexico 1.00

Petty Cash Fund 25.00
Deposit—C. & P. Telephone Company
Richmond, Virginia 100.00
Inventory—Supplies and Proceedings 6,500.00
Furniture and Fixtures 1,265.33

NET WORTH—SEPTEMBER 30, 1975 $ 17,108.75
UNITED STATES ANIMAL HEALTH ASSOCIATION
1910 BYRD AVENUE, ROOM 118
RICHMOND, VIRGINIA 23230

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR
PERIOD OCTOBER 1, 1974 THROUGH SEPTEMBER 30, 1975

ANALYSIS OF CHANGE IN NET WORTH:

Net Worth—September 30, 1974  $ 30,274.93
Increased by:
   Excess of Receipts over Expenditures  4,813.51
   ________________________________
   $ 35,088.44

Decreased by:
   Sale of one U. S. Treasury Bond  $10,000.00
   Decrease in Accounts Receivable  7,339.10
   Decrease in Inventory—Supplies and
   Proceedings  500.00
   Depreciation—Furniture and
   Fixtures  140.59  17,979.69
   ________________________________
   $ 17,108.75

NET WORTH—SEPTEMBER 30, 1975

Henry H. Budd
Accountant
UNITED STATES ANIMAL HEALTH ASSOCIATION
1910 BYRD AVENUE, ROOM 118
RICHMOND, VIRGINIA 23230

FOR PERIOD OCTOBER 1, 1974 THROUGH SEPTEMBER 30, 1975

ANALYSIS OF MISCELLANEOUS EXPENSE:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditing Books</td>
<td>$250.00</td>
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<td>Safe Deposit Box Rent</td>
<td>6.00</td>
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<td>Lettering Certificates</td>
<td>2.00</td>
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<tr>
<td>Flowers</td>
<td>33.08</td>
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<td>Printing Checks</td>
<td>8.40</td>
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<td>Dues—Dr. W. L. Bendix (A. V. M. A.)</td>
<td>75.00</td>
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<td><strong>TOTAL</strong></td>
<td><strong>$374.48</strong></td>
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</tbody>
</table>
On behalf of Governor Bob Straub, the Oregon Department of Agriculture, and its Animal Health Division, I welcome you to Oregon and our metropolis of Portland.

Portland, as you probably know, is known as the City of Roses. Unfortunately, you are here the wrong time of the year for us to display our floral wonders to you, but I hope you have noticed that some of our foliage has taken on its brightest hues for you.

And, our weather has cooperated by giving us a respite from our recent rains so you could have an opportunity to enjoy some of the beauties of the state.

I understand this is the first time in the 79 years of your Association's existence that you have met in Oregon. Of course, we think you have been missing out on a lot of very special attractions that nature has bestowed upon this state.

Our state veterinarian, Dr. Rea, being the determined and persuasive person that he is, I wonder how he's allowed you to pass us by for this long. He's been with us over 13 years. Has it taken him that long to sell Oregon to you?

As an agricultural state—agriculture is the state's second largest industry—timber leads us—and, with close to 30 percent of Oregon's income in 1974 coming from its livestock industry, we have a very special interest in the work of the U.S. Animal Health Association and the animal health programs it has promoted.

In the role of an advisory body to the federal government in establishing policies and procedures having to do with prevention, control and eradication of animal diseases through supportive action for research programs and their financing, you have been able to instigate programs that have cut losses in animals and in dollars and cents.

To name a few of the animal diseases eradicated as a result of programs recommended by your association—hog cholera; sheep scabies; foot and mouth disease (this twice); vesicular stomatitis; exotic Newcastle disease; Venezuelan equine encephalomyelitis and vesicular exanthema in swine.

Another area where great strides have been made in controlling a disease is in brucellosis eradication. This has been a tough one and we are going to have to work diligently if we are to completely eradicate it. Oregon was accredited as brucellosis free November 1, 1971, and some 90 percent of the nation now has that status.
That other 10 percent poses a continual threat and can cause reversals.

Your association has put forth a great effort in trying to push this program to complete eradication. It will take complete cooperation on the part of all of us to reach this goal.

We will have to follow an overall plan established after a “give and take” deliberation and make sure it is a plan that will be followed by all of us. We should make no exceptions.

We also need to be cognizant of the fact that federal certification as brucellosis free does not mean eradication. We have to be realistic and continue to take preventive measures.

Vaccination should not be discontinued. There are too many herds with too many unprotected cattle.

Our program needs to be one that does not strangle our beef industry by too many regulations but, on the other hand, guards against loss of the $850 million worth of progress we have already made in controlling brucellosis.

As a group representing not only livestock and animal health regulatory people but research, management, education, livestock technicians, laboratory technicians, and livestock marketing people, you have expertise to develop technically sound and workable disease control programs.

You also can lead the way in developing uniformity among states in their approach to disease control and eradication programs. Such uniformity could alleviate many frustrations and also reduce economical hardships for the livestock industry.

Regardless of your previous frustrations in this area, I urge you to continue your efforts. The livestock industry needs help. If you have problems of a political or administrative nature bring them to the National Association of State Departments of Agriculture. Let us see if we can help you.

Our northwest states have taken steps to develop uniformity on an area basis to the extent that our laws and regulations will permit. This may be a temporary answer until we can come up with uniformity that is more inclusive.

I know that the problems you face are not simple. They are complex and you may run into barriers. But your group has the expertise to develop programs and put them in a usable form.

However, one word of caution. Guard against “killing the patient with the cure”.

It has been a privilege to welcome you here and meet with you tonight. Now I leave you in the hands of Dr. Rea, Bert Hawkins, and other Oregon members of your association, who will serve as your hosts during your stay here.
RESPONSE TO ADDRESS OF WELCOME

C. L. Campbell, D.V.M., Tallahassee, Florida

President Andrews, Director Kunzman, Mr. Hawkins, members of the United States Animal Health Association and the American Association of Veterinary Laboratory Diagnosticians, ladies and gentlemen:

During the twenty-odd years in which I have been a member of this association, I have had the opportunity of hearing quite a number of Addresses of Welcome—and about an equal number of Responses to Addresses of Welcome. I use the phrase “about an equal number of Responses”, in that seldom are we privileged to being welcomed by more than one of the host state’s outstanding citizens. However, upon this occasion of the Seventy Ninth Annual Meeting of the USAHA and the Eighteenth Annual Conference of the AAVLD, I am most gratified to have been selected to respond to such a gracious twofold proffer of hospitality. In doing so, I only hope that I am capable of portraying to both of you the sincere appreciation of the membership of our two organizations as we partake of your generosity during this week’s stay in Oregon.

Tonight represents the third time that I have been honored by our program committee to assume the role of expressing the associations’ gratitude to our host, and I must say, quite candidly, that upon each occasion I have looked forward to it with greater anticipation. Let me amplify that statement somewhat. Director Kunzman asked me while we were having dinner a few minutes ago, “How does one respond, etc.” I told him, “Hopefully, with dexterity.” Except for those speeches which are impromptu in nature, I suppose that every speaker who prepares a presentation avails himself of a quantity of resource material so as to be in a position to more ably discuss his topic. A Response to an Address of Welcome, while perhaps impromptu in some respects, is really no different in that the responder, in addition to possibly wishing to advise his host of some of the purposes and accomplishments of his own organization, might also arm himself with pertinent data concerning the host’s state or community. This, then, has given me the chance to not only revisit the past history of our association, but it has also afforded me the pleasant opportunity, through various library references, to become better acquainted with your fine State of Oregon. Consequently, I dare say, with the exception of those native “Oregonians” in attendance, that I have had more exposure to the Beaver State than anyone else here tonight. In fact, it’s probably safe to say that outside of Director Kunzman, and Bert Hawkins, and possibly Glenn Rea, I’m probably the only one here who knows that over 96% of the nation’s filbert nuts are produced right here in Oregon. That’s a little gem that I picked up from the
latest American Almanac. Come to think of it, Bert, most of these flatlanders probably don't even know what a filbert is!

But speaking of filberts and, most assuredly, I am not belittling this important agricultural crop, if you'll pardon a personal reference, my first exposure to them was ten years ago when our Secretary, Dr. Bendix, and I were out here in Portland attending a meeting of the American Veterinary Medical Association. Since we had some free time on our hands one afternoon, we decided to rent a car and take advantage of viewing some of the surrounding rural scenery. Being an Arabian Horse fancier, I also had an interest in seeing some of the examples of that breed which were being produced in this neck of the woods. And let me tell you that I saw some really fine specimens of the same family lineage that I was raising back in Tallahassee, just about a stone's throw from here out in the Willamette Valley on Jim Brown's Kelvin Groves Stock Farm. But, what added so picturesquely to this setting were the hundreds of filbert trees interspersed between the various pastures housing these beautiful animals. That, then, was my first opportunity to see at least two phases of Oregon's agricultural endeavors—Arabian horses and nuts. I might add, however, that during the past couple of years I've had additional verbal exposure to the elegance of this breed from another of your state's Arab "nuts". As one who is also "hooked" on the breed, I use the term in a kindred and most affectionate context, Mr. Kunzman, in that this lovely lady of exceptional discernment, I might add, to whom I refer is Bert Hawkins' wife, Helen, who also raises Arabians here in Oregon.

As you pointed out, this year represents the first time that our associations have met in your beautiful state and, in fact, Mr. Kunzman, but the second time in the seventy nine years of the existence of the parent organization, that we have convened on the West Coast. For a number of years, three to four decades ago, our predecessors felt that it was imperative to center our deliberative activities in the hub of the country's livestock circles—namely Chicago. I am sure, at the time, that the rationale for arriving at such a decision was sound, for this location in the Mid-west not only represented the focal point of the nation's "bread-basket", but it also afforded relatively easy access to most of the participants in those days. Wisely, however, in 1948 we decided to put the association on wheels; thus, according producers from throughout the United States the opportunity of input in the deliberations of our some twenty-eight livestock health committees. Thus, as we hopefully arrive at the proper conclusions in framing national livestock health programs, the membership now has the decided benefit of nationwide, if not universal, thinking in developing these solutions. Certainly, this year, as our membership squares off to face the challenge of that seemingly omnipresent foe, "brucellosis", this meeting should be long remembered in the annals of this association. In my opinion—and I am sure that I echo the
thoughts of most of us—the United States Animal Health Association now stands at the cross-roads of decision with relation to this disease. I believe that we will determine this week whether our course will be one of eradication, or one of complete program abandonment. With producers and regulatory officials alike voicing greater dissatisfaction with the program of coexistence as usual, I look for a dramatic transformation of our present concepts within these next few days.

Nonetheless, Director Kunzman and Mr. Hawkins, for those of us who are not totally embroiled in association affairs and who will avail ourselves of the opportunity of partaking of the many pleasures offered in this fine city and great state, I am sure that by the end of our stay we will look back with great pleasure on our experiences. And, may I again express the sincere gratitude of both associations for your gracious invitation and hospitable warmth.
PRESIDENT ELECT'S ADDRESS

H. E. Goldstein, D.V.M., Columbus, Ohio

Members of the United States Animal Health Association, Members of the American Association of Veterinary Laboratory Diagnosticians, Distinguished Guests, Ladies and Gentlemen—

This has to be the most significant honor and responsibility bestowed upon me in my public career in veterinary medicine. I sincerely thank the membership of this association for the honor. I thank all of my colleagues, both within the North Central Association and outside, for their confidence and reassurance. And also my wife Ann who has been most tolerant and cooperative over the past 25 years of my leaving home for one week each year. It would be most naive for me to state that the Presidency of this illustrious association was not one of my goals and objectives, but it is with a great deal of humility that I accept this challenge and responsibility.

This humility becomes more apparent when the roster of past presidents is observed. In following in the shoes of such industrious past presidents, one is fully aware that the shoes are really large— I will try real hard to fill them. I need the cooperation and assistance of all members of this association.

The past accomplishments of this organization do not need to be reflected at this time. The livestock industries of this nation have flourished from the efforts of knowledge and program resulting from our association.

As President-Elect, my greatest concerns are for the future—What goals and objectives should be considered to provide progressive planning for the future disease programs, but even more important for the future of the U.S.A.H.A.

One of my greatest concerns over the years has been that our association for all practical purposes assumes a serious role for only one week each year. Our membership, and for the most part, our committees take on a similar role. Exceptions do exist if and when emergency situations arise, but for the most part our efforts seem to dwindle at the close of each year’s meeting.

I have had the impression that our rank and file membership, or those members representing specific livestock groups get the same impression that the U.S.A.H.A. only functions once per year. This, of course, is a most seriously mistaken impression. Our officers carry out duties all year long. Our State-Federal Relations Committee function throughout the year, and many committee chairmen continue with much effort and time. But how many are really aware of this activity?

The newsletter has been a most important vehicle in an attempt to cope with this problem which in reality is a basic communication prob-
lem. We would hope to encourage the newsletter becoming a more important tool in providing information to our members. Many individual members in my state have been most pleased with the newsletter and have been most vocal and complimentary.

We intend to put the newsletter to better use by asking the officers and committee chairmen to use it as a means of disseminating timely information.

The committee chairmen for 1976 have all been named; these chairmen have been asked to please keep the ball rolling for the entire year, rather than center on the annual meeting week. The committee chairmen have been requested to communicate with the President regularly and have been assured that the President will reciprocate.

The successful operation of this association is also dependent upon sound fiscal operations. It is no secret that our association must have additional income from membership. This will be one of our real goals and objectives for the ensuing year. We intend to use the "Key Man" approach in each state. We will designate each chief livestock disease control official with a key industry representative from the state to recruit new members. Your incoming President will ramrod this project as a pet goal and objective.

In recruitment, we must have something to sell. Our Proceedings is one of the most important disease publications printed in the world. The time of arrival for timely usage is still a great problem. We will continue the effort of reducing the lag time for this publication.

New members must have input. This will be encouraged through our committee chairman.

With industry input from new members, with an expedient time for Proceedings, with the utilization of the newsletter, and with the same excellent technical program mixed with social activities and fellowship, we can increase our membership many fold.

As President-Elect of this Association, I commend the United States Department of Agriculture for its efforts. As the State Veterinarian of Ohio, I have always been one to provide plaudits to the United States Department of Agriculture when deserving, and have always been most critical when criticism was thought necessary. This will not change.

APHIS personnel have responded most admirably in emergency disease programs, such as V.E.E. and V.V.N.D., Sheep Scabies and Hog Cholera. The livestock industries of this nation, as well as the consuming public are proud of these eradication achievements. Programs such as these point out what can be accomplished by State-Federal cooperative effort, or should I have said Federal-State cooperative effort when a crisis is paramount, when funds are available, and when industry is cooperative working for a final goal.

A special commendation should be made for the Emergency
Disease Nerve Center at Hyattsville. This complex is ready to cope with any problem, should the problem be presented. The “Nerve Center” is the most progressive regulatory disease machine found anywhere in the world.

Endemic disease programs need this same attention. Granted, the motivation is entirely different. Motivation stems from industry support, cooperative State-Federal effort and fiscal stability. Our national brucellosis eradication program needs these motivations. The State-Federal Relations Committee of this Association will continue its efforts to provide the momentum and the motivation to eradicate brucellosis.

This association has for many years recommended the use of Uniform Methods and Rules. It is not only necessary, but paramount if programming is successful.

All states must play the same game with the same rules, working toward the same ultimate goals and objectives. We have come a long way working with the principles, but it appears “we still got a long way to go.”

In my opinion, the integrity of regulatory medicine depends on our efforts to utilize Uniform Methods and Rules. We are all aware that geographical areas do indeed present distinctly different situations and problems involving herd health, incidence and attack rates, as well as mechanical and technical problems all related to the respective geographical areas or states.

Uniform Methods and Rules must take these facets into consideration. We must provide uniformity of program not only for brucellosis, but for all other programs. How confusing, for example, it is for horsemen to cope with the inconsistency of six month valid EIA Tests, or one year valid EIA Tests. Should we not be able to at least have uniformity on a requirement such as this.

Your President-Elect will charge all committee chairmen with the responsibility of providing the leadership for uniformity which will go a long way to provide industry confidence in all our efforts.

In conclusion, may I reiterate that I thank you all for your confidence. I will do my best to provide leadership, and above all will need your help.
PRESIDENT'S REMARKS


"I very humbly and gratefully accept these awards from the association and you can rest assured that I will proudly display them on every possible occasion.

"I would like to say that this has been an extra special year for me and serving you as President has been the highlight of my professional career. I would like to take this opportunity to thank the committee chairmen and committeemen for their splendid cooperation. Many of you have worked since last year's meeting on your committees and I wish to commend you for this.

"As we adjourn this annual meeting, the presidency will be turned over to Dr. Harry Goldstein, who I am sure will serve all of you very capably. Thank you."
REPORT OF THE COMMITTEE ON NOMINATIONS, RESOLUTIONS AND INTERNAL AFFAIRS

Chairman: O. H. Timm, Dixon, California

Your nominating committee convened yesterday and unanimously agreed on the following slate of officers and regional industry members for the coming year:

PRESIDENT: Dr. H. E. Goldstein—Ohio
PRESIDENT ELECT: Dr. A. E. Janawicz—Vermont
FIRST VICE-PRESIDENT: Dr. L. E. Bartelt—California
SECOND VICE-PRESIDENT: Dr. T. F. Zweigart—North Carolina
TREASURER: Dr. J. C. Shook—Pennsylvania

REGIONAL INDUSTRY MEMBERS: J. O. Pearce—Florida; Joe Finley—Texas; Francis Buzzell—Maine; E. S. Bryant—Connecticut; Bill Gallagher—South Dakota; J. R. Bishop—Indiana; Bob Lamar—Wyoming; O. H. Timm—California.

Nominations presented to general on Tuesday morning, November 4, 1975. Posted on Association bulletin board until Wednesday morning, November 5, 1975 and acted upon at Wednesday's general session as provided in the Association's Constitution.

Slate unanimously elected as presented.
Through an unprecedented joint effort between the United States and Mexico, an extensive outbreak of Foot and Mouth Disease (FMD) was contained and eradicated from the North American continent during the late 1940's and early 1950's.

Reintroduction of FMD into Panama, Central and North America would exert a disastrous effect on the entire livestock economy of this continent, with likely involvement of big game animals and other wildlife species from coast to coast.

The Federal Highway Administration's construction of a road through Panama and Colombia, to connect the North and South American continents; and in keeping with this Association's (USAHA) mutual concern for the welfare of domestic livestock and the wildlife resources of North America;

Resolution

IT THEREFORE IS RESOLVED that the U. S. Animal Health Association go on record to request an environmental impact statement adequately addressed to the effect of the proposed Darien Gap Highway on possible spread of foreign animal diseases and parasites, specifically FMD, to the countries north of the Darien Barrier.

BE IT FURTHER RESOLVED that, if the impact statement leaves any doubt regarding containment of animal diseases in the areas where they now exist, to insure positive prohibition of disease introduction into Panama, Central and North America, the Secretary of Agriculture (USDA) is urged to take such action as is necessary to be assured that the law regarding specific prohibition of entry of FMD into the United States and its territorial possessions be upheld.
Resolution No. 2

Held At: Portland, Oregon

Date: November 2 - 7, 1975

Source: Infectious Diseases of Horses Committee

Subject Matter: Official EIA Test on Horses Imported into the United States.

Background Information

The United States Animal Health Association in 1974 adopted a resolution requesting the Secretary of Agriculture to exercise interim authority to require negative tests for equine infectious anemia on equidae imported into this country.

The Secretary of Agriculture has failed to implement procedures requiring official testing of such imported animals and the problem of untested imports continues to compound the control of EIA in domestic equidae.

Resolution

THEREFORE, BE IT RESOLVED that the United States Animal Health Association again recommend that the Secretary of Agriculture immediately exercise interim authority to require that all horses and other equidae offered for importation into the United States be officially tested for equine infectious anemia and, if found negative and otherwise eligible, be allowed to enter the United States.
Resolution No. 3

Resolution

Be it resolved that USAHA recommend that USDA and NIH establish joint procedures and protocols prior to issuing permits for the importation of exotic influenza viruses and other infectious materials, and establish a laboratory security program adequate to protect the nation's livestock industry from such agents.

Background Information

The NIH Record of August 12, 1975 contains an article to the effect that a special influenza laboratory is being established by NIH in Memphis, Tennessee and that it will serve as the focus for an international network of investigators checking on influenza infections in animals and the relationship between animal and human influenza viruses. The laboratory will receive viruses from around the world which have been found in domestic and wild animals. The release does not mention precautions which should be taken with some of the virulent influenza viruses obtained from avian and mammalian species. We do not have knowledge concerning the facilities and its capability for containing exotic influenza agents and other possible contaminants that may be infectious for poultry and livestock. In addition, no mention is made of precautions to be taken when the viruses are shipped or if veterinary import permits will be used.

Resolution

Be it resolved that USAHA recommend that USDA and NIH establish joint procedures and protocols prior to issuing permits for the importation of exotic influenza viruses and other infectious materials, and establish a laboratory security program adequate to protect the nation's livestock industry from such agents.
Resolution No. 4

Held At: Portland, Oregon

Date: November 2-7, 1975

Source: Anaplasmosis Committee

Subject Matter: Increases in funds for anaplasmosis diagnostic testing and research.

Background Information

Since anaplasmosis is ranked fourth (4th) in priority for additional research by the National Cattlemen's Association, with losses estimated at 100 million dollars per year and because of interstate and international restrictions on the movement of cattle pertaining to anaplasmosis,

Resolution

Be it Resolved by the United States Animal Health Association that it is deemed necessary that the present and future budgets of the United States Department of Agriculture, including ARS, CSRS, and APHIS, provide substantial increases in funds for anaplasmosis diagnostic testing and research. Be it further resolved this resolution be directed to the Secretary of Agriculture with a request that he distribute it to ARS, CSRS, APHIS and the Agriculture Committees of the United States Congress.
Resolution No. 5

Seventy-Ninth Meeting

Held at: Portland, Oregon

Date: November 2-7, 1975

Source: Wild and Marine Life Diseases Committee

Subject Matter: Management of Wildlife

Background Matter

Proposed legislation is now being considered by numerous municipal, state, and federal governments is ultimately aimed at abolishing all forms of wild animal control and management, to create an untenable situation which neither Agriculture, Public Health, nor wildlife interests of North America can afford and the International Association of Game, Fish and Conservation Commissioners (IAGFCC) currently is assuming the major burden of responsibility relating to this frightening threat to the national welfare of this and other countries of North America.

Resolution

NOW, THEREFORE, BE IT RESOLVED that USAHA join with IAGFCC in opposing pending municipal, state, and federal legislation directed toward abolishing legal annual harvest of surplus wildlife populations;

ALSO, BE IT RESOLVED, that each State Veterinarian is urged to contact the Director of the Conservation Department of his or her respective state to offer support on this urgent issue;

AND BE IT RECOGNIZED, that cooperative efforts of this magnitude will prove highly meaningful to future relations between agricultural and wildlife interests.
Resolution No. 6

Background Information

Food products from foot and mouth disease countries are regularly carried by airplanes and ships as galley supplies and some of these vehicles are regularly arriving at airports and seaports many miles into the continental United States of America and we are informed that locker seals are not always checked until inland ports have been reached.

Resolution

THEREFORE, BE IT RESOLVED, that APHIS be requested to check in detail to determine whether existing responsibility and authority is being exercised, and further that it implement the boarding of ocean going vessels at the point where bar pilots are boarded for the purpose of adequate enforcement of existing safeguard procedures.
Resolution No. 7

Held at: Portland, Oregon  Date: November 2-7, 1975

Source: Import-Export Committee

Subject Matter: "User Fees" legislation

Background Information

There is a good chance that "user fees" legislation may be enacted by Congress to reimburse the Department for services rendered to importers and exporters of animals and animal products. There is a question of the manner in which such funds would be collected and administered now therefore

Resolution

BE IT RESOLVED, that the USAHA request that such monies be placed in a revolving fund to be administered by Veterinary Services for the express use of import-export functions.
Resolution No. 8  Seventy-Ninth Meeting
Held at Portland, Oregon  Date: November 2-7, 1975
Source: Import-Export Committee
Subject Matter: Animal Import Health Requirements

Background Information

The animal import health requirements for the several states
are more restrictive than those for entry into the United States
and importers often are uninformed of state of destination require-
ments.

Resolution

THEREFORE, BE IT RESOLVED that the Import-Export staff of
Veterinary Services be requested to develop adequate ways to advise
importers of animals that State officials at destination should be
contacted regarding their specific requirements and that a provision
be developed to similarly inform importers of animals across land
border ports.
Resolution No. 9

Seventy-Ninth Meeting

Held At: Portland, Oregon

Date: November 2-7, 1975

Source: Wild and Marine Life Diseases

Subject Matter: Hemorrhagic Disease

Background Information

In 1971 hemorrhagic disease was first confirmed as an important disease of wild cervids in the southeastern United States, at which time bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) were isolated from white-tailed deer that succumbed in seven southeastern states.

Since then, hemorrhagic disease has been diagnosed annually in wild deer of the southeastern region, which strongly suggests that BTV and EHDV are enzootic throughout the south Atlantic region.

The status of these diseases in domestic livestock, primarily cattle, remains very uncertain with current failure to recognize this disease entity in cattle attributed to several fundamental factors.

It appears that hemorrhagic disease (BT and EHD) is a rapidly emerging disease of considerable significance for the cattle industry and big game animal interests through the eastern United States.

Resolution

IT THEREFORE IS RESOLVED, that USAHA request that USDA initiate a program to define the distribution of hemorrhagic disease (BT and EHD) in cattle throughout the United States; train additional personnel in state and local laboratories in diagnostic procedures for detecting this disease entity; and use every available means to have food animal practitioners become more aware of BT and EHD infections and the various forms such can assume in cattle.
Resolution No. 10  Seventy-Ninth Meeting
Held at: Portland, Oregon  Date: November 2-7, 1975
Source: Wild and Marine Life Diseases
Subject Matter: Migratory Bird Mortality

Background Information

Dwindling migratory bird habitat augmented by current land use continue increasing concentrations of birds in a polluted environment, and the potentials for devastating native and foreign disease entities are accentuated annually.

Within the past three years this accelerating threat has been vividly manifest through loss of 40,000 mallard ducks on Lake Andes, South Dakota (1973); 20,000 coots on the Back Bay area of Virginia (1975); 13,000 coots, swans, geese, and ducks in the Sacramento Valley of California (1975); and from 30-25,000 assorted migratory geese and ducks, plus 10 to 15,000 crows in the vicinity of Phelps County, Nebraska (1975).

Future outbreaks of infectious diseases of even greater intensity appear inevitable, the consequences of which may jeopardize this nation's domestic poultry industry and wild bird resources alike.

Since neither the U. S. Departments of the Interior nor Agriculture have adequate programs to cope with such emergencies that involve migratory birds, the burden of responsibility is essentially relegated to State Game and Fish Departments for preventing spread of numerous avian diseases.

Under such circumstances few State Game and Fish Departments have the manpower resources or funding to properly deal with catastrophes of the magnitude recently experienced and predicted for the future.

A program is in operation and funded through the U. S. Department of Agriculture to assist State Departments of Agriculture in handling emergency diseases that affect domestic livestock and poultry, this authority does not extend to include migratory birds.

Since the U. S. Department of the Interior has jurisdiction of authority to assure the continued well-being of migratory birds, but provisions currently are not available to assist State Game and Fish Departments in combating native or foreign diseases that concomitantly threaten this nation's poultry industries and wild bird resources.

IT THEREFORE IS RESOLVED THAT THE UNITED STATES ANIMAL HEALTH ASSOCIATION urge the Secretary of the Interior to seek authority for declaring a state of emergency whenever significant migratory bird mortality is in progress, and develop contingency plans for funding a program to assist State Game and Fish Departments in preventing spread of highly contagious diseases of migratory birds whenever and wherever they occur;

BE IT FURTHER RESOLVED that members of this body vigorously support the Secretary of the Interior in acquiring such authority to include provisions for funding efficient means to protect this nation's migratory birds and poultry industries alike.
Resolution No. 11

Held At: Portland, Oregon

Source: Brucellosis Committee

Subject Matter: Feasibility study

Background Information

Cooperation from the livestock industry, federal and state legislatures, and various animal health officials is essential in deterring the spread of brucellosis.

Resolution

Therefore, be it resolved that a feasibility study to determine all possible programs for the control or eradication of brucellosis shall be conducted. In order that maximum credibility be accorded this study, an impartial team composed of the following national recognized scientific authorities is requested:

N. B. McCullough, M.D., Ph.D.
R. K. Anderson, D.V.M., Ph.D.
D. T. Berman, D.V.M., Ph.D.
W. T. Berry, Ph.D., Ag. Economist
J. L. Hopkin, Ph.D., Ag. Economist
Wm. Goodwin, Ph.D., Ag. Economist (alternate)

The investigative team shall include evaluations of cost-benefit ratios, economic, social, hygienic and other factors in their study. The study shall include recommendations on various courses of action to be taken and shall be concluded in the shortest possible time. The conclusions of this study shall be delivered to the Congressional committees responsible for the funding of the Cooperative State-Federal Brucellosis Eradication Program and to the USAHA Brucellosis Committee for their consideration.
Within the past three years two exotic diseases, viz., VEE and VVND, have been introduced into the United States and have threatened this nation's equine and poultry enterprises, with national emergencies subsequently being declared.

At any time a multitude of other devastating foreign animal diseases can be accidentally or purposefully introduced, which will take place in grave jeopardy this country's entire livestock economy and wildlife resources alike.

The next national emergency may intricately involve wildlife as unrestrained carriers of numerous foreign diseases transmissible to domestic animals, e.g., white-tailed deer as carriers of Foot-and-Mouth Disease (FMD).

In order for successful eradication to be accomplished, it may become necessary to exterminate countless thousands of big game animals thereby involved, at which time State and Federal Wildlife Agencies will be expected to cooperate.

Provisions are available for indemnity paid to livestock producers who suffer the consequences of such drastic but essential measures, similar arrangements have not been established for replacement of wildlife so involved.

The individual States hold in public trust resident wildlife as defined by individual States and payments for the extermination, clean up, and restoration of big game populations should be made to the respective State or States involved.

NOW, THEREFORE, BE IT RESOLVED that the United States Animal Health Association urges the Secretary of Agriculture to develop a program to provide funding to State Wildlife agencies for relocation costs associated with replacement of wildlife species that may have to be depopulated by virtue of declared national emergencies in order to prevent establishment of a major foreign animal disease in the United States.
Resolution No. 13

Held at: Portland, Oregon

Date: November 2-7, 1975

Source: Salmonella Committee

Subject Matter: Safe, effective, and economical procedures for Chemosterilization

Background Information

There is an urgent need to develop information on safe, effective, and economical procedures for chemosterilization, or other methods, to inactive salmonella in animal feeds and feed ingredients at the manufacturing and proprietary levels and it is equally important to develop safe, effective, and economical procedures to prevent recontamination of feeds; and decontaminate feeds that may be recontaminated.

Resolution

NOW THEREFORE BE IT RESOLVED that the U. S. Department of Agriculture be urged to request the appropriation of such funds, or redirect such funds, as may be necessary to support research for the achievement of this objective.
Resolution No. 14

Held at: Portland, Oregon

Source: Committee on Salmonella

Subject Matter: Reference Assistance

Background Information

There has been a significant decline in the availability of reference assistance to local diagnostic and research laboratories for salmonella serotyping of cultures from agriculture and related sources and the availability of such reference assistance is essential to the maintenance of the proficiency of these laboratories as well as the well being of the livestock and poultry industries.

Resolution

NOW THEREFORE BE IT RESOLVED that the U. S. Department of Agriculture be urged to request such monies from the Congress to re-establish a Salmonella Reference Center to provide the needed technical support at a level commensurate with the needs to meet this national problem.
Resolution No. 15

Held at: Portland, Oregon

Date: November 2-7, 1975

Source: The Evaluation and Development of State-Federal Programs Committee

Subject Matter: Mutual Economic benefits

Background Information

The Evaluation and Development of State-Federal Programs Committee, U. S. Animal Health Association, in meeting at Portland, Oregon, on November 6, 1975 to evaluate State-Federal Programs, and contractual agreements entered into by both State-Federal Agencies for cooperative record keeping and providing working space for personnel and laboratories are vital to continuation and efficiency of cooperative programs and an inconsistency of approach to on-going existing agreements prevailed in the Spring of 1975 and time has proven these contractual arrangements are efficient and essential for accomplishment of the program goals.

Resolution

THEREFORE BE IT RESOLVED, that these contracts be continued and possibly refined to obtain the maximum in mutual economic benefits;

BE IT FURTHER RESOLVED that this Resolution be directed to the Secretary of Agriculture for action.
Resolution No. 16

Held At: Portland, Oregon
Date: November 2-7, 1975

Source: Sheep and Goats Committee

Subject Matter: Background Information

The officers and membership of the U. S. Animal Health Association are vitally interested in the health and vitality of the sheep and goat industry and certain diseases of both public health and economic concern to the industry, and biologics and therapeutic drugs have been developed and marketed for many years as an aid in the prevention and treatment of certain specific disease conditions and recent regulations have been promulgated by the U. S. Department of Agriculture, Bureau of Biologics and the Federal Drug Administration as regard development, production, and marketing of biologics, and drugs for treatment of animals, and the market potential for said biologics and drugs for certain diseases in the involved species do not permit an economical cost to benefit ratio to the biologic and drug manufacturers, and

The lack of availability of safe, uniform, and proven efficacious biologics and drugs will result in non-scientific home production of products to fulfill the demands of the industry, and such products may in fact increase the public health hazard and possibly jeopardize the well-being of the industry.

Resolution

BE IT HEREBY RESOLVED, that we, the USAHA, encourage the U. S. Department of Agriculture, Bureau of Biologics and Bureau of Veterinary Medicine Federal Drug Administration, to give serious consideration to adoption of measures which will insure continuing availability of high quality, safe biologics and drugs to be utilized by the industry in the interest of public health and economical production of high quality wool, red meat, and by-products for the needs of the hungry world.
Resolution No. 17  

Held at: Portland, Oregon  

Date: November 2-7, 1975  

Source: Committee on Sheep and Goats  

Subject Matter: Funds for Foot-Rot  

Background Information  

The Committee on Diseases of Sheep and Goats of the USAHA has long stressed the need for Foot-Rot research. The National Wool Growers Association, and the National Lamb Feeders Association in their priority list has placed Foot-Rot as the disease most needing research. USDA, ARS funding has not reflected the needs of the industry, and adequate research is not being conducted.

Resolution  

THEREFORE; be it resolved that the United States Animal Health Association recommends to the USDA that funds for Foot-Rot research be increased and given top priority.
Resolution No. 18  Seventy-Ninth Meeting
Held at: Portland, Oregon  Date: November 2-7, 1975
Source: Livestock Identification Committee
Subject Matter: Funds for a better system of livestock identification

Background Information

A better system of livestock identification and disease detection is needed by the livestock industry to enhance its management practices and efforts to eradicate diseases and

Significant progress has been made in developing an electronic livestock identification and disease detection by LASL and

Another eighteen months and two hundred twenty thousand dollars is needed to complete the work;

Resolution

Be it resolved that the United States Animal Health Association requests the Director of Animal and Plant Health Inspection Service of the United States Department of Agriculture to make available at the earliest time the above mentioned funds so that the work in progress will be completed.
Mr. Chairman:

The Committee on Livestock Commerce met as scheduled with 15 members and guests in attendance.

The committee again reviewed recommendations made in previous years and communications received during the year.

Prospective legislative proposals concerning the humane handling of livestock in commerce and the resultant undue burden on the livestock industry was discussed. The committee recommends the U. S. Department of Agriculture inform the USAHA Livestock Commerce Committee of any legislation relating to handling procedures of livestock in interstate commerce.

Information concerning the availability update and need for re-publication of the “Requirements for Interstate and International Movement of Livestock and Poultry” (Pub. 91-17-6) was requested. The committee was informed that a revision process is underway now with a probable target date of September 1, 1976 for completion.

Utilization of para-professionals in livestock commerce health related fields was considered. The committee recommends that standards for veterinary accreditation be changed to provide for utilization of veterinary technicians for sub-professionals functions while in the employee of accredited veterinarians who are responsible for their actions.

Procedures for implementation of the livestock transportation certificate were considered. It was noted that the Livestock Commerce Committee has recommended adoption of the Uniform Livestock Transportation Certificate since 1972 and this recommendation has been approved and accepted by the USAHA. However, there has been no action taken by any state regulatory agencies toward the implementation of the use of this form.

Therefore, the Livestock Commerce Committee recommends that
the USAHA create a task force to spearhead necessary action on the state level to accomplish acceptance of this livestock inspection certificate requested and needed by the livestock industry to facilitate movement of certain classes of livestock.

Should the USAHA be unable to create the task force, the president should immediately contact interested national associations seeking their involvement to create said task force to accomplish this goal. For example such associations might be the American Cattle Growers Association, National Livestock Feeders Association, Livestock Conservation, Inc., National Certified Livestock Marketing Association, National Livestock Dealers Association, and National Association of State Departments of Agriculture.

The chairman of the USAHA Rabies Committee reported on the progress of the recommended standardized small animal health certificate and rabies vaccination certificate. The Livestock Commerce Committee has previously concurred with the recommendations and again recommends that all states consider adoption of the standardized small animal health certificate.

This constitutes the report of the Livestock Commerce Committee.

I respectfully submit the report for approval by the Executive Committee.

L. N. Butler
Co-Chairman
REPORT OF THE WILD AND MARINE LIFE DISEASES COMMITTEE

Chairman: Frank A. Hayes


This newly created committee held its first meeting from 1:30 to 5:30 p.m., November 3, 1975, at which time the perimeters of responsibility were considered and partially defined. On this occasion, five resolutions were composed, which hopefully reflect in part, the jurisdiction of this committee. These resolutions subsequently were submitted November 5, 1975, for consideration by USAHA’s Committee on Nominations, Resolutions, and Internal Affairs. Abbreviated titles of pending resolutions are as follows: DARIEN GAY HIGHWAY, MIGRATORY BIRD MORTALITY, HEMORRHAGIC DISEASE, RELOCATION COSTS FOR WILDLIFE, and MANAGEMENT OF WILDLIFE.

During the course of deliberations that ensued, two other pertinent items relating to the welfare of domestic livestock and the world’s wildlife resources received considerable attention. The text of these with accompanying recommendations are as follows:

Regulatory Needs

During the era of surface transportation, most animal diseases become manifest during shipment as sequela of time and stress. This is not the case today, when any port of entry in the world is within twenty-four hours from any point of origin. With the advent of today’s accelerated jet airline transportation, potentialities for animal disease dissemination are multiplied manyfold.

With exception of a few diseases of grave implications for domestic livestock, e.g., foot-and-mouth disease . . . forty years ago little attention was given to infectious agents in free-living wild animals. It was generally assumed that wild animals were healthier than their domestic counterparts, augmented by a dilution factor that made it highly unlikely for epizootic diseases of any consequence to exist in such populations. Within the past four decades, research and surveillance have shown that this line of reasoning fails to be in keeping with reality.

It now is known that many species of wild birds and mammals harbor a multitude of devastating infectious agents capable of severely affecting other species of wildlife and wrecking havoc with a nation’s domestic livestock economy. For example, it was through the importation of psittacine birds that Exotic Newcastle Disease was introduced into southern California during the summer and fall of
1971. Through recent transmission studies conducted at the Plum Island Animal Disease Center of the Agricultural Research Service of USDA, it now is known that white-tailed deer are highly susceptible to foot-and-mouth disease and rinderpest. Numerous workers also have disclosed the fact that many cloven-hoofed animals of Africa are reservoir hosts for such etiologic agents as East Coast Fever, malignant catarrhal fever, and rinderpest. These same animals harbor a large number of arthropod vectors capable of transmitting a multitude of other viral, rickettsial, and protozoan diseases contagious for wild and domestic animals alike.

Protecting a nation’s economy from foreign animal disease introduction therefore is accomplished basically by two methods: a) outright prohibiting importation, and b) establishment of rigid, scientifically sound, protective importation requirements. In considering the first of these options, it should be recognized that an embargo policy forges a double-edged sword, whereby the end may not justify the means. In fact, inflationary costs of the commodity in question often encourages illegal entry, to be sustained and magnified by the profits realized. This approach also imposes deleterious restraints upon international trade with consequent adverse effects upon commerce and ultimately the economy of both the exporting and importing countries. It can similarly indicate an inadequate understanding of the scientific resources that might permit controlled importation. Nevertheless, for some of the more devastating, highly contagious disease entities, outright prohibition of importation is an only recourse for protection of a nation’s livestock economy and wildlife resources. For example, the United States does not import cloven-hoofed animals or uncooked meat and places severe restrations on uncooked by-products from any country in which foot-and-mouth disease is known to occur. Australia has a similar policy relating to foot-and-mouth disease, which also includes an embargo on cattle or even semen from any nation where bluetongue is known to exist. England recently stopped the importation of swine and pork products from the United States as a result of a diagnosis of hog cholera in the State of Texas. Hardships that this approach imposes on both nations reflect a foregone conclusion, but under some circumstances it is an only alternative to assure protection of a multibillion dollar livestock economy.

Therefore, in lieu of an embargo, under most circumstances importation regulations are a preferred measure for reducing the likelihood of disease introduction. Many problems can be anticipated that have not been associated with movement of domestic livestock if wild animals are included as part of existing importation requirements. Present facilities and regulations are not adequate in most countries of the world today. Almost anything suggested at this time should reflect improvement, so perhaps it is best to consider a few basic needs.

*First,* a permit system should be inaugurated to regulate entry of
any and all terrestrial wildlife species. This would be a multi-purpose device, that would prevent importation of undesirable species as well as reduce the likelihood of disease introduction. Many State Game and Fish Agencies in the United States have adopted such requirements, but enforcement is inadequate. Nevertheless, it represents a beginning, which certainly should be considered by all countries that import or export wild birds or mammals. Requirements obviously will differ in relationship to intranational and international trade.

Second, a complete history should be available on each lot of wild animals received at or in the vicinity of a port of embarkation. This should include speciation, numbers, method of capture, point of origin, and the length of time that the animals have been kept in captivity. At that time, each bird or mammal should receive a permanent identification in the form of a number or some other descriptive marking and an identification record should be maintained with the animal for life.

Third, adequate facilities should be available in the country of origin where a quarantine of sixty days can be imposed on all wild animals to be exported. During this interim, each animal should be inspected at the beginning of quarantine by a trained animal health official and reinspected and certified before release for shipment at the end of sixty days. For certification, the quarantined animals should be carefully inspected for ecto and hemoprotozoan parasites, with appropriate laboratory tests conducted that are in keeping with requirements established by the importing country. It should be emphasized, however, that when an individual or nation desires to import animals, some risks automatically must be assumed. On the other hand, if a negative laboratory test for everything is required, an embargo policy essentially is in effect.

Through inauguration of the suggested principles of preventive medicine, the burden of responsibility thus far will be on the exporting nation. Responsibility then will shift to the importing nation, which should have the means of duplicating similar quarantine measures at the port of entry. During this second quarantine period, added precautions would be realized, with invaluable information accumulated for future reference on the spectrum of pathogens or potential pathogens that could undermine the ultimate welfare of an entire nation.

Recommendations:

It is most timely for the U. S. Animal Health Association to consider the merits of this line of reasoning and evaluate the desirability for developing standards or guidelines for an international program to be enacted through cooperative efforts with the Food and Agriculture Organization of the United Nations (FAO).

An Ad Hoc Subcommittee designated to explore this possibility is
DEVELOPMENT OF LIAISON

Within the past decade it has become well established that a multitude of foreign and some native diseases represent an increasing threat to the continual well-being of domestic livestock and wildlife resources of North America. A few examples of these are: Venezuelan Equine Encephalomyelitis (VEE), Viscerotropic Velogenic Newcastle Disease (VVND), Duck Virus Enteritis (DVE), Vesicular Exanthema of swine (VES), Foot-and-Mouth Disease (FMD), Rinderpest, Africa Swine Fever, Piroplasmosis (Cattle Fever), Brucellosis, Tuberculosis, etc. Each of these diseases and numerous other infectious entities are capable of wreaking havoc on the livestock economy of this and other countries of the continent.
When prevention and/or eradication efforts involving these diseases in domestic livestock are contemplated, it is immediately apparent that wildlife often may be intricately associated with the problem. The success or failure of most livestock disease control or eradication programs therefore is directly dependent upon whether or not the infectious agent becomes established in wildlife and upon the availability of resources for early detection. Few animal disease control efforts can be successful without essential epizootiologic information on the status of the disease in wild animal populations. Monitoring and surveillance of disease entities in wildlife subsequently comprise necessary prerequisites to prevention, control, or eradication of exotic or native diseases of major consequence to domestic animals and man. In the event of foreign disease introduction, or under circumstances where an indigenous disease assumes emergency proportions, maximum cooperation between those responsible for the well-being of this and other nations' domestic livestock and wildlife resources will be imperative for success in control or eradication to be realized.

For example, concern and cooperation from wildlife interests of the United States were vividly manifest during the last National Test Exercise conducted by USDA in May, 1971. During a one-week Test Exercise, coordinated from the National Emergency Disease Ready Room at Beltsville, Maryland, 38 State Game and Fish Departments were contacted—32 responded—and more than 2,200 highly trained men with comparable numbers of two-way radioed vehicles were committed as part of a nationwide effort in combating a hypothetical introduction of Foot-and-Mouth Disease. In addition, airplanes, helicopters, bulldozers, drag lines, four-wheel drive vehicles, etc., were offered by numerous State Conservation Agencies. Under real circumstances of such direful implications, even more support and cooperation can be anticipated if the proper groundwork has been established.

Although much progress has been made along these lines, to date it represents only token security. Efforts must be intensified, for until better understanding between domestic livestock and wildlife interests is realized, all of the elaborate measures currently planned for combating Foot-and-Mouth Disease, Rinderpest, African Swine Fever, etc., may evolve into an exercise in futility.

Minimizing the awesome consequences of such a national catastrophe depends upon realizing the current flaw in communications, with subsequent development of better liaison and rapport between agricultural and conservation interests throughout the United States. Such an investment today will pay unprecedented dividends tomorrow.

Recommendations:

It is respectfully requested that the Executive Committee of USAHA go on record via open letter in the Proceedings of the 79th
Annual Meeting of the United States Animal Health Association in recognition of the need for better understanding and liaison between wildlife and domestic livestock interests, with previously forwarded copies to the President and Executive Vice President of the International Association of Game, Fish and Conservation Commissioners; Director of the U. S. Fish and Wildlife Service, USDI; the Deputy Administrator of Veterinary Services, APHIS, USDA; Executive Vice President of the National Wildlife Federation; and President of the Wildlife Management Institute.

Dr. John S. Gottschalk  
Executive Vice President  
International Association of Game, Fish  
and Conservation Commissioners  
1412 16th Street, N.W.  
Washington, D. C. 20036

Mr. Lynn A. Greenwalt, Director  
Fish and Wildlife Service  
U. S. Department of the Interior  
Washington, D. C. 20240

Dr. John M. Hejl, Deputy Administrator  
Veterinary Services  
Animal and Plant Health Inspection  
Service  
U. S. Department of Agriculture  
Washington, D. C. 20250

Mr. Thomas L. Kimball, Executive Vice President  
The National Wildlife Federation  
1412 16th Street, N.W.  
Washington, D. C. 20036

Mr. John E. Phelps, President  
International Association of Game, Fish  
and Conservation Commissioners  
Director, Utah Division of Wildlife  
Resources  
1596 W. N. Temple  
Salt Lake City, Utah 84116

Mr. Daniel A. Poole, President  
Wildlife Management Institute  
709 Wire Building  
1000 Vermont Avenue  
Washington, D. C. 20005
A brief resume of the following items was presented by Drs. Geyer, Madin, and Kistner: SEA LION VIRUS STUDIES, APPRAISAL OF MONK PARAKEET INTRODUCTION, AQUACULTURE AND AGRICULTURE, SARCOCYSTIS IN DOMESTIC AND WILD ANIMALS, and PREDATOR MANAGEMENT. Information is being compiled on these subjects for more in depth consideration by the committee.
REPORT OF THE COMMITTEE ON PARASITIC DISEASES AND PARASITICIDES
USAHA 1975

Chairman: J. L. Hourigan, Hyattsville, Md.
Co-Chairman: R. L. Pyles, Albuquerque, N. M.

J. C. Augustine, Brighton, Co.; D. W. Baker, Albuquerque, N. M.;
S. J. Cougar, Austin, Tx.; Irwin Elliott, Littleton, Co.; F. G.
Hamilton, Austin, Tx.; L. W. Hinchman, Indianapolis, Ind.; John
Holcombe, Oklahoma, City, Ok.; A. C. Newman, Opeleokia, Al.;
I. H. Roberts, Albuquerque, N. M.; G. O. Schubert, Hyattsville, Md.;
R. H. Singer, Frankfurt, Ky.; H. R. Smith, Cincinnati, O.;
R. K. Strickland, Beltsville, Md.; W. C. Tobin, Denver, Co.; W. W.
Utterback, Austin, Tx.; J. H. Womack, Hyattsville, Md.; J. B.
Young, Austin, Tx.; D. E. Zinter, Bowie, Md.

This committee met in open session on Monday, November 3, 1975, and discussed the following:

SCREWORM PROGRAM STATUS REPORT

There have been 8,547 laboratory-confirmed cases of screwworms reported as of September 20, 1975, from the United States during this calendar year. There were 7,267 cases reported during the entire calendar year 1974. Confirmed cases were as follows:

<table>
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<tr>
<th>State</th>
<th>CY 1974—Cases</th>
<th>CY 1975—Cases*</th>
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<tr>
<td>Texas</td>
<td>6,900</td>
<td>8,023</td>
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<td>Arizona</td>
<td>197</td>
<td>297</td>
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<td>New Mexico</td>
<td>99</td>
<td>161</td>
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<td>Arkansas</td>
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<td>Oklahoma</td>
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<tr>
<td>California</td>
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<td></td>
<td>7,267</td>
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<td>Mexico (6 Northern States)—11,374</td>
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Laboratory-Confirmed Screwworm Cases in the United States

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<tr>
<td></td>
<td>50,850</td>
<td>7,168</td>
<td>400</td>
<td>1,062</td>
<td>1,898</td>
<td>872</td>
<td>9,877</td>
<td>219</td>
<td>153</td>
<td>473</td>
<td>95,642</td>
<td>14,976</td>
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Weather favorable for screwworms continues to pressure the United States. Wounds from the Gulf Coast ear tick (*Amblyomma maculatum*), while unimportant for many years, has become an im-
important factor in the development of screwworms in south Texas. Pasture improvement and cattle rotation may be responsible for the tick population increase. In July 1975, less than 3 percent of the cases were from tick-wounded ears. In August 1975, almost 30 percent were associated with tick wounds.

Another tick sometimes associated with screwworm infestations, though of lesser importance, is the Lone Star tick, *Amblyomma americanum*. Research is needed to develop a good control for both of these ticks when they build up to large populations. We will continue to experience screwworm outbreaks associated with them until better control measures which are economically feasible for the ranchers are available.

Screwworms have been eradicated from Puerto Rico and the Virgin Islands following a four-year effort. The last reported case was November 5, 1974.

The Joint U. S.-Mexico Program is progressing satisfactorily. The production facilities at Tuxtla Gutierrez are nearing completion. Production is expected in early 1976. Reduction of screwworms in Mexico should eliminate the pressure on the livestock industry in the United States.

**CATTLE FEVER TICKS**

Parts 72 and 73, 9 Code of Federal Regulations (CFR), were amended several times during the year.

Effective March 17, 1975, an amendment deleted the requirement that a vatside or field test be available when proprietary brands of pesticides are specifically approved by the Department as permitted dips for use against ticks or scabies.

Amendments to Part 72, 9 CFR, released portions of Duval, Webb, Kleberg, McMullen, Nueces, Live Oak, and Jim Wells Counties (effective September 24, 1974) and portions of Duval, Kleberg, and Sims Wells Counties (effective April 9, 1975) in Texas from Federal quarantine because of *Boophilus* spp. ticks. Additional portions were released from Federal quarantine in Jim Wells, Kleberg, and Nueces Counties effective July 22, 1975.

A tick identification and eradication procedures training course was held at Beltsville, Maryland, October 15-29, 1974. Trainees included 11 State or Federal employees from Puerto Rico, New York, Texas, and New Mexico. Immediately following (October 21-23), a pesticide application training course was held for 16 State or Federal employees representing the same States.

During FY 1975 *Boophilus* ticks were collected from 47 herds of 283 head of native livestock located within the area under State and Federal quarantine along the Rio Grande River in southern Texas.
and from 9 herds of 105 livestock north of this quarantined area. In addition to these infestations, 93 cattle and 15 horses, smuggled or strayed from Mexico, were apprehended in the quarantined area—15 of the cattle and 2 of the horses were found to be infested with _Boophilus_ ticks. Two non-infested Mexican hogs, sheep, or goats, illegally in the United States, and 83 United States livestock straying or being smuggled into Mexico and returning, were also apprehended. A total of 28,732 certificates involving the inspection and intrastate movement of 180,264 livestock and 15 certificates involving the interstate movement of 1,529 livestock were issued.

In the quarantined area, 796,482 cattle and 38,750 horses or mules and outside the quarantined area, in Texas, 447,190 cattle and 22,681 horses or mules were inspected for ticks. In the quarantined area, 156,676 cattle were treated (151,663 dipped and 5,013 sprayed) and 24,699 horses or mules were treated (858 dipped and 23,841 sprayed).

Outside the quarantined area, 67,271 cattle were treated (62,473 dipped and 4,798 sprayed) and 11,854 horses were treated (653 dipped and 11,219 sprayed).

Delnav was used to treat 1,633 cattle and 33,235 horses or mules. Co-Ral® was used to treat 235,357 cattle and 2,171 horses or mules.

On June 1, 1975, ticks were collected from a roping steer at the rodeo area in Carrizo Springs, Texas. Investigation disclosed heavy infestation in a feedlot. Some 50,000 acres in Dimmit County were placed under State quarantine.

Two collections of _Boophilus annulatus_ were made in July 1975 involving new areas of infestation outside the quarantine zone. The first was at Cotulla in La Salle county, the second near Laredo in Webb county. The La Salle county outbreak was approximately 60 miles north of the quarantine line.

New findings of _Boophilus microplus_ ticks were made in Kleberg county in October 1975.

_Amblyomma variegatum_, the tropical bont tick, infestations reported from Puerto Rico during June 1974 have increased from the 26 premises reported in July 1974 to 59 premises as of September 9, 1975. Veterinary Services and the Commonwealth of Puerto Rico Department of Agriculture are developing plans for the eradication of this tick. The program is necessary if _Amblyomma variegatum_ is to be kept from the continental United States.

Tropical bont ticks were eradicated from the Island of St. Croix, U. S. Virgin Islands.

Dr. W. W. Utterback presented some preliminary information on a study being made on the stability of Co-Ral® wettable powder in dipping vats by collecting samples for laboratory analysis and bioassay as the vats are being used in the fever tick eradication program in south Texas.
The goals of the study are:
1. Determine the length of time, up to one year, that Co-Ral® remains effective against fever ticks.
2. Determine the environment and management factors which may alter the stability of Co-Ral®, such as amount of vat use, pH of water, soil types, weather conditions, etc.
3. Determine the potential of extending the life of Co-Ral® by filtering the bath.

The preliminary study indicates that there is no measurable decrease in effective tick control in vats which had been sitting idle for up to 20 months.

Three heavily used baths are being filtered with Bauer Hydrasieve to keep contamination low enough to continue the bath use for the one year duration of the study.

Methods of bath agitation are also being studied. The methods studied are: (1) mechanically, with a paddle; (2) air compressor and a T-bar; (3) water circulation pump.

An emergency effort is being made to strengthen tick surveillance, outside the quarantined area, in south Texas. The Texas Animal Health Commission has, with emergency funds, employed 30 temporary inspectors to inspect cattle moving through auction markets in south Texas. The purpose of this added effort is to detect new tick infestations more promptly.

CATTLE SCABIES

This committee has been involved with scabies eradication for many years and played an active part in the successful program to eradicate psoroptic sheep scabies. Unfortunately, and despite all our efforts, psoroptic cattle scabies remains with us. Although more than 20 years ago, in 1953, it appeared we were nearing success, the following year a series of outbreaks began and since there have been only two years, 1963 and 1967, when no outbreaks were found. Numbers of infected herds were 91 in FY 1972; 53 in 1973; 39 in 1974; 49 in 1975; and as of today 15 in FY 1976.

This Association's committee on Evaluation and Development of State-Federal Programs requested that a review be made of cattle scabies programs. In March 1972, a meeting to develop more effective means for fighting the disease was held in Denver, Colorado. Representatives involved in the problem included those from 14 states. The programs in the states most involved were reviewed and distributed to other states.

A second cattle scabies meeting was held in Denver in April 1975. This was an ad hoc meeting of our Parasitic Diseases and Parasiticides Committee and included 13 committee members in addition to industry representatives and others.
Notes and recommendations developed at the meeting were distributed to our committee and to others.

Essentially these were: The geographic area of greatest problem included Texas, New Mexico, Oklahoma, Kansas and Colorado; officials representing these states were uniformly resolved to eradicate scabies; that funds appropriated for scabies should be used for scabies eradication; the required number of persons should be assigned full-time to scabies and have no other responsibilities; the position of Scabies Regional Epidemiologist should be established promptly; and uniform eradication procedures should be used.

Following the meeting the proposed "Recommended Procedures — Cattle Scabies Eradication" was distributed. Earlier copies of a draft had been sent to committee members in April 1974, and discussed during our annual meeting in Roanoke in October 1974 and at the April 1975 meeting in Denver.

At the request of the USAHA President, copies of the proposal were sent to each member of the Executive Committee. That committee adopted the proposal while convened here in Portland, and it appears as an addendum to the report of our committee.

In addition to the above meetings, a Scabies Research and Control Work Conference was held at Bushland, Texas, in August 1973. This meeting was attended by representatives of the Texas Animal Health Commission; Texas Agricultural and Mechanical University, College Station, Texas; Texas Agricultural Extension Service; New Mexico Livestock Board; Veterinary Services, APHIS, USDA; Agricultural Research Service, USDA; and industry.

Many of the recommendations in regard to cattle scabies eradication have been effected. These include establishing and filling the position of Regional Scabies Epidemiologist; adding pesticides to list of permitted dips which are effective against cattle grubs and scabies mites (Coumaphos and Prolate); and developing Standard Recommended Procedures — Cattle Scabies Eradication.

Federal Quarantines

The policy of promptly placing Federal quarantines on areas involved in psoroptic cattle scabies outbreaks was placed in abeyance pending amendment to Part 73, 9 Code of Federal Regulations (CFR). The most recent Federal quarantines were placed January 20, 1975, on areas in Childress, Hansford, and Swisher counties in Texas.

Federal quarantines were lifted in December 1974 from areas in Seward and Stevens counties in Kansas and Cimarron county, Oklahoma. During FY 1975 quarantines were lifted from Texas counties as follows: January - El Paso; February - Childress, Cochran, Moore, and Swisher; and May - Hansford county. The latter completed lifting of all Federal quarantines.
Additional Permitted Dips

Effective November 11, 1974, Part 73, 9 CFR. was amended to add approved proprietary brands of coumaphos (Co-Ral®) 25 percent wettable powder used at a concentration of 0.30 percent to the list of permitted dips for use against cattle scabies. Coumaphos had been on the list of permitted dips for use against ticks since 1968, and was approved by the Environmental Protection Agency on October 4, 1974, for use against cattle scabies. This product was also recommended by our Committee last year.

An amendment effective September 11, 1975, added proprietary brands of a second organophosphorous product, Prolate® (GS-118) used a concentration of 0.20-0.25 percent, to the list of permitted dips for use against cattle scabies. Trials were carried out by the U. S. Department of Agriculture in 1973, 1974 and 1975. The results of these trials and the trials carried out by Thuron Industries and representatives of Texas A and M University were the basis for the Environmental Protection Agency granting approval to a label on July 16, 1975, for the product and its use against scabies mites in official programs. All these trials were carried out to demonstrate the stability and efficiency of the product in the vat. Before a dip will be specifically approved as a permitted dip, Veterinary Services requires that the product be registered under the provisions of the Federal Insecticide, Fungicide and Rodenticide Act, as amended (7 U.S.C. 135 et seq.) GX-118 is an emulsifiable concentrate. Triple super phosphate must be added to the vat to maintain proper pH levels. Dipped animals should be withheld from slaughter for 21 days.

CATTLE GRUBS


Systemics used as pour-ons, feed additives, sprays, and dipping vat products are produced by four major companies. During recent years there have been few significant changes. Costs have represented one of the prime reasons for lack of greater development. Due to expiration of patent rights new products have appeared, but most of these include some of the original chemical compounds.

The effort against cattle grubs was to achieve practical economic control. The estimated loss of $15 million in 1974 was only one-fourth the loss in 1958. Feed lot operators are affected directly by grub losses seen at slaughter and treat with available materials more consistently than do range cattle operators.

The name of the Cattle Grub Committee (one of the National Standing Committees of Livestock Conservation, Inc.) has been
changed to the Livestock Parasite Committee, and the Committee’s activities thus expanded. The Chairman of the Committee, Mr. Irwin C. Elliott, is, of course, also a member of our own Parasites and Parasiticides Committee.

The following report was presented before the Committee:

**BOVINE THELAZIASIS (EYE WORMS)**

Dudley B. Sisk, D.V.M., Ph.D., Central Kentucky Animal Disease Diagnostic Laboratory, R.R. 6, Lexington, Kentucky 40505

Until this year, bovine thelaziasis has been considered a foreign disease, enzootic in Europe, Asia and Africa with occasional reports from Australia. The discovery of eye-worms in native Kentucky cattle in February, 1975, should alert the livestock industry to the potential significance of this parasite in the pathogenesis of bovine ocular disease.

On other continents, especially Northern Europe and Southern Africa, thelaziasis is associated with a disease syndrome which ranges from lacrimation, mild conjunctivitis and photophobia to a progressive keratoconjunctivitis with corneal opacity, ulceration and occasional panophthalmitis leading to permanent blindness. In these areas, many apparently normal animals are also infected with the parasite.²

The most common eyeworms of cattle are *Thelazia gulosa*, *T. rhodesi* and *T. skrjabini*. The face fly (*Musca autumnalis*), which was introduced to the North American continent in 1951, is considered an intermediate host.³ *Thelazia gulosa* has recently been collected in California from a giraffe imported from South-West Africa, received in New Jersey and shipped across the United States to the West coast.⁴

A limited survey of cattle presented for necropsy at the Central Kentucky Animal Disease Diagnostic Laboratory (Lexington, Ky.) suggests an infection rate of more than 20% with apparently no associated eye lesions. Diagnosis is difficult in live animals as the small (5.0 to 21.0 mm), white nematodes usually inhabit the lacrimal gland and nictitans gland ducts. Eye washings with physiological saline and manipulation of the lids and membrana nictitans is suggested for clinical diagnosis. Demonstration of the worm is not difficult on post mortem dissection, assuming careful examination. Exeneration of the lids and eye with histologic sectioning of the tear ducts provides the most thorough approach to demonstrating the parasites, especially the larval forms which range from 0.2 mm to 5.0 mm in length depending on the stage of the life-cycle. Precise morphologic descriptions of the genus *Thelazia* are available.⁵ Successful treatment includes ocular instillation or subcutaneous administration of levamisole.⁶ Control is directed toward the intermediate host.
REFERENCES

I. Definitions

For the purpose of these recommended procedures, the following definitions apply:

Scabies—Scabies is a disease caused by mites of the genus *Psoroptes* or the genus *Sarcoptes* and does not include infestations caused by *Chorioptes*, *Psorergates*, or *Demodex* mites.

Infected animal—An animal from which mites have been collected prior to official treatment or a member of an infected herd or lot.

Infected lot—Any lot of cattle in which one or more cattle have been disclosed to have scabies mites prior to official treatment.

Lot—Any group of cattle which are gathered together in any manner whether it be in a pasture, on a range, on a farm, in a market, or concentration point.

Herd—A group of cattle maintained for any purpose on common grounds, or two or more groups of animals under common ownership or supervision, geographically separated, but which have an interchange or movement. Herd decision must be based on sound epidemiological evidence.

Feedlot—Any location in which animals are gathered together for purposes of fattening for sale and where the feed is carried to them.

Range—Grassland on which cattle may forage for their food.

Ranch—Any operation where cattle are maintained and from which animals, both breeders and/or feeders, are sold.

Farm—A premises which carries out more than livestock operations. This may be either a dairy farm in which cattle are cared for and in which milk is sold, or it may be a farm in which grain is raised and fed to livestock which were purchased or raised on that farm.

Farm feedlot—A farm which, as part of its operation, takes animals raised on that farm or purchased and places them in a drylot on the farm. This is compared to a feedlot operation where the feedlot is the sole or primary operation.

Dairy—This may be where milking cows are kept for the production of milk or where a dairy herd raises its own replacements. All cattle involved are primarily of the dairy breed and maintained for the production of milk.

Exposed—An animal which is known to have had an opportunity to be in contact with animals which may have been infected or to have been in contact with trucks, pens, chutes, and alleys through which infected animals have moved within the preceding 10 days.

Official treatment—That treatment which makes use of a pesticide listed as a permitted pesticide by Veterinary Services, APHIS, USDA, and which is used in a manner prescribed by Veterinary Services and where such treatment is carried out under the direct super-
vision of an employee of the USDA or State livestock health agency.

**Free area—**That area in which scabies is not known to exist and which is not under a State or Federal quarantine because of scabies.

**Permitted pesticide—**A pesticide which is listed in Part 73 or 74, Title 9, Code of Federal Regulations.

**Change of animals' status—**Infected animals and exposed animals shall be considered free of scabies when official treatment, as listed above, is completed on all animals on the infected or exposed premises. The treatment shall be two times for infected animals and either one or two times for exposed animals, according to the permitted pesticide used.

II **Guidelines for Handling Cattle Scabies**

A. Psoroptic Cattle Scabies

1. **Handling of Infected and Exposed Cattle and Premises**

   Infected and exposed cattle should be placed under State quarantine until properly treated under supervision. Only permitted dips should be used.

   Every animal in an infected herd, both beef and dairy, should be treated twice. This includes both those with lesions and those not showing visible signs. Herds in which no mites can be demonstrated but from which cattle have moved that are found to be infected within 30 days should be treated twice as infected herds. Herds through which these animals have passed should be treated as exposed. Every animal in an exposed beef or dairy herd should be treated according to the directions for the pesticide used.

   Cattle previously moved from infected herds during the period they logically could be considered exposed to scabies, i.e., up to 120 days, should be treated as exposed and the entire herd into which the animals have gone or through which they have passed should be treated as exposed herds.

   Herds not showing any evidence of scabies but which are adjacent to infected herds should be treated as exposed herds. This includes all adjacent herds separated from the infected animals by a fence only.

   Following treatment of infected or exposed cattle, the premises on which they were located should be treated with the acaricide used to treat the cattle.

2. **Handling Adjacent Herds**

   Adjacent herds separated from the infected cattle by a road with a fence on both sides or other appropriate barriers, should be placed under surveillance and inspected regularly but do not need to be treated unless otherwise indicated.
3. Handling Scabies in Feedlot Cattle

When scabies is found in a feedlot all cattle in the feedlot should be treated as infected. An exception may be made if the feed-yard operation is so divided that there is no mixing or exposure between sections, and a separate sick pen is used for each section.

Pesticide residues in cattle treated for scabies and moving to slaughter present a problem and owners should be advised concerning the possibility of residues in treated animals. It is desirable to work out arrangements to permit infected cattle to move to immediate slaughter without dipping. The following is recommended:

When feedlot cattle are found to be infected they may move intrastate upon State concurrence, for immediate slaughter. Cattle should leave the feedlot for slaughter within 21 days from the time the mite was identified in order to take advantage of this provision. Those not to be slaughtered during this period require official treatments.

Previous arrangements, approved by State and Federal officials, must be made with the slaughtering establishment. The animals must move in placarded trucks. They should go directly to slaughter pens and should not pass through other public facilities. The vehicles used must be treated with a permitted acaricide before being used to haul cattle from other premises.

Cattle from infected premises may be moved interstate for immediate slaughter after one treatment, provided they move in placarded trucks. They should go directly to slaughter pens and should not pass through other public facilities. The vehicles should be treated with a permitted acaricide before being used to haul cattle from other premises.

4. State Quarantines

Infected and exposed herds and lots should be immediately placed under State quarantine and remain in this status until they receive proper treatments and post-treatment inspections. Cattle in such herds should be identified.

When State quarantines are necessary they should be promptly placed and properly enforced. Quarantines must be practical and operate against the infected and exposed herds while protecting neighboring herds and herds in other areas and States. Quarantines should not be released until all animals have been treated or slaughtered.

5. Federal Quarantines

Preliminary geographical survey of the areas recommended for quarantine to be received by Regional Director in 7 calendar days from the finding of the outbreak. The Federal quarantine will be applied on confirmation of mite at first point with trained identifiers.
Quarantine will be released when the infection has been eliminated from the quarantined area.

6. Methods of Treatment of Infected or Exposed Cattle
The dipping vat is the superior and certainly the preferred method of treatment. However, it is recognized that a dipping vat is not always available nor is it possible in every case for owners of small herds to construct one. The spray-dip machine can be used in such instances. It is an effective method if treatment is closely supervised by qualified inspectors. Close supervision must be given to the use of the dipping vat also. Infected or exposed animals must be held in the spray-dip machine or dip vat for the minimum period to assure that each animal is wetted to the skin. Cattle, either dipped or spray-dipped, should be examined to determine if the acaricide is wetting the animal to the skin. The pesticide selected will determine whether one or two treatments of exposed animals are required.

7. Assembled Cattle on Pasture
Assembled cattle on pasture, wheat field, etc., handle as herd.

B. Sarcoptic Cattle Scabies
Treat the same as psoroptic, including epidemiological investigation.

C. Other Mite Infestations
1. Chorioptic—Cattle in infected herds may not be shipped interstate until treated under procedures outlined for psoroptic cattle scabies. Epidemiological investigation may be omitted.

2. Psorergatic—Cattle in infected herds may not be shipped interstate until treated in heated lime-sulfur. Two treatments, with a 21-day interval between treatments, are required to eliminate the infection. Epidemiological investigations may be omitted.

3. Demodectic—Inform owner of diagnosis and no satisfactory treatment. Epidemiological investigations may be omitted.

III Tracing Movements from Infected or Exposed Herds
Every effort should be made to locate any infected cattle and trace animals moved to and from infected herds in order to locate all foci of the disease.

Officials should immediately notify other States of any movements from infected or exposed herds that involve them. This should be done by wire or telephone (followed by a letter giving further details) if this will aid in locating the animals more promptly and thus prevent exposure of additional herds and spread scabies.

All pertinent information available should be furnished. This includes description of animals, vehicles (breed, age, sex, color, weight, brands, eartag numbers, car numbers, waybill numbers, truck and
trailer license plate numbers, truckers, owners, shippers, and commission company names, etc.) date shipped, and other information that will aid in locating the cattle and/or herds concerned. Veterinary Services should also be notified.
REPORT OF IMPORT-EXPORT COMMITTEE

Chairman: Glenn B. Rea
Co-Chairman: C. K. Jewel

The Committee on Import-Export is privileged after years of frustration to report that at long last negotiations have been made to abandon the inadequate import facilities at Clifton, N.J. in favor of new quarters at Stewart Airport, Newberg, N.Y. We have been informed that hopefully this facility will be in operation within 3 years. In the interim remodeling of buildings at Clifton has provided additional space to accommodate more animals. Whereas inadequate funding delayed development of the Fleming Key facility adequate monies are available for the completion of the new facility at Stewart Airport, which has not yet been officially named.

The delay in developing Fleming Key is at an end. Funds are now available and plans are being finalized and contracts are soon to be let. Construction is expected to begin in the spring of 1976 with anticipated completion in the spring of 1978. Prior to the opening of the Fleming Key Animal Import Center a European embarkation quarantine facility must be designated and approved. The matter of allocation of permits for cattle is still being considered.

Last year the question of private facilities at Payne Field near Everett, Washington for the importation of cattle from non-Foot and Mouth countries was considered and tentatively approved. However, the necessary private funds were not forthcoming and the activity was dropped. Subsequently it was decided that private facilities were not after all a satisfactory answer to the problem of inadequate space.

This committee recommended in 1974 that a user fee be implemented for financing animal import-exports. We have been advised that proposed legislation has been developed and chances for enactment appear to be good. The application of such funds is presently undecided. They may be channeled to the Treasury or they may be administered as a revolving fund within Veterinary Services. This committee has resolved that if and when user fee legislation is enacted that such funds should be administered as a revolving fund within the Veterinary Services. (Resolution Attached)

The question of third country imports was raised. The import-export staff outlined the disease control procedures in force, and pointed out some communication problem between foreign regulatory
officials and USDA. It is anticipated that with the opening of the Fleming Key facility there will be little use for this type of importation.

We have been asked "What about St. Pierre and Miguelon"? The Import-Export Staff have informed us that such import movement will be administered in the same manner as animals coming through Canada.

Our 1974 request for regulations pertaining to importation of Equine species with regard to EIA and piroplasmosis has been answered by proposed rule making which will be presented for public comment within the next 15 days.

The success of the Avian Import Program through private facility is manifest by the detection of infection in 24% of the lots of birds offered for entry.

This committee regrets that a decision by the office of General Council makes mandatory the approval of any privately owned bird import facility meeting this minimum standard of Veterinary Services. This will pose an unacceptable financial and personnel burden on the program.

Fears expressed a year ago regarding the importation of semen were not realized. A point was made that certification of disease control procedures, by the USDA would enhance exportation of this product. It was pointed out that there is international competition in this field and that the U.S. commodity is discriminated against because of the lack of such certification. This committee recommends that Veterinary Services seek authority to make such certification.

The fact that state requirements for importation often supersede Federal requirements was pointed out. This causes complication for state officials when importers have not had adequate and timely information regarding these facts. This committee has resolved that the Import and Export Staff be requested to develop methods whereby importers may be so informed (Resolution attached).

The situation as pertains to the surveillance of illegal imports on the Mexican border is neither new nor has a ready and adequate answer been presented. However, it was suggested that an improvement to the problem would be the creation of a separate area office in the same manner as that provided for the screwworm program to deal specifically with all border problems. This could allow for a closer liaison with customs officials and give closer direction to the personnel involved. It is felt that this result cannot be as effectively obtained with headquarters in Austin and Sacramento.

Last year your Committee questioned the lack of adequate controls on imported milk and milk products from foot and mouth disease infected countries. We have been informed that on September 25, 1975, Part 94, Title 9, CFR, was amended to provide increased restrictions on the importation of such products to the end that they
will be prevented from incorporating in livestock feed. This Committee is pleased that restrictions have been expanded to this end. However, we recommend that the USDA continue investigation of procedures to assure that present accepted processing methods will in fact eliminate foot and mouth disease virus from the imported product.

The Committee was challenged regarding the adequacy of surveillance of galley supplies carried by both airlines and ocean going vessels arriving from F & M countries. We resolved to seek specific action byAPHIS in this regard. (Resolution attached.)
Rabies Committee Report

Chairman: R. Keith Sikes, Atlanta, Ga.
Co-Chairman: E. A. Carbray, Ames, Iowa


The Rabies Committee met on November 4, 1975 with a total of seventeen members and guests present. Two new members, Drs. James Glosser and Victor Cabasso, worked with the committee during 1975.

The Rabies Committee reviewed the recommendations made at the 1974 meeting and acknowledged the following accomplishments:

1. That there are no longer any rabies vaccines being sold to unlicensed veterinarians in the United States. The two companies who were previously selling their vaccines to non-veterinarians, have now discontinued that practice at the request of members of our committee and certain governmental agencies.

2. The standardized rabies vaccination and small animal health certificates developed by our committee in 1974 were endorsed and recommended for national use by the Association of State Public Health Veterinarians at their annual meeting in June 1975. Also, the Livestock Commerce Committee meeting in Portland reviewed these certificates and recommended their use to standardize such certificates throughout the United States.

During our 1975 meeting, the committee discussed reports presented by the following subcommittees:

A. The Animal Rabies Vaccine Compendium Subcommittee reported that progressively more states are using the Compendium as a basis for animal vaccination and standardization. Dr. Jerry Peacock kindly presented some of the USDA's policies which Veterinary Biologics requires for testing and he answered several questions posed to him by our members. The committee recognized that a sufficient number of changes are needed in the Compendium and recommended that it be revised as soon as possible. Dr. Jerry Winkler, Chairman of this Sub-
committee, agreed to initiate efforts with members of his group to revise the Compendium and have it published by February 1976. The committee recommended that CDC be the agency responsible for printing the Compendium and making it available to all of the states.

B. The Subcommittee on the Standardization of the Health/Rabies Vaccination Certificate recognized that it is now time to request assistance from the 50 State Veterinarians to implement the use of this certificate developed by this committee. A resolution to that affect is being presented to the Executive Committee, USAHA.

C. The Subcommittee on Stray Animal Control, headed by Dr. Bruce Kaplan, presented a summary of a National Animal Control Package prepared by his group. The committee recommended adoption of that package of recommendations and that these be made available by the USAHA Rabies Committee to the CDC and to the ASPHV as well as others who can help implement stray animal control procedures at the local level.

D. The Subcommittee on the Development and Promotion of Television and Educational Material announced successful efforts in getting some recent national TV coverage on rabies control through ABC. Further plans for more national TV coverage were announced. The committee recommends that all states work closely with their Veterinary Extension Service to coordinate local educational material on rabies control.

E. The Subcommittee on Unethical Rabies Vaccinations acknowledged the excellent cooperation from vaccine manufacturers to prevent the sale of their rabies vaccines to non-veterinarians. However, it was recognized that certain distributors still manage to obtain and sell rabies vaccines to “lay” personnel. The committee recognized the difficulty in obtaining complete control of this practice and therefore recommends that states which continue to have this problem be advised to pass state-wide legislation to prevent such sales of rabies vaccines to persons other than licensed veterinarians.

F. The Subcommittee on Rabies Research presented interesting studies now underway. The one study concerned with age-specific efficacy for canine rabies vaccination being conducted by Dr. Strating at Ames, Iowa will undoubtedly assist in making better recommendations for canine vaccination in the
The committee recommend that these and similar studies be continued.
Mr. Chairman, this concludes the 1975 Rabies Committee Report.

Respectfully submitted,
R. Keith Sikes, Chairman
REPORT OF COMMITTEE ON FOOD ANIMAL HYGIENE & INSPECTION

Chairman: Walt Fechner, Little Rock, Ark.
Co-Chairman: J. K. Payne, Washington, D. C.

Your committee wishes to reiterate its renewed support and additionally comment on those topics of our 1974 report which are still viable.

1. The Proposed National Meat & Poultry Inspection Program

Your committee wishes to re-emphasize the need for efforts by the National Meat & Poultry Inspection Advisory Committee to continue to explore the concept for developing a more closely integrated state and federal inspection system, in the Interest of: (1) Greater uniformity of program application and increased efficiency in inspection manpower utilization; and (2) To continue efforts to improve communications from state to state and between the states and the Federal Government.

2. Tissue Residue Reports

The committee was encouraged to learn that progress had been made in the drug residue program, especially in regard to: (1) Supplying drug withdrawal time information to producers by the Bureau of Veterinary Medicine of FDA; and (2) The development of an integrated program for the state and federal agencies for use in the investigation of reported, violative drug residues.

FDA officials reported that a memorandum of understanding is in the final stages of preparation which will provide for the coordination of states with FDA and USDA in residue tracebacks.

The committee urges that every effort be made by all three agencies to implement the coordinated state-federal traceback program as soon as possible to hopefully achieve a uniform investigational procedure without duplication of effort.

3. Swine Tuberculosis

The committee recognizes that although swine tuberculosis is primarily of the Avian or Atypical groups, there is support the theory that infections may be due to atypical soil borne organisms.

Whereas there may be some advantage in clarification of such etiological agents as swine mycobacteriosis because of an apparent stigma attached to the term swine tuberculosis; and

Whereas the public health significance of swine mycobacteriosis has not yet been clearly determined;

Be it therefore resolved that a rapid development and implementation of a protocol for the investigation of the thermal death point of the causative organisms of swine mycobacteriosis, and the results
of such a study, if of positive value, be made available for use through the rule making process to amend meat inspection regulations.

New recommendations emanating from this 1975 meeting are:

1. Your committee recommends that all states with ongoing "equal-to" federal meat and/or poultry inspection programs exert every effort to continue to manage and operate their respective state-federal cooperative inspection programs for as long as they continue to be acceptable as "equal-to" operations and shall continue to receive up to 50% of the total costs through federal funding.

2. Animal Disease Reporting
Whereas inspection of food animals at slaughter results in detection of many animal diseases; and
Whereas there is insufficient data presently available in the United States on the prevalence, geographic and temporal distribution of animal diseases; and
Whereas data is presently being compiled on numbers of animals slaughtered and numbers retained and condemned for various conditions;
It is recommended that the USAHA recommend to USDA/APHIS that such data on animal diseases as is being generated daily by inspection of food animals at slaughter be analysed for prevalence and incidence rates, temporal and geographic distribution, and secular trends, and that this information be made available to veterinary services and other agencies and individuals in a timely manner to evaluate disease control programs and needs for control programs or disease control research.

3. Microbiological Guidelines
Your committee is concerned with the proliferation of states or local governments that have enacted statutes implementing microbiological standards for meat and poultry products. Your committee recommends that caution should be exercised and to this end we support the National Meat and Poultry Inspection Advisory Committee motion "that USDA continue their investigations in the microbiological area and that APHIS establish a structure with broad based technical expertise to begin developing model microbiological guidelines, and should keep the states apprised of developments."

4. Inspection Priorities and Manpower Utilization
Your committee recommends that meat and poultry inspection resources be utilized in inspection of areas of greatest public health concern or need, specifically focusing more on slaughter inspection and increased use of statistical and laboratory sampling of processed products.
Your committee further recommends that "a task force or action committee," as selected by this committee, be confirmed to evaluate inspection areas and establish a priority order of public health significance consistent with insuring a continuing supply of wholesale human foods. Concentration shall be on inspection areas that may be de-emphasized without affecting the wholesomeness of human foods.

Thank you, Mr. Chairman.

Members of this group who were named to the Action Subcommittee Chairmen are: Dr. Ray McFarland, California; Dr. Dave Miller, Virginia; Dr. David Bedell, Georgia; Mr. John Winterbauer, Illinois; and Dr. Walt Fechner, Arkansas; Coordinator.

Individual reports are to be received by the Chairman not later than August 1, 1976.
The committee wishes to alert members of USAHA that throughout the bicentennial year, many organizations will be utilizing horse and oxen teams for methods of profit to celebrate the bicentennial. We wish to caution all persons concerning the proper handling of these animals during long and extended trips.

The committee strongly recommends the passage of the dog and cock fighting bills by Congress, however, we also recommend this bill be placed under the auspices of the Federal Bureau of Investigation or other law enforcement agency, in cooperation with USDA in so far as USDA personnel will be utilized for reporting of problem areas only. All law enforcement activity must be pursued by the FBI. This committee does favor the Foley-Magnusen bill in emphasizing the need of two separate agencies in enforcing this act.

Wild Horse Round Up

It is, at this time, apparent that the wild horse and burros act needs amending to allow for better management of the protected herds. The law is restrictive to a point that it places both animals and man in considerable danger in the collection of excess horses and burros. It is recommended that an amendment be considered to allow the use of aircraft and land vehicles for spotting purposes only, by qualified personnel. Catch horses are now being run into the ground in an attempt to locate and separate animals that must be removed from the herd. Consideration should be given to other uses of the excess animals, but not for commercial purposes.

It is the recommendation of the Committee on Animal Welfare that the USAHA continue to support good legislation to improve conditions and add additional protection for animals in air transportation as well as support extension of the Laboratory Animal Welfare Act through amendment to include transportation by air under the jurisdiction of the USDA, the work of the National Council on Animal Transportation and the hearings being held by the CAB on air transportation. We also support the USDA in the consideration of the need for international regulations to protect horses being shipped to Europe and Africa by both sea and air as food and breeding animals.

Consideration must be given to bringing livestock haulers within the 28 hour law to afford animals transported by truck the same pro-
tection now afforded animals transported by rail. Consideration should also be given to bringing this same group of livestock haulers within the jurisdiction of the ICC, exempting farmer owned vehicles transporting their own livestock. In extension of this subject we need to consider the mismanagement of livestock at livestock auctions.

The Committee on Animal Welfare submits the following recommendations for presentation to the Secretary of Agriculture:

1. To require photographic identification of each dog and cat sold to random source dealers for resale, and of the license plate of the car of the persons offering the animals for sale. Purpose of the documentation: to discourage theft of animals for resale and to facilitate successful prosecution in the event of a violation.

2. To promulgate final regulations to provide opportunity for daily exercise for laboratory dogs.

3. To place a higher priority on complaints of violations of the Animal Welfare Act to reflect increased FUNDING AND PERSONNEL and to provide for immediate direct action to achieve compliance with the act in a prompt and orderly fashion.

The Animal Welfare Committee further recommends that the Animal Welfare Act of 1970 be amended to:

1. Improve enforcement capabilities of the Department of Agriculture by amending Section 16(c) of the Act to include the following: "An action against dealers, exhibitors, research facilities, and operators of auction sales may be brought before any United States Magistrate in any judicial district in which such person is found and such magistrate shall have jurisdiction to hear and decide such action," and;

2. Because hunting and other working type dogs moving in commerce are often shipped under substandard conditions, they should be included. The words, "hunters and other working type" should be inserted in the definitions of "dealer" and of "animal" and throughout the Act wherever required to insure coverage.

The Committee on Animal Welfare commends the U. S. Department of the Interior for the promulgation of regulations February 24, 1975 on the Injurious Wildlife Act consistent with recommendations made last year by the U. S. Animal Health Association and urges that these regulations be made final.
The major interest of the Professional Relations Committee is to activate USAHA to inform the segments of the livestock and allied industries and the veterinary profession of the activities and accomplishments of the USAHA.

Increased emphasis needs to be directed towards disseminating this information in that the deliberations and resolutions of the USAHA are not completely reaching all segments of the industry.

More effective distribution of USAHA activities would result in greater support for the organization of this objective, the committee recommends that the Board of Directors of USAHA consider methods and means of publicizing USAHA activities:

Recommendations are:

a. The use of a trained Information Specialist for a period prior to and after the meeting to aid in publicizing the oncoming meeting and the organization's activities during and following the meeting.

b. The continuation of the newsletter on a monthly basis, circulation to include livestock organizations not now members of USAHA.

c. To finance the above, the committee recommends that $2.00 of every member's dues be earmarked for this purpose.

The committee was pleased that the American College of Veterinary Preventive Medicine has received probationary status from the Council of Education and the House of Delegates of the American Veterinary Medical Association and recommends that the USAHA support the College.

The Committee recommends that the USAHA commends Emergency Programs, Veterinary Services and APHIS for planning and developing Regional Emergency Animal Disease Eradication Organizations to handle outbreaks of Foreign Animal Disease.

Mr. Chairman, these recommendations and deliberations constitute the actions of the Committee on Professional Relations and they are respectfully submitted. We recommend their adoption.
REPORT OF THE STATE-FEDERAL RELATIONS COMMITTEE

Chairman: H. E. Goldstein, Columbus, Ohio
J. F. Andrews, Atlanta, Ga.; A. E. Janawicz, Montpelier, Vt.;
W. L. Bendix, Richmond, Va.; J. C. Shook, Mechanicsburg, Pa.;
D. H. Spangler, Olympia, Wash.; T. A. Ladson, College Park, Md.;
H. Q. Sibley, Austin, Tex.; L. E. Bartelt, Sacramento, Calif.

The Committee wishes to express appreciation to all Federal personnel who gave freely of their time and efforts at this meeting and we hope that mutual benefits will result for all agencies and associations concerned. The State-Federal Relations Committee presents the following statement for mutual consideration and guidance.

Veterinary Services

This committee commends Veterinary Services for the efficiency of the Emergency Disease Programs. This group has demonstrated the ability to mobilize attack problems and render results. The Emergency Disease Program staff has demonstrated the "urgency motivation" to provide the necessary programming to accomplish their purpose.

This committee recommends that steps be taken to provide the necessary budget to expand and diagnostic reference laboratory services needed by the respective states to adequately service the livestock and poultry industries of the nation. The committee urges an additional two million dollars to be included in the 1977 fiscal budget.

This committee recommends that veterinary services review all private laboratories approved to perform EIA tests from the standpoint of techniques and regulatory communications. It is recommended that no more private laboratories be approved and to provide a dephasing of existing approved laboratories.

The committee recommends proper funding of the E.I.A. regulatory program to prevent the movement of E.I.A. reactors in interstate movement.

We commend and encourage veterinary biologics in their efforts to provide safe and efficacious biologics. It is recommended that all steps be taken to provide the necessary budget to insure the time and facility programs as were presented to the committee. We recommend that the laboratory and building program be expanded approximately 20% beyond the 1977 projection to meet the needs of this nation by 1978.

The committee notes the expanded activity of the Import-Export staff. The committee points out the need of this mandated program and urges adequate financing of this effort.

This committee recommends an all out effort in the control of privately owned pet bird quarantine stations, be put into effect.

We recommend the completion of the Fleming Key facility at the
earliest possible time, and the building of the Clifton, New Jersey replacement. Laboratory facilities at Plum Island must be available in the same time table.

The committee points out the need for consideration to a swine tuberculosis eradication program. Additional research is necessary to provide diagnostic techniques for field use. An identification and indemnity program should receive serious consideration.

The committee recommends a continuing budget for hog cholera in compatibility with the Secretary of Agriculture National Hog Cholera Eradication Committee recommendation for a three year surveillance after declaration of Hog Cholera Free Status. The committee suggests review of this budgetary item for reprogramming in more vital programs in the 1977 budget.

This committee commends the Secretary of Agriculture for his apparent interest in the proposed National Poultry Disease Advisory Committee. This committee urges the formation of this important advisory committee, and will recommend to the U.S.A. H. A. Executive Committee, that this body officially seek membership on this committee.

The brucellosis eradication program is in a most critical status. This committee was asked in the 1974 meeting with A.P.H.I.S. if the committee felt that staff members of veterinary services appreciated the urgency of this problem. The committee’s reply was an emphatic negative. The committee related that the integrity of regulatory veterinary medicine was in jeopardy.

At a called meeting of the Executive Committee of the U.S.-A.V.M.A. in December, the group petitioned the Secretary of Agriculture to declare a national emergency to give the program a status of urgency. It is now May, 1975 and this committee observes no changes in program or policy. This committee in reviewing the policy, the program and the budgeting requests feel that this program is going “down the drain.”

We appreciate the small increase in funding as projected for 1976, amounting to approximately four million dollars. Veterinary services proposal to continue the program for an additional ten years at that rate is most unrealistic, but will represent the expenditure of an additional three hundred and twenty million dollars to little purpose, and will not achieve eradication, but will so disorganize the effort that it will all but disappear including what industry support we still maintain.

We insist that a realistic program be developed for financing fiscal year 1977 designed to eradicate bovine brucellosis in a four to five year time, and that proper change in uniform methods and rules be developed for recommendations to apply and enforce the necessary sanction for those areas of the nation that decline to support this type of program.
We further insist that A.P.H.I.S. develop this type of program and present it to the Executive Committee of U.S.A.H.A. at the Anaheim meeting of the A.V.M.A. in July.

We pledge total support through the office of Budget and Management, the Secretary's office, the respective states support and the industry support through all our effort of the U.S.A.H.A.

If this type approach cannot be realized, this committee has no other recourse but to recommend to the U.S.A.H.A. that it consider withdrawing its support to the program and recommending its abolishment.

Veterinary A. R. S.

This committee has reviewed with the ARS staff, the obligations and projections for the research program for diseases and parasites of livestock and poultry. We compliment your agency on the persistence they have demonstrated in many of these projects often with limited funds and restrictions. These research programs are needed by the various livestock and poultry industries and have benefited them economically. Your agency should also be applauded for the rather small percent increase between funds obligated for 1974 and those estimated for 1975. It is gratifying to see a federal agency that is not escalating spending at a blinding pace.

The committee appreciates the important of everyone of these research projects, however we feel that a close study of priorities needs to be made in view of our present economy.

We point out the example of bovine brucellosis. Several years ago the Department of Agriculture staff recommended deemphasis of the use of Strain 19. Many states who had gained free status or had come to a point in their eradication efforts where incidence was very low, followed this recommendation. Right now we are realizing the fallacy of this recommendation. The department itself has now turned about fact on the need for brucellosis vaccination. Had adequate research data been available, this mistake might not have been made and the resulting delay in program progress may have been avoided or at least minimized. Even now we are unable to give straightforward answers to the industry regarding benefits of Strain 19 vaccination. The eradication of brucellosis in our country is now number one in priority yet we note that only $152,500 was spent in 1975 by your agency for bovine brucellosis research. This committee recommends that research for brucellosis in testing techniques and use of newer immunizing agents be given top priority. We urge serious considerations be made to put into abeyance some research programs which are of less economic importance to the industry and to public health and redirect vast increased funds into brucellosis research. This support to the cooperative eradication program is needed urgently if we are going to complete our efforts in reasonable time. If combined
research and regulatory agencies fail to complete eradication now, the millions of dollars spent over the last several decades in this endeavor will be lost and the disease will persist.

Another area of concern at the present time are those diseases of wildlife which effect both wild and domestic species and in some cases humans. We recommend that ARS work closely with other federal, state and private agencies in researching these diseases so that outbreaks can be prevented from occurring in our country and if they do occur will not create economic disaster for any of our domestic food producing industries. The utilization and/or disposal of products from animals involved in emergency situations is an area where hard facts are drastically needed, for example milk from dairy animals infected or exposed to FMD.

We recommend that ARS, HEW, and EPA work cooperatively in the establishment of guidelines for human tolerance of various residues detected in food products derived from livestock and poultry.

Another area of immediate concern is needed for further study on equine infectious anemia involving diagnostic methods and possible therapeutic measures.

We would welcome representatives from your agency presenting to the Executive Committee meeting of U.S.A.H.A. at Anaheim, California, in July your recommendations and budget projections for fiscal year 1977. Our Association pledges support to those areas where it is needed and desired.

Meat Inspection

USDA Meat Inspection Program as a whole when compared to those of other nations, continues to show outstanding leadership in obtaining compliance for Consumer Protection.

Current financing is tight and in many areas inadequate as indicated by the following:

(1) Provide timely proof of slaughter of branded reactors. Currently many indemnity payments are months behind waiting for such reports.

(2) Not only in submitting laboratory specimens and placing emphasis on such submissions, but providing adequate available identification (now often lost in the slaughter process) in order that herd traceback may be possible.

(3) Residue testing increased or changed to include (possibly under separate statistical standards) special consideration for animals showing mastitis or other lesions where there is increased likelihood that individual animal treatment may have occurred.

The above items will be difficult to correct in high volume plants without adequate staffing that must be oriented to correction of the problem source in addition to removal of the end product when unwholesome from consumer channels of trade.
This committee concurs with the proposed efforts of Meat Inspection to develop a consumer education effort to reduce salmonella outbreaks as well as processing techniques designed to reduce the amount of salmonella contamination.

This committee sees a problem if states continue to set microbiological standards on their own without guidance for achieving uniformity on a national basis. We recommend that USDA take the leadership to obtain research needed to formulate these guidelines if need is determined.

This committee would like to see USDA study the above recommendations with the thought of proposing changes in their 1977 proposed budget. An up-to-date or progress report to the U.S.A.H.A. Executive Committee during the A.V.M.A. convention in Anaheim, California may prove helpful for this Association to lend assistance if needed.

This committee recommends that continued efforts to establish a single state-funded system in states wishing to participate be researched if USDA desires continued state participation. An efficient effective system demands that the roadblocks be removed from the development of a single system in each state.
REPORT OF THE COMMITTEE ON EVALUATION AND DEVELOPMENT OF STATE-FEDERAL PROGRAMS

Chairman: J. L. O'Harra
Co-Chairman: J. G. Milligan


The USAHA Evaluation and Development of State-Federal Programs Committee met at 1:30 p.m., Thursday, November 6, 1975, with 12 members and 20 visitors present. The 1974 Committee report was reviewed and the members unanimously commend APHIS, Veterinary Services, for their response to the USAHA request for relocation of District Veterinarians and other suggested refinements to correct problems resulting from the realignment program. The maintenance of District Veterinarians in the field is correcting many of the problems in the field operations.

Discussions revealed that there was lack of uniformity in the contracts and no provision for increased workloads and necessary salary adjustments. The Committee developed a resolution concerning this problem which has been forwarded to the Resolutions Committee for action.

The Committee recognizes the past complacency among State-Federal and livestock groups concerning brucellosis eradication and commends recent acceptance of responsibility by these groups and the spirit of urgency exhibited in the eradication of this disease.

It was brought to the Committee's attention that many months elapse before federal positions can be filled or the Civil Service Register is received so that a selection can be attempted. These delays are not consistent with efficient program management and we urge APHIS to take necessary action to speed up selection and hiring procedures.

Your Committee recognizes and supports the commitment by the U. S. Department of Agriculture and by this Association that brucellosis has first priority in our activities. However, we also recognize, in certain instances, miscellaneous disease activities are essential in maintaining the health of our livestock population and that in the interest of efficient use of manpower and other resources the locally assigned employee, be he State or Federal, should be available to conduct this work provided this does not interfere with the eradication of brucellosis.

Mr. Chairman, the Committee respectfully submits this report to the Executive Committee for Consideration.
The Committee on Public Health and Environmental Quality met Wednesday afternoon, November 5, 1975; 24 members and guests attended. Dr. Erskine Morse of Purdue University presented a paper entitled "The Status of Bovine Salmonellosis—Prevalence and Epizootiology, with Public Health Clinical and Control Aspects" which is appended to this report for inclusion in the proceedings. Several in attendance commented on the problem from their experience, it was the consensus that bovine salmonellosis constitutes a more serious than commonly recognized public health hazard to those in contact with infected animals, secondary cases have also been noted in the household contacts of such cases. Anecdotal accounts from several states were reported, in some, the sources of the infecting organism was unknown, but in most instances, the same serotype was recovered from cattle (or calves) and the associated human cases.

The need for facilities for serotyping of salmonella organisms as an aid to epidemiologic investigations of the relationship of outbreaks of bovine disease and associated human illness was emphasized by the committee members.

The committee discussed items for inclusion in the agenda for next year. Animal systems useful for monitoring the environment for specific pollutants, the value of this identification, \(^{30,31,32}\), and methods of including them in appropriate systems will be discussed. The committee will be given an opportunity to submit additional suggestions for subject matter.

* * * * * * * *

The Status of Bovine Salmonellosis — Prevalence and Epizootiology, with Public Health, Clinical and Control Aspects

Erskine V. Morse,* Margo A. Duncan,* John S. Baker† and Kenneth Weinland†

Purdue University, Lafayette, Ind. 47907.
PREVALENCE

Salmonellosis represents the most prevalent zoonotic infection among cattle in the U.S. Of over 1700 identified Salmonella serotypes, at least 111 have been isolated from cattle according to surveys reported in the world literature during 1933-1973. Some 81 have been isolated in the U.S. and 60 have been cultured and typed from bovine outbreaks by laboratories outside the continental U.S. Approximately 30 serotypes have been found in Bovidae in both the U.S. and other countries.

The most common salmonellae isolates found in the U.S. and other lands are summarized in Table 1. S. typhimurium is the predominant of the group (72.27%) found in the U.S., while S. dublin represents the most prevalent (40.73%) of the pathogens reported from abroad.

SEE PAGE 47 FOR TABLE 1

Less common isolates found in cattle during 1933-1973 have been S. cholerae suis var kunzendorf, S. cholerae suis, S. Pullorum, S. meleagridis, and S. paratyphi B. SEE PAGE 48 FOR TABLE 2

The authors estimate that approximately 10% of the U.S. cattle are infected or have been infected with salmonellae. Rothbenbacher theorized that 13% of all Michigan cattle have been carriers. The premise of 10% infection for U.S. livestock probably is realistic.

EPIZOOTIOLOGY/EPIDEMIOLOGY

S. typhi, has been isolated only once from animals according to Dack. The animal species was not specified. S. paratyphi B transmission from cattle to man has been documented. Serotypes typhi and paratyphi B are usually accorded host-specificity for man, especially the former. S. typhi is a true host-adapted enteric pathogen. Milk borne typhoid epidemics occur when the milk is contaminated by human carriers. This epidemiologic situation should be emphasized to the lay public for cattle do not harbor the typhoid organism!

One may theorize that cattle infections with S. cholerae suis resulted from transmission of the agent from swine. S. pullorum, S. meleagridis, and S. gallinarum may have been acquired from contact with poultry. There is little doubt of the susceptibility of cattle to

*Dept. Microbiology, Pathology and Public Health, School of Veterinary Medicine & the Environmental Health Institute.
†Dept. of Large Animal Clinics, School of Veterinary Medicine. Published with the approval of the Director of the Agricultural Experiment Station. Journal Paper 6057.
all *Salmonella* serotypes under proper environmental conditions or exposures.\(^{18}\)

Edwards indicates\(^{21}\) that on occasion a salmonella-mastitis may occur and that *S. typhimurium* may be the cause of outbreaks due to consumption of infected milk.\(^{22}\) Food poisoning outbreaks due to cheese prepared from “pasteurized” milk have been reported by Tucker, *et al.*\(^{23}\) Gibson states: “Human infection with salmonellae can be derived from cattle by contact, direct or indirect, with infected cattle on the farm, by contamination of the milk supply, or from contaminated or infected meat or offal”. A complete discussion of *S. typhimurium* transmission from cattle via milk is given.\(^{18}\)

Epizootics of *S. typhimurium* infection in calves resulted in 3 outbreaks of salmonellosis in farm workers which spread to their families.\(^{17}\) Dogs also were sero-positive.\(^{17}\) Sirmon, *et al.*\(^{4}\) described episodes of *S. typhimurium* and *S. enteritidis* infection which spread from carrier cows to calves and to people. The probable transmission of *S. typhimurium* from infected dairy cows (7 in herd) to seven members of a farm family has been reported in Wisconsin.\(^{25}\) The agent was isolated from the feces of a dairy cow which died. A second animal expired and in due time the five remaining cattle developed diarrhea and fever. *S. typhimurium* was cultured from the stools of the seven human patients who experienced the typical symptoms of acute salmonellosis.

The authors have investigated two episodes of possible *Salmonella* transmission from cattle to human beings. A 24 year old male was milking salmonellae-infected cows. He routinely consumed the unpasteurized milk over a 3 week period. The sequence of events was as follows:

*5/17* he felt “ill” and experienced malaise. Penicillin was self-prescribed. He then “felt better”.

*5/19* at 2 a.m. a violent diarrhea commenced. Stools were watery and contained mucus, but no blood. Evacuations occurred every 15 to 30 minutes. Vomiting was experienced every 30 minutes. He did not eat, but partook of fluids. Reported to the hospital and received “symptomatic treatment”.

*5/20* diarrhea continued; vomiting ceased.

*5/21* diarrhea continued but severity diminished.

*5/22* diarrhea ceased. Patient commenced eating and retaining food. On 5/19 a *Salmonella* was isolated from the patient’s stool. It was serotyped as “group B: 4,5” according to the hospital laboratory. Unfortunately, the culture was discarded. Group B: 4,5 contains a number of serotypes, one of which is *S. typhimurium*. This agent was present in the cattle during the time the patient consumed unpasteurized milk. The pathogen may have been secreted in the milk of an infected cow or the milk could have been contaminated with salmonellae contained in feces.
In the second episode, Weinland and colleagues, investigated a S. typhimurium bovine epizootic involving 20 calves of which 12 had a severe diarrhea and fever. Nineteen calves died within 3 weeks after purchase. S. typhimurium was isolated from fecal material from one and S. newport was cultured from the intestinal tract of another. An open bag of commercial milk replacer which was fed to the calves contained S. typhimurium. Three daughters in the family who cared for the animals, developed a paratyphoid-like syndrome shortly after the diseased cattle were purchased from a sale barn. The children were hospitalized for approximately one week. S. typhimurium was cultured from the stools of all three. S. newport was found only in one calf; however the probability exists that some of the bovine infections were dual serotype salmonellosis.

CLINICAL ASPECTS/TREATMENT

The signs of bovine salmonellosis are well known and documented. They do not differ markedly from those observed in other livestock. The peracute form, with bacteremia/toxemia and sudden death in a matter of 2-4 days, is not uncommon in calves and debilitated, mature animals. Among 5 peracute infections observed by the authors, 2 died and 3 recovered. Those that recovered were calves under 1 year of age. Such is an unusual situation. Mortality in calves on dairy farms where enzootic salmonellosis exists may exceed 70% as already indicated in this paper (second episode). The acute form of the infection has a course of 1 to 3 weeks and occurs in cattle of all ages. Eight of 10 patients recovered in a series we observed. The chronic form may last for several weeks to three months. Of 4 such cases, 3 recovered and 1 died; all were mature animals. Salmonellae have been shown to persist for months in the bovine rumen and may be present and shed in the feces as well.\textsuperscript{11,15,27}

The treatment of bovine salmonellosis has been frustrating and discouraging to clinicians. Furacin compounds, chloramphenicol, polymyxin B, neomycin, ampicillin, kanamycin, tetracyclines, streptomycin, various sulfonamides as well as penicillin compounds have all received attention. The authors have isolated salmonellae from the feces of cattle, prior to, during, and following various antimicrobial therapies. Some isolants have been sensitive, while others were resistant to the drug(s) employed at these times. It has been concluded, following evaluation of 20 cases of bovine salmonellosis, that supportive and symptomatic therapy are the most significant factors in successful patient recovery.\textsuperscript{27} The value of ascertaining antimicrobial sensitivities of salmonellae in the laboratory is open to question. The results seem to bear little relationship to the \textit{in vivo} elimination of the infecting agents.

Occult or subclinical carriers are not unusual in cattle. Multiple
infections with several serotypes do occur and should not be overlooked. In the environment of a veterinary clinic, occult carriers are admitted regularly as patients for treatment of medical or surgical conditions other than salmonellosis. The cattle's resistance is lowered by transportation, change of diet, and the presence of other disease conditions. Under the stress of surgery for abomasal displacement, cattle appear to be more susceptible to *Salmonella* infections. In addition the subclinical carrier state may be precipitated and progress to the acute phase of the disease. Infected horses in the hospital complex may serve as sources of bovine salmonellosis. Therefore the inter-species transfer of the agent in the facility is a factor that should be considered.

The control of bovine salmonellosis is important from a human and animal health standpoint. Cattle apparently harbor a wider variety of serotypes than do swine, horses and dogs. Infected adult cattle may become permanent carriers and shed large numbers of salmonellae in their feces. As many as 1 million salmonellae may be found in 1 gram of lung tissue from an infected cow.

Factors which contribute to enzootic bovine salmonellosis, and complicate the control of infection are:

* Early weaning of calves and colostrum deprivation.
* Shipment of calves under 1 week of age for over 100 miles.
* Congregation of calves under 1 week of age at salebarns and collection points.
* Crowding in feedlots, salebarns, and dairy barns.
* Unsanitary conditions, i.e. damp barns with manure accumulation, unsanitized stock transport trucks, contaminated feed trucks, etc.
* Inclement cold, wet weather with inadequate shelter, feed and water availability, especially for calves.
* Inadequate treatment and care of respiratory, gastrointestinal diseases etc. in calves in the presence of a *Salmonella* contaminated environment.
* Stresses of calving, metritis, mastitis, milk fever, cystitis, ketosis, surgical operations, etc. in carrier animals may precipitate clinical salmonellosis.
* Stresses involving prolonged hospitalization for medical or surgical problems in a salmonellae contaminated environment.
* Improper disposal of human as well as animal wastes thus allowing pollution of water supply and premises.
* Improper disposal of dead animals which can serve as sources of salmonellosis or any other infectious disease.
* Permitting cattle access to hog and poultry lots, as well as streams, ponds, and rivers.
* Failure to isolate newly purchased stock — especially if they originate from a salebarn or collection point.
Lack of quarantine for cattle which have diarrhea or fever, and failure to establish the etiology.

Storage of feeds, milk replacer and premixes in rodent, pigeon, and wild bird infested areas, failure to keep dogs and cats from such places as well.

Measures to prevent transmission of salmonellae from cattle to human beings are:
1) Refrigerate milk as soon as possible.
2) Pasteurize all milk for home consumption.
3) Prevent fecal contamination of hands and other body surfaces — particularly when in contact with cattle which have diarrhea.
4) Keep children away from “sick” calves and cattle.
5) The custom or home slaughtering of cattle and calves should be conducted under sanitary conditions. Fecal contamination of the meat should be avoided. Obviously, only healthy animals should be consumed. Proper and adequate refrigeration of meats is necessary.
6) Persons with persistent diarrhea and concurrent fever should be referred to the family physician. If cattle on the premise are also sick, the physician should be so informed.

In summary, salmonellosis is a serious disease in beef and especially dairy cattle. Any serotype of Salmonella may be responsible for infections in cattle and may also be acquired by man. Human beings may conceivably infect cattle as well. The local veterinarian and family physician are the most important links in the prevention, education and control chain. Through their combined efforts, economic losses in livestock and human suffering due to salmonellosis can be reduced and hopefully minimized.
### Table 1

Prevalence of Serotypes Identified in the U.S.\textsuperscript{1-9} and Foreign Countries\textsuperscript{10-18}

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>U.S. 1933-73</th>
<th>FOREIGN 1939-1971</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{typhimurium}</td>
<td>6872 (72.27%)</td>
<td>574 - second (36.19%)</td>
</tr>
<tr>
<td>\textit{newport}</td>
<td>967 (10.2%)</td>
<td>10 - eighth (6.31%)</td>
</tr>
<tr>
<td>\textit{dublin}</td>
<td>713 (7.50%)</td>
<td>646 - first (40.73%)</td>
</tr>
<tr>
<td>\textit{anatum}</td>
<td>224 (2.36%)</td>
<td>33 - fourth (2.08%)</td>
</tr>
<tr>
<td>\textit{heidelberg}</td>
<td>167 (1.76%)</td>
<td>8 - tenth (0.504%)</td>
</tr>
<tr>
<td>\textit{saaintpaul}</td>
<td>104 (1.09%)</td>
<td>11 - seventh (0.694%)</td>
</tr>
<tr>
<td>\textit{enteritidis}</td>
<td>99 (1.04%)</td>
<td>9 - ninth (0.567%)</td>
</tr>
<tr>
<td>cholerae-suis (both)</td>
<td>37 (0.389%)</td>
<td>0</td>
</tr>
<tr>
<td>muenchen</td>
<td>18 (0.189%)</td>
<td>37 - third (2.33%)</td>
</tr>
<tr>
<td>brandenburg</td>
<td>0 0</td>
<td>23 - fifth (1.45%)</td>
</tr>
<tr>
<td>give</td>
<td>6 (0.063%)</td>
<td>19 - sixth (1.20%)</td>
</tr>
<tr>
<td>oranienburg</td>
<td>9 (0.095%)</td>
<td>19 - sixth</td>
</tr>
</tbody>
</table>

**Total:** 9216 (96.92\%) 1389 (87.58\%)

**Grand Total:** 9509 1586
REPORT OF THE COMMITTEE

TABLE 2

Salmonella Serotypes and Isolations from Cattle 1933-1973(1-18)

<table>
<thead>
<tr>
<th>UNCOMMON SEROTYPES ISOLATED</th>
<th>U.S.</th>
<th>FOREIGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL SEROTYPES CULTURED FROM CATTLE ... 111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FROM U.S. CATTLE .... 81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FROM FOREIGN CATTLE .... 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAME SEROTYPES CULTURED FROM BOTH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.S. AND FOREIGN CATTLE .... 30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| S. cholera-suis, kunzendorf                     | 26   | 0       |
| S. cholera-suis                                 | 11   | 0       |
| S. pullorum                                     | 3    | 3       |
| S. meleagridis                                  | 0    | 6       |
| S. paratyphi B                                  | 0    | 3*      |
| S. typhi                                        | 0    | 0**     |

* Only occasionally in animals; 1 transmission cow to man. (21)
** Typhoid bacillus found just once from animals (20)

REFERENCES

27. Morse, E. V. and Duncan, M. A. unpublished data, Purdue University, 1973-74.
EARLY PROTECTION AGAINST INFECTIOUS BOVINE RHINOTRACHEITIS WITH INTRAMUSCULARLY ADMINISTERED VACCINE

Dale E. Bordt, PhD, Phillip C. Thomas, BA, and Richard F. Marshall, DVM, MS

INTRODUCTION

Effective intramuscularly administered Infectious Bovine Rhinotracheitis (IBR) vaccine was first reported in 1957 by Schwarz, et al., and since that time field evaluation and extensive usage has demonstrated prophylactic effectiveness of parenteral vaccines in preventing IBR.

More recently Todd, et al., reported on the use of an IBR vaccine by the intranasal route after which protection against challenge was observed by 72 to 96 hours post vaccination. Interferon was detected in the nasal secretions and sera of the vaccinated calves and the authors concluded that interferon was associated with early resistance to challenge with virulent virus.

These same authors in a second experiment reported that detectable nasal interferon was not induced by intramuscular IBR vaccination. However, early resistance to challenge after intramuscular vaccination was not evaluated in the experiment.

PURPOSE

The purpose of the work reported here was to determine the earliest time following intramuscular vaccination that resistance to virulent IBR challenge develops and to gain possible insight as to the mechanisms involved.

MATERIALS AND METHODS

Experimental Animals

Angus steers and heifers weighing 700-750 pounds and devoid of serum neutralizing (SN) antibodies by the plaque reduction test were purchased from a single herd. They were transported to our facility and housed in two buildings designed for holding cattle in isolation.

Vaccine

The vaccine used was a commercial serial of bivalent IBR, parainfluenza virus type 3 (PI3) vaccine,* and was administered according to the manufacturer’s recommendations.

Challenge Virus

Virulent IBR challenge virus, Cooper strain, was obtained from the

*Respacine-2® Diamond Laboratories, Inc., Des Moines, Iowa 50304
INFECTIOUS BOVINE RHINOTRACHEITIS

Veterinary Biologics Division, Animal and Plant Health Inspection Service, United States Department of Agriculture. This was supplied as frozen cell culture virus fluid containing approximately $10^7$ TCID$_{50}$ per ml.

**Serum Collection and Processing**

Blood was collected by venipuncture from the jugular vein into sterile glass tubes. Blood was allowed to clot at ambient temperature, held at 4°C overnight, and serum separated by centrifugation. Serum was inactivated at 56°C for 30 minutes and stored at -20°C until tested.

**Nasal Secretions**

Nasal secretions were collected by careful insertion of a tampon into one nasal cavity, where it was allowed to remain for two hours. Tampons were carefully removed and placed into sterile petri dishes for transportation to the laboratory. Secretions were expressed into sterile vials and held frozen at -60°C until tested for interferon or antibody activity. Prior to testing for antibody, nasal secretions were inactivated at 56°C for 30 minutes.

**Nasal Swabs**

Sterile cotton swabs were carefully inserted deep into the nostrils of each animal. After swabbing the mucosal surface, swabs were immersed in virus stabilizing medium, frozen, and held at -60°C until tested for presence of virus.

**Tests for Presence of Viruses**

Thawed specimens were tested for presence of viruses by inoculation of embryonic bovine lung (EBL) monolayer cultures and observation for cytopathogenic effect (CPE) for seven days. After seven days the fluids from each culture were subcultured into fresh EBL cultures and incubated an additional seven days. After the second passage cultures were examined for CPE, culture fluids were removed, the cultures were rinsed with phosphate buffered saline (PBS), and a mixture of chicken, human type “O”, and guinea pig erythrocytes was added to each tube. After 30 minutes at 4°C, the monolayers were rinsed free of non-adherent erythrocytes using PBS, and the monolayers were examined microscopically for evidence of hemadsorption.

**Plaque Reduction Tests for IBR Antibody**

Plaque reduction tests were performed in petri dish cultures of EBL cells incubated under 5% CO$_2$ tension at 37°C.

Serum-virus mixtures were incubated for 60 minutes at room temperature, three cultures were inoculated with 0.2 ml aliquots of each serum-virus mixture, and placed under 5% CO$_2$ tension for 120 minutes for virus absorption, with periodic mixing to ensure even
distribution of the inoculum. After adsorption, each culture was overlaid with a mixture of growth medium and agar and returned to the CO₂ incubator.

After three days incubation, the monolayers were stained with neutral red, the plaques were counted, and mean plaque counts calculated. Nasal secretion plaque reduction antibody tests were conducted similarly.

Endpoint dilutions of antibody activity were considered those that caused at least 80% reduction of the mean plaque count. The test was considered valid if the mean plaque count in the negative antibody control plates was in the range of 40-80 plaque forming units.

**Interferon Assay**

Interferon assays on nasal secretions and serums from selected animals were performed by Dr. Bruce Rosenquist of the University of Missouri, Department of Veterinary Microbiology. The plaque reduction assay method employing bovine kidney monolayer cultures challenged with vesicular stomatitis virus was used.

**Experimental Design**

Thirty cattle were randomly assigned to six groups of five animals each.

Temperature and clinical observations were taken daily for three days prior to vaccination to accustom the animals to the handling procedures and to establish baseline values.

When all groups were judged clinically normal, one group of five animals was vaccinated intramuscularly, each with a single 2.0 ml dose of vaccine. Additional groups of five animals each were vaccinated similarly two, four, five, and six days later.

The five animals in the sixth group served as non-vaccinated controls.

All animals were challenged intranasally on day 7 with 10⁷.¹ TCID₅₀ of virulent IBR virus given 2.0 ml in each nostril by means of an atomizer. The challenge thus was conducted one, two, three, five, and seven days after vaccination of the respective vaccinated groups. The non-vaccinated control animals were also challenged on day 7.

Rectal temperature readings were taken twice daily on all animals from the day the first group was vaccinated until 14 days post challenge. Clinical observations were recorded for all animals over the same time period.

Nasal swabs for virus isolations were taken on the day of vaccination of the first group and again immediately prior to challenge of all groups.

Nasal secretions were collected daily from each group starting with the day of vaccination and continuing until the day of challenge.
RESULTS
A. Clinical Response to IBR Challenge
1. Non-Vaccinated Control Animals
   Figure 1 is a graphical presentation of the average rectal temperature of the non-vaccinated control animals.

   Following challenge, a marked temperature rise was seen. Temperature elevations began on day two post challenge, peaked to an average of 106°F on day three, and persisted for approximately five days before returning to normal.

   Clinical observations revealed that nasal discharge occurred in all five animals from day two post challenge and persisted throughout the test. Nasal lesions (encrusted mucus and erythema) developed in four of the five animals and concurrent labored breathing and anorexia occurred in three of the five animals.

2. Animals Vaccinated One Day Before Challenge
   Figure 2 presents temperature responses in animals vaccinated one day prior to challenge. A pronounced febrile reaction was also seen in this group after challenge that was only slightly less severe than that of the non-vaccinated control group.

   Nasal discharges occurred in all five animals from day two post challenge, persisting throughout the observation period. Nasal lesions, labored breathing, and increased respiratory rates were noted in four of the five animals, and anorexia was observed in two of the five animals.

3. Animals Vaccinated Two Days Before Challenge
   Figure 3 presents temperature observations in animals vaccinated two days prior to challenge. A somewhat less pronounced febrile reaction was observed after challenge as compared with the previous two groups. Clinically, nasal discharge occurred in all five animals from day two post challenge. In two animals lesions were noted that were healed by day 12 post challenge. Appetite was slightly depressed in three animals. Labored breathing or increased respiratory rates were not observed in this group.

4. Animals Vaccinated Three Days Before Challenge
   Figure 4 presents average temperatures of the five animals vaccinated three days prior to challenge. The magnitude and duration of febrile response following challenge in this group was further suppressed as compared with previous groups. Clinically all animals in the group showed nasal discharges and one showed an irritation of the nasal mucosa. Some anorexia was seen in three of the animals. No labored breathing or increased respiratory rate was observed.

5. Animals Vaccinated Five Days Before Challenge
   Figure 5 shows average temperatures of animals vaccinated
five days prior to challenge. In this group temperatures remained normal following challenge. Four of the animals had some nasal discharge, and two animals showed some conjunctivitis with ocular discharge. Some loss of appetite was noted in three animals. No labored breathing or increase in respiratory rate was observed in this group.

6. Animals Vaccinated Seven Days Before Challenge

Figure 6 presents temperature observations on cattle vaccinated seven days prior to challenge. Temperatures remained in the normal range throughout the post-vaccination and post-challenge periods. Clinical observations revealed that the animals remained essentially normal following challenge.

Figure 7 is a composite of temperature responses of all six groups for comparison purposes.

B. Plaque Reduction Antibody

Plaque reduction tests were performed on pre-vaccination and pre-challenge serums and nasal secretions from all six experimental groups. In no case was a significant level of antibody detected in undiluted serum or nasal secretion.

C. Interferon

Nasal secretions taken on days 0, 2, 4, and 7 post vaccination and sera taken on days 0 and 7 post vaccination from two cattle vaccinated seven days prior to challenge were tested for interferon-like activity. All nasal secretions and serum specimens tested were devoid of interferon activity at a 1:3 dilution.

D. Virus Isolation Attempts

Nasal swab specimens from all test animals taken on the day of vaccination of the first group of cattle and again immediately prior to intranasal challenge were tested for presence of IBR or other CPE or hemadsorbing viruses. In no case was IBR or other CPE or hemadsorbing virus observed.

DISCUSSION AND CONCLUSIONS

The results of this study indicate that intramuscular vaccination of susceptible cattle with the recommended field dose of IBR, PI, live attenuated vaccine induced resistance to a massive challenge with virulent IBR virus administered by the respiratory route.

A degree of resistance, as determined by temperature and clinical responses, was evident as early as 48 hours following vaccination. Resistance was progressively stronger as the time between vaccination and challenge increased and was essentially complete by five days post vaccination, as evidenced by febrile reactions.

Plaque reduction antibody tests revealed no significant levels of IBR neutralizing antibody in the serum or nasal secretions either before vaccination or at the time of challenge up to seven days later.
Interferon assays on serum and nasal secretions from selected animals revealed no detectable interferon activity in resistant animals during the seven-day post-vaccination interval.

Virus isolation attempts on nasal swab specimens obtained at initiation of the test and immediately prior to challenge revealed no virus in the nasal secretions. This is consistent with our previous unpublished observations that the vaccine administered intramuscularly is not shed from the respiratory tract and does not spread to non-vaccinated contact control animals.

This study did not reveal the mechanism involved in the observed early resistance to challenge following intramuscular vaccination. It is clear, however, that resistance was not correlated with in vitro detectable neutralizing antibody or interferon activity of either serum or nasal secretions.

Mechanisms that could possibly be operative, but which were not investigated in this study, include:

1. Development of early complement dependent antibody as suggested by the work of Rossi and Kiesel  and Potgieter.
2. Early development of cellular immunity as suggested by the work of Davies and Carmichael and Rouse and Babiuk.
FIGURE 1
Mean Rectal Temperatures
Non-Vaccinated Controls

FIGURE 2
Mean Rectal Temperatures
Cattle Vaccinated One Day Prior to Challenge
FIGURE 3
Mean Rectal Temperatures
Cattle Vaccinated Two Days Prior to Challenge

FIGURE 4
Mean Rectal Temperatures
Cattle Vaccinated Three Days Prior to Challenge
FIGURE 5
Mean Rectal Temperatures
Cattle Vaccinated Five Days Prior to Challenge

FIGURE 6
Mean Rectal Temperatures
Cattle Vaccinated Seven Days Prior to Challenge
FIGURE 7
Mean Rectal Temperatures
Composite of All Groups

KEY:
- Non-vaccinated controls
- Vaccinated one day prior to challenge
- Vaccinated two days prior to challenge
- Vaccinated three days prior to challenge
- Vaccinated five days prior to challenge
- Vaccinated seven days prior to challenge

Temp. (F)
REFERENCES

Dr. John Gorham reported on "Reversion to Virulence in Canine Distemper Living Virus Vaccine". (See accompanying paper)

Dr. Robert Bushnell, Extension Veterinarian, California, reported that the incidence of clinical bluetongue is increasing. The disease is diagnosed with increased frequency in feeder lambs. Black faced sheep seem to be more severely affected. Four to five percent of lambs born to ewes vaccinated with currently available vaccines are abnormal. Bluetongue virus has been isolated from these lambs.

In the bovine, bluetongue virus has been incriminated as causing premature births, weak calves, and hydrocephalus calves.

In Oregon, bluetongue virus has been isolated from feeder lambs with laminitis.

A critical evaluation of currently available bluetongue vaccines resulted in 50% morbidity and high mortality in vaccinates. The bluetongue viruses isolated in these cases represented strains differing from strains used in vaccine production, suggesting the need for polyvalent vaccines.

Extension veterinarians representing sheep industry states further explained the problems of exporting sheep relative to bluetongue. Vaccinated sheep are not, in fact, fully protected against the field strains of bluetongue, based on recent epidemiological evidence.

In consideration of the preceding report, the Biologics Committee makes the following recommendation:

That the Administrator of the Agricultural Research Service be informed of the Biologics Committee's concern over the increased incidence of bluetongue in the United States, and of the occurrence of new strains, and of the apparent need for vaccines with a broader antigenic spectrum. The Committee recommends that the Administrator make available the research capabilities of the Agricultural Research Service for the development of such vaccines.

The committee considered the problem of mixed bacterins removed from the market. In most instances, only certain antigens were removed from the formula and the product has been given a new name. Those antigens which had been removed were those which were least likely to be shown effective. Effective products are available for the major disease problems in most species.
The Committee was requested to review the status of current research efforts in the development of an improved vaccine for use in the brucellosis eradication program. It was reported that Veterinary Services is currently conducting trials of the 45-20 and H-38 bacterins. Also, it was noted that 4 reports evaluating 45-20 vaccine have been published in the USAHA Proceedings in recent years. The Committee deferred any action on this subject as it was the consensus that further deliberation or recommendations on this subject should be referred to the Brucellosis Committee.

State regulations for biologicals were discussed. Dr. R. Holt of the California Department of Agriculture reported that as of July 1975 all biologics registration of licensed and experimental products will be under the control of the Agriculture Department. Biologics must comply with safety and efficacy requirements outlined by the state, exempting biologics having Federal approval.

The size of printed labeling on biologics, including inserts, was discussed. Printing should be large enough to allow a clear understanding of instructions, especially precautions and directions for use.

The problem of efficacy and duration of immunity of Clostridium hemolyticum bacterin was confirmed and discussed. This microorganism is known to be a relatively weak antigen and there are no presently known methods to improve the bacterin. Currently, the only solution to this problem is frequent administration of the product, up to four times per year, if necessary.

The problem of the non-availability of biological products with limited sales potential was brought out by Dr. R. A. Gessert in reviewing the report of the Committee on Pharmaceuticals and Toxicology.

Some of the low volume products involved are Ram Epididymitis Bacterin, Ovine Ecthyma Vaccine, Enzootic Ovine Abortion Vaccine, and Bluetongue Vaccine. The Biologics subcommittee was assigned the task of developing a complete list of such products and making these needs known to appropriate officials of the Agricultural Research Service, Animal and Plant Health Inspection Service, State experiment stations, colleges of veterinary medicine, and members of Congressional agriculture appropriations committees.

This problem is especially important for diseases in sheep and leptospirosis in horses. In order to partially resolve this problem, this committee strongly recommends that the Animal and Plant Health Inspection Service permit recommendations for minor species based on extrapolations from data obtained from other species when safety of the product can be established.

Dr. Donald Bailey, veterinary practitioner from Roseburg, Oregon, reported that the sheep industry in his area is endangered by the present lack of, or possible future withdrawal of, biological products. These products included mixed bacterins, Ram Epididymitis
vaccine, vaccine for enzootic abortion of ewes, and bluetongue vaccine.

It was decided that future studies should be made by the committee on the possibilities of classifying biologic products. These classifications would indicate the degree of efficacy required for licensure. These avenues will be explored in the ensuing year by the committee.

The committee received a report that a U.S. veterinary license is pending for a snake antivenin with animal dosage recommendations. It is anticipated that the product will be marketed within the next year.

The special subcommittee will continue to act as a liaison between Animal and Plant Health Inspection Service, Veterinary Services, and keep the industry and the veterinary profession appraised of new developments relating to biologic products. Problems relating to these needs should be forwarded to this subcommittee. Members are J. W. Glosser, L. E. Hanson, D. A. Fuller, G. V. Peacock, Skip Thayer, and R. F. Hall.

*Report to the Biologics Committee by John R. Gorham,*
Pioneering Research Laboratory, Agricultural Research Service, Pullman, Washington

Reversion to Virulence in Canine Distemper Living Virus Vaccine

Dr. John West adapted a naturally occurring pathogenic fox distemper isolate to the chorio-allantoic membrane of the developing chicken embryo. Dr. Joan Budd determined that at the 50-53rd fertile egg passage, the virulence was lost for the ferret. Dr. Gorham and his coworkers passaged the fertile egg (64 passage level) virus in ferrets at 6-day intervals to select for a virulent virus population. After the 22nd ferret back passage, all ferrets succumbed to distemper. Also, contact ferrets were infected and died of distemper.

When the adapted Rockborn strain of distemper was similarly passaged in ferrets, no reversion was evident by the 14th ferret back passage.

These data suggest that it is unlikely that the FXNO and Rockborn strains (at passage levels used in current vaccines) revert to virulence under field conditions. The purpose of the Pullman experiments was not to revert viral vaccines to virulence. The aim was to study the mechanisms of virulence using distemper as a model.
LATENT ANAPLASMOSIS INFECTION IN IDAHO MULE DEER DEMONSTRATED BY CALF INOCULATION

R. A. Magonigle, M.S., Harland W. Renshaw, DVM, Ph.D.,
Erik Stauber, DVM, Ph.D., Harry W. Vaughn, DVM,
and F. W. Frank, DVM, Ph.D.

From the Department of Veterinary Science, Agricultural Experiment Station, University of Idaho, Moscow, ID 83843. Published with the approval of the director of the Idaho Agricultural Experiment Station, Moscow as Research Paper No. 75811.

Supported by funding from the Idaho Agricultural Experiment Station, Idaho Beef Council, and Cooperative Agreement No. 12-16-4-149 from the US Department of Agriculture, Animal and Plant Health Inspection Service. The authors acknowledge the assistance of personnel from the Idaho Fish and Game Department in conducting this study.

SUMMARY

The anaplasmosis carrier status of Idaho mule deer (Odocoileus hemionus hemionus) was investigated. Blood samples collected from 87 mule deer, which were trapped along the Idaho-Utah border near Snowville, Utah, were inoculated into susceptible splenectomized calves. Acute anaplasmosis occurred in 2 of the 3 inoculated calves and blood from the 3 calves caused anaplasmosis when subinoculated into nonsplenectomized cattle. The significance of latent infections of wild ruminants, and the problems that wildlife reservoirs pose for anaplasmosis control and eradication programs are discussed.

Introduction

Since elk (Cervus canadensis canadensis) and a number of species of wild deer can be experimentally infected with anaplasmosis, considerable effort has been devoted to determining the role wild ruminants may play in the natural spread of the disease. Experimental transmission of Anaplasma marginale from cattle to elk has been demonstrated in studies conducted in Wyoming and Idaho. Experimentally infected elk undergo subclinical disease and become carriers. White-tailed deer (Odocoileus virginianus) are susceptible to anaplasmosis and investigators have transmitted the disease from deer to cattle. Studies conducted in the southeastern United States and in Wyoming have not demonstrated natural infection or anaplasmosis carrier status in free-roaming white-tailed deer. In the coastal range areas of California the Columbian black-tailed deer (O. hemionus columbianus) is naturally infected with anaplasmosis and serves as a reservoir of the disease for cattle.

The Rocky Mountain mule deer (O. h. hemionus) is experi-
mentally susceptible to anaplasmosis. The disease can be experimentally transmitted from cattle to mule deer to cattle. Limited studies on the carrier status of wild native mule deer have been inconclusive. In a study conducted in Wyoming, one of five pooled samples of mule deer blood caused anaplasmosis when inoculated into splenectomized calves. When blood samples from 31 Oregon mule deer were inoculated into 5 nonsplenectomized calves, latent anaplasmosis infection of the deer was not demonstrated.

The carrier status of wild ruminants is of the utmost importance to any type of program aimed at control of the disease. If wild ruminants are reservoirs of anaplasmosis in western range states like Idaho, it certainly makes it a more difficult task to control, let alone eradicate, the disease. In rangeland areas where infected deer and cattle live and feed in close proximity it would be possible for arthropod vectors to effect interspecies transmission of the disease. The anaplasmosis carrier status of wild native mule deer in Idaho has not been studied.

The purpose of the current study was to determine if natural infection and latent carriers occur in the mule deer population ranging in areas of southeastern Idaho where anaplasmosis is endemic in the cattle population.

Materials and Methods

Trapping of Mule Deer and Sample Collection

The study area was located in southeastern Idaho and northern Utah along the Idaho-Utah border approximately 2 miles north of Snowville, Utah. 87 mule deer, both adults and fawns, were trapped by the Idaho Fish and Game Department with a drop net. This area consists of hill and mountain grazing lands. Samples were collected in January and February of 1975 as deer moved through the area on their winter migratory route. Two blood samples were collected from each deer by venipuncture of the jugular vein: 1) serum for use in the rapid card agglutination (RCA) test and the compliment-fixation (CF) test, and 2) 10 ml of heparinized (10 U/ml) blood for calf inoculation.

Experimental Calves and Subinoculations

Splenectomized 8- to 10-month old mixed breed calves were obtained from the anaplasmosis-free experimental herd maintained at the University of Idaho. Calves were splenectomized 8 weeks before inoculation with mule deer blood. Animals were transported to the trapping area for inoculation. The average time interval between collection of deer blood and calf inoculation was 2½ hours. Each calf was inoculated intramuscularly with deer blood. Blood samples were inoculated at random with individual calves receiving blood from both adults and fawns.
After inoculation, microscopic examination of Wright's-stained blood films, microhematocrit determination of PCV, and the RCA test were conducted twice weekly. When signs of anaplasmosis appeared in a calf, subinoculations were made into susceptible non-splenectomized adult cows. All animals were maintained until signs of anaplasmosis appeared or until 180 days elapsed since inoculation. The calves and subinoculated cows that did not develop anaplasmosis were challenged with blood from a known bovine carrier to determine their susceptibility.

Serologic Methods

The RCA test was performed on deer and cattle samples according to the procedure recommended for cattle. Both the plasma and serum RCA tests were used to evaluate the bovine samples; deer samples were evaluated only with the serum RCA test. The CF test was performed by the USDA Disease and Parasite Laboratory, Beltsville, Maryland.

Results

Pertinent data on the 87 mule deer blood samples are presented in Table 1. The total volume of blood inoculated into calves ranged from 190 to 390 ml. Of the blood samples inoculated, calf 1 received 4 that were serum RCA test positive, calf 2 received 6, and calf 3 received 3. All RCA test positive deer serums with an equal number of negative samples were evaluated with the CF test. None of the RCA test negative deer serums were reactive in the CF test.

The results of inoculations of deer blood into splenectomized calves and the subinoculations into nonsplenectomized susceptible cows are summarized in Table 2. Calf 1 developed peracute anaplasmosis 39 days after inoculation and died 72 hours after the onset of clinical signs. The hematocrit fell from 30% to 9% with 50% parasitemia present at the time of death. During this phase, 50 ml of blood was collected and subinoculated into a susceptible non-splenectomized adult cow. This cow developed acute anaplasmosis after an incubation period of 30 days. The cow became RCA and CF test positive, the hematocrit fell and parasitemia was present.

Calf 2 showed signs of acute anaplasmosis 44 days after inoculation of deer blood. The hematocrit dropped from 32% to 14% and a 35% parasitemia was observed; the RCA and CF tests were positive. After clinical infection was observed, 50 ml of blood was subinoculated into a susceptible adult, which developed acute anaplasmosis after an incubation period of 28 days. The cow became RCA and CF test positive and parasitemia was documented.

Calf 3 did not exhibit clinical signs of anaplasmosis. At 68 days post-inoculation and thereafter for a period of 6 days, transient anemia (a 4% decrease in hematocrit) and a low degree of para-
sitemia (<1%) were observed. Neither the RCA nor CF test became positive. During this period, 50 ml of blood was subinoculated into a susceptible adult. This animal developed anaplasmosis after an incubation period of 31 days. The cow became RCA and CF test positive and parasitemia was documented. Observation was continued on calf 3 through 180 days post-inoculation. No further evidence of anaplasmosis was noted during this period. At this time, blood was again subinoculated into a susceptible cow and calf 3 was challenged with 50 ml of blood from a known anaplasmosis carrier. The subinoculate did not develop anaplasmosis during a 60 day observation period. However, after an incubation period of 36 days, clinical anaplasmosis developed in calf 3. Parasitemia was documented and the RCA and CF test became positive.

At no time during the study period was eperythrozoonosis noted in any of the primary inoculates or subinoculates.

TABLE 1. Mule Deer Blood Samples Inoculated into Splenectomized Calves

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Number of Samples Inoculated*</th>
<th>Number of Samples Collected From Adults</th>
<th>Fawns**</th>
<th>Number of Serologic Positive Samples RCA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>16</td>
<td>23</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>13</td>
<td>16</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Inoculums consisted of 10 ml of blood from each deer.
**Yearlings were classified as fawns.

TABLE 2. Inoculations of Mule Deer Blood into Splenectomized Calves and Subinoculations into Susceptible Cows

<table>
<thead>
<tr>
<th>Deer Blood into Splenectomized Calf</th>
<th>Calf Blood into Susceptible Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of Incubation Period (days)</td>
<td>Severity of Clinical Response</td>
</tr>
<tr>
<td>Calf No.</td>
<td>percaute</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
</tr>
</tbody>
</table>

*RCA test reactions are expressed as positive (+) or negative (−) agglutina-
tion; CF tests are expressed as reactors (+) or suspects and non-reactors (−).
**+clinical response indicates presence of parasitemia, decreased packed cell
volume, and clinical signs of illness.

Discussion

The results from this study indicate that free-roaming mule deer in southeastern Idaho are infected with \textit{A. marginale}. Since anaplasmosis can be experimentally transmitted from infected mule deer to cattle, it is not difficult to visualize natural circumstances whereby they could act as a reservoir of this important bovine disease. After inoculation with mule deer blood, 2 of the 3 test calves developed...
typical cases of bovine anaplasmosis. The onset of clinical signs, the
pathogenesis of the infection, and the immunologic response were
analogous to that observed in calves inoculated with blood from a
bovine carrier.

Consideration was given to the possibility that *A. marginale*
might not be the causative organism involved in this study. However,
clinical, hematologic, and serologic evidence indicates that the infe-
tions seen were in fact bovine anaplasmosis.

Results from the serologic tests in this study revealed little corre-
lation between the number of serums positive for the RCA test (13)
and reactor or suspect by the CF test (3). However, 13 serums nega-
tive for the RCA test were also negative by the CF test. The lack of
correlation between the RCA and the CF test has been noted in other
studies on wild ruminants.8,13 Each of the 3 calves received blood from
mule deer positive for the RCA test.

The data on calf 3 are difficult to interpret. Definite hematologic
changes, although they were transient, were documented 68 days after
inoculation. At this stage subinoculated blood was infective and the
recipient developed clinical anaplasmosis. However, blood subinoculat-
ed from calf 3 at 180 days was not infective and at that time the calf
was susceptible to challenge. The reason for this phenomenon is un-
clear, but a subacute transmissible infection may have existed at day
68 which did not acutely infect the principal and did not stimulate a
detectable immunologic response. Adaptation of the agent in one host
could lead to attenuation of the microorganism for a different host.
Infection with such an attenuated strain might not elicit acute disease
and immunologic responsiveness. The lack of a detectable immuno-
logic response and acute signs in calf 3, though subinoculation was
positive, leaves the status of this animal in question.

The data from this study amplifies the need for further studies
of the anaplasmosis carrier status of wild ruminants. The occurrence
and prevalence of anaplasmosis in wild populations must be thorough-
ly investigated if attempts to control the disease in various geographic
locales are to meet with success. Control programs that are aimed
only at the disease in cattle, and which fail to consider wildlife res-
ervoirs and arthropod vectors, would be of limited value in certain
areas.
LATENT ANAPLASMOSIS INFECTION

REFERENCES


ANAPLASMOSIS: PREVALENCE, TREATMENT, AND CATTLE TO ELK TRANSMISSION STUDIES

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Summary

The rates of anaplasmosis infection in areas of Idaho and Washington range from a low of 0.4% to a high of 47.4%. The incidence of anaplasmosis was higher in open herds than closed herds and in cattle on open valley ranges than in cattle on high country forest ranges. Serums from 27 free-roaming elk (Cervus canadensis canadensis) from the Clearwater National Forest in North Central Idaho were tested by the rapid card agglutination test and complement-fixation test for the presence of antibodies against Anaplasma marginale. The serum card test and complement-fixation test gave incomplete and false positive reactions. Blood from all elk, including 2 that were serum card test positive, failed to produce clinical, hematologic, or serologic evidence of infection in 3 anaplasmosis susceptible bovine calves. Anaplasmosis was experimentally transmitted from cattle to elk and back to cattle. Infected elk became serologically positive by the plasma and serum rapid card agglutination test. Infected elk did not show clinical signs of disease, although they became latent carriers. Of 97 reactor cattle treated with oral tetracyclines (11 mg/kg/day for 45 days), 86 were negative to the serum rapid card agglutination test when retested 120 days after completion of treatment. Intravenous administration of oxytetracycline hydrochloride at the rate of 22 mg/kg daily for 5 days was effective in rendering parasite-free, 11 adult cattle that were naturally infected anaplasmosis carriers. Through 12 post-treatment months the efficacy of the treatment procedure was evaluated by serologic tests and subinoculation of blood into susceptible splenectomized calves. After subinoculation of blood into splenectomized calves (at 4 and 12 months after chemotherapy), serologic, hematologic, or clinical evidence of infection with A. marginale was not seen during a 60-day observation period.
ANAPLASMOSIS: PREVALENCE TREATMENT

Introduction

Anaplasmosis is an infectious, parasitic disease of cattle caused by the microorganism *Anaplasma marginale*. Anaplasmosis may result in abortion, poor reproductive ability, severe weight loss, decreased milk production, and sometimes death of the infected animal. Clinically recovered animals remain carriers with a low degree of parasitemia and it is this carrier status which poses the greatest problem in control of the disease. These chronically infected animals act as a reservoir of the disease, which can be transmitted from the carrier to a susceptible by mechanical or biological vectors. The continuing spread of the disease has aroused increasing concern among cattlemen, regulatory officials, research workers and veterinary practitioners. Until recently anaplasmosis was not regarded as an important disease in northern Idaho and southeastern Idaho. However, the uncontrolled movement of cattle imported as herd replacements or for seasonal grazing has introduced cattle from areas with heavy anaplasmosis infection into this area. Since one of the basic agricultural pursuits in these areas is cattle ranching, investigations have been conducted to determine the prevalence and distribution of bovine anaplasmosis.

In the western United States there are range land areas where domestic and wild ruminants intermingle. In addition to feeding on cattle in these regions, arthropod vectors also feed on wild ruminants that are experimentally susceptible to anaplasmosis. Species of wild ruminants that have been experimentally infected with *A. marginale* include the big-horn sheep (*Ovis canadensis canadensis*), the pronghorn antelope (*Antilocapra americana americana*), Columbian black-tailed deer (*Odocoileus hemionus californicus*), Rocky Mountain mule deer (*O. h. hemionus*), white-tailed deer (*O. virginianus*), and elk (*Cervus canadensis canadensis*). Since numerous species of wild ruminants can be experimentally infected with *A. marginale*, there are many unresolved questions about the reservoir status of wild ruminants. In California the Columbian black-tailed deer (*O. h. columbianus*) are naturally infected with anaplasmosis. Infected deer become carriers of the disease and transmission between cattle and deer frequently occurs. Although mule deer can be experimentally infected, evidence from studies conducted in Oregon and Wyoming suggests that in those states mule deer are probably not important reservoirs of anaplasmosis. However, recent studies in Idaho demonstrated infected mule deer. In studies of 262 white-tailed deer (*O. virginianus*) in 9 southeastern states and 49 in Wyoming, attempts to demonstrate natural infection or anaplasmosis carrier status were unsuccessful. The status of elk regarding their susceptibility to infection with *A. marginale* and role as carriers has not been adequately investigated. Elk can be experimentally infected and become asymptomatic carriers. Blood from experimentally infected elk
can produce disease in splenectomized bovine calves. Studies have been conducted to determine, 1) whether natural infection and carriers occurred among elk ranging in the Clearwater National Forest of Idaho, and 2) the susceptibility of elk by inoculating them with infected bovine blood.

An estimated 50,000 to 100,000 animals die of anaplasmosis each year, with an annual economic impact up to $100 million. Because of the magnitude of these losses and the continuing spread of the disease, serious attention is being given to need for control and eventual eradication of anaplasmosis. "Test and treatment" programs have been proposed and implemented to determine the feasibility of eliminating the carrier status of A. marginale in cattle raised under different management practices. Although preimmunization, vaccination, blood transfusions, and chemotherapy have been utilized to treat and control anaplasmosis, broad spectrum antibiotics at present are the most widely used treatment regimen. The tetracyclines are the only antibiotics approved for use in food animals by the US Food and Drug Administration that are effective in treating the disease. Tetracyclines can be used to treat both the acute and latent infection, the acute infection being controlled primarily by destruction of many of the organisms, the latent infection by elimination of the organism. We have conducted studies to determine the effectiveness of 1) intravenous administration of oxytetracycline hydrochloride (22 mg/kg/day for 5 days) and, 2) oral administration of chlortetracycline (11 mg/kg/day for 45 days) in eliminating carrier anaplasmosis infections.

Materials and Methods

**Cattle Survey**—The survey areas for the study of anaplasmosis in cattle comprised 1) all Idaho counties north of the Clearwater River except Kootenai, and four southeastern counties of Washington State (Asotin, Columbia, Spokane, and Whitman), 2) the Salmon-Challis area of southeastern Idaho, 3) the Snake River canyon areas in southern Idaho, and 4) the south central area of Idaho. Herds throughout the survey areas were selected randomly and categorized according to age of individual animals and geographic location of grazing ranges. The reactor (carrier) status of each animal was determined by the standard complement-fixation (CF) test and the serum rapid card agglutination (SRCA) test. The SRCA test was conducted with equipment supplied by Hynson, Wescott, and Dunning, Inc., Baltimore, Maryland. The CF test was performed by the USDA Disease and Parasite Laboratory, Beltsville, Maryland.

**Elk Survey**—In the Clearwater National Forest in Idaho, 27 female elk were trapped along a 60 km stretch of the Lochsa River. The animals were trapped in the spring while they were still on their winter and spring range. The elk were tranquilized with aceproma-
zine maleate (Ayerst Laboratories Inc., New York, New York) at the rate of approximately 1 mg/6 kg of body weight and restrained in a wooden headgate chute for collection of blood. Heparinized (10 U/ml) and unheparinized blood samples were collected by venipuncture of the jugular vein. Blood films prepared from unheparinized elk blood were stained by Wright’s method and examined by light microscopy for anaplasma bodies. A 10 ml heparinized blood sample was centrifuged at 2000 X g for 10 minutes to obtain plasma for use in the plasma rapid card agglutination (PRCA) test. The 15 ml unheparinized blood sample was allowed to clot. Serum was separated from the clotted blood and stored at -20 C. A 4 ml serum sample which had been treated with 0.4 ml of 2.5% phenol was used as the serum source for the complement-fixation (CF) test. The remaining serum was used in the SRCA test.

Subinoculation of Elk Blood—Three anaplasmosis susceptible nonsplenectomized bovine calves, 3 to 5 months of age, were subinoculated with blood from 11 of the trapped elk. Individual calves were inoculated with blood from elk trapped within a 5 day period, because anaphylactic reactions were observed when elk blood was administered after this length of time. Blood samples from individual elk were not pooled before subinoculation into calves. Calves were inoculated intravenously with 50 ml of heparinized elk blood within 20 minutes after the blood was collected. Two calves received blood from 3, and 1 calf received blood from 5 elk.

Preinoculation and postinoculation data on body temperature, packed cell volume (PCV), hemoglobin (Hb), stained blood smears, CF and SRCA tests were obtained from the calves. Rectal temperatures, blood samples, and blood smears were taken at bi-weekly intervals for a period of 96 or more days on the 3 calves. At varying intervals the CF and SRCA tests were applied. After a 120 day observation period the calves were challenged with 10 ml of infected bovine blood.

Cattle to Elk Transmission Studies—Infected bovine blood was inoculated into 8 elk to ascertain their susceptibility. Blood from a known anaplasmosis carrier cow was either used after freezing at -70 C for 4 weeks or fresh. Two elk were inoculated with 0.5 ml of frozen blood, 2 with 0.5 ml of fresh blood, and 4 with 50 ml of fresh blood. The elk were examined at intervals for reactor status with the SRCA and CF tests for clinical signs of disease. At intervals after inoculation blood from the elk was inoculated into splenectomized calves to determine their carrier status.

Treatment of SRCA Test Positive Cattle with Chlortetracycline Administered Orally—Reactor animals (97) from selected herds were fed a medicated feed for 45 days at the rate of 11 mg chlortetracycline/kg/day. Two formulations were used for oral administration of the chlortetracycline. Each animal was fed 1.1 kg/day of either
aureomycin crumbles or local grains pelleted with molasses and aureomycin 50 (aureomycin furnished courtesy of American Cyanamid Co., Princeton, New Jersey). Treated animals were retested with the SRCA test no sooner than 120 days after completion of drug treatment;20,35 this time is necessary to allow for subsidence of antibody levels prior to reevaluation of the anaplasmosis carrier status of the animal.

Treatment of Carrier Cattle with Oxytetracycline Hydrochloride Administered Intravenously—Eleven 2- to 3-year old, rector Hereford cows were purchased from a herd where anaplasmosis was enzootic. Before antibiotic therapy the rector (carrier) status of each cow was determined by the standard complement-fixation (CF) test, the serum rapid card agglutination (SRCA) test, and inoculation of blood into susceptible non-splenectomized 8- to 12-month old Holstein-Friesian calves. After therapy, rector status was determined by the CF test, SRCA test, and inoculation of blood into susceptible splenectomized 8- to 12-month old Holstein-Friesian calves. Calves were obtained from the anaplasmosis free experimental dairy herd maintained at the University of Idaho. Before calf inoculation Wright’s-stained blood films were examined to determine there was no parasitemia; hematocrit values were determined to establish that the packed cell volume (PCV) was within the normal range, and CF and SRCA tests were conducted to determine there was no serologic evidence of anaplasmosis. Susceptibility of calves was determined after inoculation of blood from a known carrier by observing for clinical, hematologic, and serologic evidence of infection.

Oxytetracycline hydrochloride (Liquamycin (50 mg/ml), Pfizer Inc., Terra Haute, Indiana was diluted 50% with sterilized physiologic saline and administered intravenously to each cow at the rate of 22 mg/kg/day for 5 days. Each treatment was administered in 5 to 8 minutes. The total amount administered per cow ranged from 36.7 to 50.1 g (mean, 43.0 g).

The serums of all cattle were examined with the CF and SRCA tests for anaplasmosis reactor status. The SRCA test was conducted at 2, 4, 6, 8, and 12 months, and the CF test at 4, 8, and 12 months after treatment. At 4 and 12 months after treatment, 10 ml of blood from each cow was subinoculated into splenectomized calves. Microscopic examination of Wright’s-stained blood films, microhematocrit determination of PCV, and the SRCA test were conducted twice weekly for 60 days after inoculation. After the 60 day observation period, the calves were inoculated with 10 ml of blood from a known anaplasmosis carrier to verify their susceptibility to the disease. Efficacy of treatment was confirmed when the subinoculated splenectomized calves that failed to manifest evidence of anaplasmosis during the observation period developed anaplasmosis after inoculation with blood from the known carrier.
ANAPLASMOSIS: PREVALENCE, TREATMENT

Results

Cattle Survey—The rates of anaplasmosis infection in northern Idaho and southeastern Washington State ranged from a low of 0.4% to a high of 47.4% in individual reactor herds. There were 5514 animals evaluated in 1973-1975 and the incidence of infected cattle in the test population was 6.7%. There were reactor animals in 23 of the 33 herds examined. Environmental management factors were evaluated, and in agreement with previous reports, it was found that the incidence of the disease was higher in cattle on open valley ranges than in cattle on high country forest ranges. Closed herds had a lower incidence of infection than open herds. There were some areas where anaplasmosis transmission did not readily occur even though infected animals were present in the herd. A high incidence of infection occurred among cattle which graze during the winter and spring in low lying areas along the Snake River canyons.

Based on a limited number of tests the incidence of anaplasmosis was low in the Salmon-Challis area of southeastern Idaho (1.4% of 1,021 animals tested), higher along the Snake River in southern Idaho (7.5% of 1,242 animals), and substantially higher in the south central area of Idaho (14.5% of 1,852 animals).

Elk Survey—The results of serologic tests conducted on the 27 elk sera are presented in Table 1. There were no positive reactions with the CF test, but 7 suspect and 10 incomplete reactions were reported. No positive reactions occurred with the PRCA test, whereas 2 positive reactions were noted with the SRCA test. Serums that gave suspect CF reactions did not always give positive SRCA test results. No anaplasma bodies or other blood parasites were observed in stained smears of elk blood.

Because the 3 indicator calves became sensitized to elk blood it was only possible to make subinoculations from 11 elk. However, 2 elk with a positive SRCA test, 7 with an incomplete and 1 with a suspicious CF test reaction were included in the 11 donor elk. No clinical, hematologic, or serologic evidence of infection with A. Marginalae was noted in the calves inoculated with elk blood during a 120 day observation period. When the 3 calves were challenged with 10 ml of blood from a known anaplasmosis carrier they developed clinical signs of anaplasmosis. After challenge the calves became SRCA test positive, PCV and Hb levels fell, and parasitemia was present.

Cattle to Elk Transmission Studies—At 2 months after inoculation the 2 elk inoculated with 0.5 ml of fresh blood from an anaplasmosis carrier reacted positively to the PRCA and SRCA test, however actual clinical anaplasmosis was never documented. During this period, no changes in normal blood values (Hgb, PCV) were encountered with bi-weekly testing, and no anaplasma bodies were noted. The 2 elk inoculated with frozen blood did not develop a serologic response and no clinical or hematologic signs were observed. A splenectomized
calf was inoculated with 10 ml of pooled blood from the 2 SRCA test positive elk 60 days after the elk were exposed. This calf died 22 days post-inoculation with acute anaplasmosis. A splenectomized calf was inoculated with 10 ml of blood from the 2 elk that had been inoculated with the frozen blood. No evidence of infection was noted in this calf through a 60 day observation period. When 50 ml of blood from the elk was inoculated into splenectomized calves 1 year after the elk were initially exposed it was found that those receiving the fresh blood were carriers and those receiving the frozen blood were not. The 4 elk inoculated with 50 ml of fresh blood developed a positive PRCA and SRCA test, but acute clinical anaplasmosis was not observed. Blood inoculated from these elk into splenectomized calves, 60 days after the elk had been exposed, caused typical cases of anaplasmosis in the calves.

Studies designed to adapt the bovine card test to elk serum are in a preliminary stage, but using the accepted 48 hour bovine time span, many false-positive reactions occur. This appears to be due to differences in the time period necessary for clotting factors to disappear from elk serum when compared to bovine serum. Normal serum samples tested at specified time intervals after collection indicate that 72 hours is an acceptable end point.

**Treatment of SRCA Test Positive Cattle with Chlortetracycline Administered Orally**—After treatment with oral tetracyclines 86 of 97 reactor animals were negative to the rapid card test when retested; a treatment efficacy of 86.8%. Owners of the cattle that remained reactors after treatment maintained that the cattle did not eat the medicated feed well enough to get the full treatment amount of drug. In a number of cooperating herds all reactor cattle have been either treated and retained in the herd or sold for slaughter; several documented anaplasmosis-free herds have been established. Several cooperating herds are currently under observation to determine whether reinfection will occur under field conditions present in northern Idaho.

**Treatment of Carrier Cattle with Oxytetracycline Hydrochloride Administered Intravenously**—Intravenously administered oxytetracycline hydrochloride was very effective in eliminating carrier anaplasmosis infections. The experimental data and their interpretation are given (Table 2). At 2 months after treatment, 5 of the cows were SRCA test-positive. All cows were SRCA test-negative at 4, 6, 8, and 12 months. However, CF titers were variable and transient during the post-treatment period. At 4 months after treatment, 2 cows (No. 1 and 11) had a CF titer of 1:5, which disappeared on remaining tests. Cow 10 had a CF titer of 1:5 at 8 months, but not at 4 and 12 months. Cow 6 had a CF titer of 1:5 at 8 and 12 months.

The splenectomized calves, inoculated with blood from the 11 adult cattle at 4 and 12 months after chemotherapy, did not develop
serologic, hematologic, or clinical evidence of infection with *A. marginale* during the 60 day observation period. When these calves were inoculated with blood from the known carrier, however, they developed clinical signs of anaplasmosis after an average incubation period of 22 days. The calves then became SRCA test-positive, the mean PCV decreased from 31% to 10%, and parasitemia developed.

**Discussion**

The prevalence of anaplasmosis was not high in most of the herds studied. But the higher than anticipated percentage of reactors, the high incidence of reactor herds, the widely accepted practice of mixing herds, and a definite vector transmission capability has been interpreted to imply that once anaplasmosis becomes established in the area that it could become extremely difficult to control.\(^4\) Indiscriminate buying and selling of cattle in the study area appears to be the most important factor in causing the spread of disease from herd to herd and region to region. The variation in percentage of reactor animals from 47.4% to no reactors in survey herds illustrates the complexity of controlling the incidence of disease in a limited geographic area. Presently anaplasmosis is considered the most important bovine disease in Idaho.\(^4\)

Results from the elk survey study confirmed and extended the observation that bovine serologic tests for anaplasmosis may be inadequate when used with elk serum.\(^4,9,13,15\) Positive reactions occurred with the SRCA test in 2 elk, but blood from these animals failed to cause disease in susceptible calves. Similarly, incomplete suspicious CF reactions were observed with elk serums, but the blood was not infectious for calves. Howe and Hepworth\(^11\) noted that nonspecific and suspicious CF reactions were frequent in both elk calves and adults. Several precautions were taken to minimize the risk of the loss of infectivity in elk blood samples that might contain only a few infectious units. Blood samples were not pooled before subinoculation and the elk blood was subinoculated into the susceptible calves within 20 minutes after collection. In a previous study subinoculations of pooled blood samples were not completed until 1 to 5 days after collection.\(^15\)

The area where the elk were trapped is thought not to be enzootic for bovine anaplasmosis. Although the sampling of this wild elk population was small, approximately 1% of the estimated elk numbers ranging in the area, the results suggest that this elk population has very little, if any, infection with *A. marginale*. Further studies are needed with elk that cohabit rangeland areas with cattle where anaplasmosis is known to be enzootic in the cattle population.

Anaplasmosis was experimentally transmitted from cattle to elk and back to cattle. All elk inoculated with fresh blood from a known bovine carrier became latent anaplasmosis carriers, although clinical
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disease was not observed in the elk. Infected elk became PRCA and SRCA test positive and 2 have remained serologically positive for more than a year after exposure. These studies imply that under appropriate circumstances free-roaming elk could become infected and act as a reservoir of the disease.

Numerous experiments have been conducted to evaluate the ability of tetracyclines in eliminating carrier infections. These experiments have indicated that oral therapy can eliminate carrier infections, but only after the prolonged administration of large amounts of these drugs and only when care is taken to insure full consumption by all animals. The less than 100% treatment efficacy observed in this study was probably attributable to lack of consumption of medicated feed by some animals. For effective eradication or control programs in range areas, a wider choice of treatment methods needs to be developed so that the disease can be feasibly treated at any time of the year. In range cattle operations, it is not always possible to collect the cattle for testing and then set up separate operations for treatment of reactors. Carrier animals would be eliminated more rapidly if a short-term treatment could be administered at the time of testing and detection of carriers. Therefore, short-term methods of chemotherapy, with drugs approved for use in food animals, are necessary. Without such a treatment procedure many carriers are not treated for months following the test, if at all. The effectiveness of the treatment with intravenous oxytetracycline employed in the present study suggests that this procedure may have value under certain field conditions. Although this treatment procedure (22 mg/kg/day for 5 days) utilized an antibiotic approved for use in cattle, it exceeds the present recommended (label) maximum dosage of 11 mg/kg/day. Cattle treated intramuscularly with oxytetracycline hydrochloride have to be withheld from slaughter for at least 18 days. A withholding time has not been established for cattle treated intravenously, but tissue residue concentrations may be of less concern than with the intramuscular route. The parenteral use of oxytetracycline in lactating dairy cattle at the maximum recommended dosage causes milk residues to occur for at least 96 hours after the last treatment. At the 22 mg/kg level a longer withdrawal period than 96 hours could be required, thus the therapy used in the current study could not be used in lactating dairy cattle until a withdrawal period is established for milk at that dosage. Approval by the US Food and Drug Administration of product label dosages that would include the treatment regimen used in the current study will be necessary before it can be considered for state or federal sponsored field application as a method of eliminating the carrier status of bovine anaplasmosis. Management situations where this treatment program could have potential application include: 1) range cattle operations where the rancher wants to shorten the treatment period; 2)
cow-calf and other operations where minimal handling of the infected animal is prudent; and 3) dairy operations where the dairyman wants to treat non-lactating carrier dairy cattle.

Tetracycline treatment regimens that will effectively eliminate latent infections with *A. marginale* are compared (Table 3). When labor and cost of medication are considered, the intravenous treatment regimen utilized in the present study compares favorably with others currently employed to eliminate the carrier status of anaplasmosis. The total time involved in administration of the medication for the 5 day period varied from 25 to 40 minutes, and this could possibly be reduced. At the current price of $7.00 per 500 ml (50 mg/ml) of oxytetracycline hydrochloride, the cost of medication for treating a 400 kg animal would be $12.32. Medicated feed that contains 100 g of chlortetracycline per 22.7 kg sack is currently priced at $9.33. The total cost of administering 11 mg/kg/day to a 400 kg animal for 30 to 60 days would range from $12.28 to $24.56. It is difficult to estimate the time involved in administering the oral medication, but it would probably be similar to that for intravenous therapy. Those “test and treatment” programs, which have been implemented with most success, have utilized oral administration of chlortetracycline at the rate of 11 mg/kg daily for 45 days. Although certain proprietary product were employed in this study, other generic products of equal or greater concentration may also be suitable.

Blood from the treated cattle was shown to be non-infective when subinoculated into susceptible splenectomized calves at 4 and 12 months after treatment. As reported by other investigators, a CF titer persisted in some cattle after they had been shown to be free of anaplasmosis. In our study, however, all cattle were SRCA test-negative by 4 months after treatment. These results suggest that the SRCA test may be more accurate than the CF test for determining the success of treating carrier animals.
Table 1—Serologic Test Results on 27 Elk Serums for Anaplasmosis

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<tr>
<th>Elk No</th>
<th>Complement Fixation Reaction</th>
<th>Serum Card Test Agglutination Reaction</th>
<th>Plasma Card Test Agglutination Reaction</th>
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* The complement fixation reactions are designated: (-) negative, (± or 1) incomplete, (1+ to 3+) suspicious, and (4+) reactor.

**Agglutination in the Serum or Plasma Card Test is (+) reactor or (-) non-reactor.

† Blood samples from these elk were subinoculated into susceptible bovine calves.

‡ Not tested.
### Table 2: Effect of Oxytetracycline Hydrochloride Administered Intravenously (22mg/kg/day) for Five Days on the Carrier Status of Bovine Anaplasmosis, as Indicated by Rapid Card Agglutination and Complement-Fixation Reactions and Calf Inoculation Tests.

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Total drug dose (kg)</th>
<th>Rapid Card Agglutination/ Complement Fixation Reaction*</th>
<th>Calf subinoculation test results (months after treatment)</th>
<th>Calf susceptibility test results (months after challenge)</th>
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<td>Body admin. dose (g)</td>
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<td>+/20</td>
<td>-/NT</td>
</tr>
<tr>
<td>9</td>
<td>377</td>
<td>41.5</td>
<td>+/10</td>
<td>+/NT</td>
</tr>
<tr>
<td>10</td>
<td>375</td>
<td>41.3</td>
<td>+/20</td>
<td>+/NT</td>
</tr>
<tr>
<td>11</td>
<td>334</td>
<td>36.7</td>
<td>+/40</td>
<td>+/NT</td>
</tr>
</tbody>
</table>

RCA test reactions are expressed as positive (+) or negative (-) agglutination; CF titers are expressed as the reciprocal of the serum dilution; CF tests performed by USDA Beltsville Laboratories, Beltsville, MD.

** NT = Not tested
** - Negative results
+ = Positive results

### Table 3: Successful Treatment Regimens Utilizing Tetracycline Drugs for Elimination of Anaplasma marginale Carrier Status

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Route</th>
<th>Number of daily treatments</th>
<th>Dosage (mg/kg/day)</th>
<th>Total drug (g) to eliminate infection in 400-kg bovine animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>Oral</td>
<td>41</td>
<td>2.2</td>
<td>36.1</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>Oral</td>
<td>120</td>
<td>1.1</td>
<td>52.8</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>Oral</td>
<td>60</td>
<td>3.3</td>
<td>79.2</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>Oral</td>
<td>45</td>
<td>5.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>Oral</td>
<td>60</td>
<td>5.5</td>
<td>132.0</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>Oral</td>
<td>30-60</td>
<td>11</td>
<td>132.0-264.0</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>IV</td>
<td>16</td>
<td>33</td>
<td>211.2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>IV or IM</td>
<td>10</td>
<td>11</td>
<td>44.0</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>IV or IM</td>
<td>12-14</td>
<td>11</td>
<td>52.8-61.6</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>IV</td>
<td>5</td>
<td>22</td>
<td>44.0</td>
</tr>
</tbody>
</table>

* Chlortetracycline: Aureomycin, Lederle Laboratories, American Cyanamid Co, Princeton, NJ
  Tetracycline Hydrochloride: Polyotic, Lederle Laboratories, American Cyanamid Co, Princeton, NJ
  Oxytetracycline: Terramycin or Liquamycin, Pfizer Inc, Terra Haute, IN

** Reference number
REFERENCES


ANAPLASMOSIS: PREVALENCE, TREATMENT


REPORT OF THE COMMITTEE ON ANAPLASMOSIS

Chairman: B. W. Hawkins

Co-Chairman: W. E. Brock


The Anaplasmosis Committee met at 1:30 p.m., November 4, 1975, in the Hilton Hotel, Portland, Oregon. Thirty-one persons were in attendance. The following reports were presented:

Dr. K. J. Peterson reported on the continuation of research on transmission of anaplasmosis in eastern Oregon. Tentative results during the 1975 anaplasmosis season indicates that there was no transmission of anaplasmosis between herds when the herds are separated by a 20 to 35 foot space. Also there is indication that infected vectors did not carry through the previous winter. Continuation of the study of transmission in cattle on tick-free platforms showed no transmission of the disease to the tick-free cattle.

Dr. Harlan Renshaw reported that anaplasmosis can be transmitted from carrier cattle to elk and one year later could be transmitted from the elk back to cattle. Screening of captured mule deer showed approximately 16% positive on subinoculation of pooled samples into three calves. Tetracycline given orally at the rate of 5 mg. per lb. of body weight for 45 days to anaplasmosis carrier cattle resulted in 90% of the cattle showing negative to the card test 120 days after completion of the treatment. Treatment of carriers with 10 mg. per lb. of body weight intravenously for five days showed 100% card test negative after 120 days.

Dr. James Trace in answer to a question from Dr. F. W. Frank said that fewer cases of neonatal isoerythrolysis are being reported possibly due to more accurate diagnosis or reduced occurrence.

Dr. J. Lee Alley reported successful control of anaplasmosis in Georgia by low-level oral dosage of tetracycline in feed and the elimination of the carrier state with high level oral dosage.

The committee agreed that there should be additional funding for anaplasmosis research and diagnostic testing for anaplasmosis.
PIKE COUNTY, ILLINOIS, SWINE BRUCELLOSIS PROJECT

Paul L. Spencer* and James R. Mattison**

In 1973 regulatory officials in the Illinois Department of Agriculture became aware of an increasing number of swine brucellosis infected herds in Pike County, Illinois. These herds were disclosed through the Market Swine Testing program and from blood tests conducted by local practicing veterinarians in herds experiencing abortions or breeding problems. Subsequent investigations by Dr. Arthur L. Starkey, supervisor of a Department market swine testing and swine disease project at a large hog slaughtering plant at Beardstown, Illinois, revealed that practically all of these herds were located in a two township area of eastern Pike County.

To the best of our knowledge, a swine brucellosis eradication program based on complete area testing of all breeding swine and depopulation of infected herds with indemnity had not been utilized previously in the United States. The decision was made to explore the possibility of such a project and our results are the basis of this presentation.

Pike County is located in west-central Illinois (Slide 1), bordered on the east by the Illinois river and on the west by the Mississippi river. Only two bridges cross the rivers at the points indicated. One small county lies to the south of Pike County, occupying the area down to where the two rivers join. We therefore consider this county to be fairly well isolated on three sides. Pike County is one of the largest Illinois counties and is listed as having 316,500 head of swine as of December 1, 1973.

(Slide 2) This slide pictures what is known locally as the "Pike County Pigcasso" and is entrenched on a corner of the courthouse lawn. While several other counties may dispute it's claim, it does symbolize the general attitude and pride of the citizens of Pike County in their greatest asset, the pork industry.

Our swine brucellosis problems were concentrated in Detroit and Montezuma townships located on the eastern edge of the county. The general terrain is rough, hilly land (Slide 3), much of it covered by timber (Slide 4), and with the area divided into a multitude of farms with small acreages of tillable land. The general swine producing practices are open shed rearing (Slide 5), but with a considerable number of central units used for farrowing and nurseries only, then finishing in small pastures and lots (Slides 6 & 7). Swine are usually

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turned into corn fields following picking (Slide 8), and fences in the area range from average to poor.

The next two slides, the first being a copy of the plat map of Detroit township (Slide 10) and the second of Montezuma township (Slide 11), show the extensive division of ownership of land in the townships as the land has been passed down from generation to generation, since the area was initially settled around the time of the Civil War. Only 9 farms in the 57 square miles composing the two townships are larger than 300 acres in a solid block. This factor contributed greatly to the spread of swine brucellosis, as I will explain later.

Mr. Harry S. Wright, Extension Agricultural Advisor of Pike County, scheduled a meeting for area residents interested in learning more about swine brucellosis at the Milton, Illinois, town hall at 2 p.m. on January 16, 1974. At 1:45 it appeared that 5 regulatory officials and the extension advisor would constitute the sole attendance. By 2:15 the small town hall, built to hold about 100 persons, was completely jammed and the walls were lined with standing persons. In my 24 year career in regulatory Veterinary Medicine, this was the most quickly assembled audience I have ever observed.

The meeting was opened by Mr. Wright, followed by a discussion of swine brucellosis and a question and answer session led by Dr. Paul B. Doby, Superintendent of the Division of Meat, Poultry and Livestock Inspection, Dr. Arthur L. Starkey and myself. It soon became apparent that many of the producers in the area had encountered problems similar to those noted with swine brucellosis, had voluntarily disposed of many sows because of breeding problems, and had a sincere interest in eliminating the disease.

The following program was then developed and put into action:

(1) Advance publicity on swine brucellosis and what we planned to do. This was capably directed by Mr. Wright, the Extension Agricultural Advisor. Mr. Neal Black, editor of the "National Hog Farmer" later spent a day with us and we sincerely commend him on his fine articles in the March and May, 1974 issues. In addition, each herd owner in the area was personally contacted by a regulatory veterinarian to explain the program.

(2) Establishment of a field headquarters and testing laboratory in Pittsfield, the county seat of Pike County, in early February. The project was placed under the supervision of the co-author of this paper, Dr. James R. Mattison, while Dr. Charles D. Hertich, a Department field veterinarian with considerable laboratory experience, operated the laboratory.

(3) Complete area brucellosis testing of all breeding swine 6 months of age or over in Detroit and Montezuma townships, utilizing state employed field veterinarians or fee-basis practicing veterinarians.
Identification of reactors, depopulation of infected herds and obtaining post mortems when the animals were slaughtered. Whenever possible, arrangements were made to collect tissues for bacteriological examination. Herds were approved for depopulation, based on either a 10% or greater herd infection rate, on positive isolation of Brucella suis from tissues collected from reactors, or on the basis of professional judgment when only 1 or 2 reactors were disclosed and all factors were weighed. Any feeder swine under market weight on infected premises were ear tagged and maintained under quarantine until they attained normal slaughter market weight and were then moved off the premises without indemnity. Sows with small pigs were permitted to remain on infected premises, in isolation, for a few weeks until their pigs were weaned. In our opinion this delayed depopulation procedure reduced indemnity payments without jeopardizing the effort to eradicate swine brucellosis.

Animals depopulated in infected herds were appraised at their value for breeding purposes. Indemnity was paid by the State of Illinois in the amount of the difference between appraisal and salvage value up to a maximum of $50 for grade females and $100 for registered purebred animals.

Through cleaning and disinfection of infected premises after all swine had been depopulated. Power sprayers were used in the disinfection process. No repopulation was allowed until at least 30 days following disinfection.

The entire project was originally scheduled to be completed in 3 weeks but eventually took 5. A total of 5855 head of breeding swine were tested and 329 reactors were disclosed. The number of herds is difficult to calculate due to joint ownership and fragmentation of farms mentioned previously.

Two infected farm complexes were voluntarily depopulated prior to the start of the project. Fourteen additional infected complexes were disclosed just prior to or during the course of the project. Thirteen were depopulated with indemnity. One herd which revealed only a single reactor on two consecutive herd tests was not depopulated and was finally cleared by retesting when an isolation could not be made on tissues collected from either of the reactors. The herd depopulations resulted in a total indemnity of $59,017.26 being paid by the State of Illinois on this project. A further breakdown will appear as Table 1 when this paper is published but I will not discuss it due to time limitations.

The one transparency I will show (Table 2) is a mock-up of the lower half of Detroit township and the upper half of Montezuma township. Premises where known infected swine were located at one time are marked in color and you can readily see the wide dispersion
of infected groups in the area, brought about by constant interchange of swine between fields and farrowing houses, poor fences, and loaning of boars between relatives.

Two of the depopulated herds were located out of this area. One herd (Chamberlain) was approximately 6 miles north of Detroit. No relationship between this herd and the infected area could be established despite an extensive epidemiological investigation. The second herd (McIntyre) was located across the Illinois river in Scott County but was known to have been in Montezuma township earlier for a period of time and MST reactors had been detected in cull sows removed from this herd prior to the start of the project.

Summary

1. Since the completion of the testing program, we have continued to monitor the area through the MST program and random testing of breeding swine in the area. No additional infected herds have been detected since April, 1974.

2. Drs. Mattison and Hertich devoted several days in May, 1975, to a resurvey of the swine producers whose herds were depopulated, the extension advisor and practicing veterinarians. All parties were in complete approval of the manner and speed with which the project was conducted, allowing for as little "down time" as possible which was necessary for them to maintain their livelihood. One of the owners of depopulated swine has since moved out of the area, all of the remainder except 2 have restocked after remaining out of the hog business for 1 month to a maximum of 6 months after depopulation.

3. Our conclusions are that a project of this type will work in a heavily concentrated swine area provided that it is accompanied by sufficient advance publicity, indemnity funds, realistic appraisals, utilization of competent personnel, prompt testing and depopulation and thorough cleaning and disinfection.
PIKE COUNTY, ILLINOIS, SWINE BRUCELLOSIS PROJECT

INFECTIONED AND DEPOPULATED HERD SUMMARY

<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Test Dates</th>
<th>Results</th>
<th>Negative Reactors</th>
<th>State Indemnity Paid</th>
</tr>
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<tbody>
<tr>
<td>Battershell, R.</td>
<td>Pearl</td>
<td>1-31-74</td>
<td></td>
<td>18 5</td>
<td>$1,100.00</td>
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<td>Campbell, R.</td>
<td>Pearl</td>
<td>1-23-74</td>
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<td>Chamberlain, A.</td>
<td>Pittsfield</td>
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<td></td>
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<td>4,800.00</td>
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<tr>
<td></td>
<td></td>
<td>1-30-74</td>
<td></td>
<td>47 43</td>
<td></td>
</tr>
<tr>
<td>Graham &amp; Colvin</td>
<td>Pearl</td>
<td>1-25-74</td>
<td></td>
<td>24 2</td>
<td>1,250.00</td>
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<tr>
<td></td>
<td></td>
<td>1-25-74</td>
<td></td>
<td>4 21</td>
<td></td>
</tr>
<tr>
<td>Hayden Farms 1</td>
<td>Milton</td>
<td>9/13 thru 11/30/73</td>
<td>227 1</td>
<td></td>
<td>11,250.00</td>
</tr>
<tr>
<td>Hoover Bros.</td>
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<td>1/30 thru 2/11/74</td>
<td>115 48</td>
<td></td>
<td>3,750.00</td>
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<td>Hoover, J.O. 3</td>
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<td>0 6</td>
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<td>Hoover, J.B. 4</td>
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<td>2-1-74</td>
<td></td>
<td>28 11</td>
<td>14,500.00</td>
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<tr>
<td>Lacy, P.</td>
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<td></td>
<td>72 1</td>
<td>Herd was not depopulated</td>
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<tr>
<td></td>
<td></td>
<td>4-4-74</td>
<td></td>
<td>71 1</td>
<td></td>
</tr>
<tr>
<td>Lemons, S. 5</td>
<td>Milton</td>
<td>Voluntarily depopulated before project was started.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McIntire, H. 6</td>
<td>Winchester</td>
<td>3-9-74</td>
<td></td>
<td>85 37</td>
<td>4,000.00</td>
</tr>
<tr>
<td>Miller, L.</td>
<td>Pittsfield</td>
<td>3-29-74</td>
<td></td>
<td>12 12</td>
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<td></td>
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<tr>
<td>Parks &amp; Gress 7</td>
<td>Pearl</td>
<td>9/26/73 thru 1/23/74</td>
<td>158 26</td>
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<td>6,234.07</td>
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<td>Wheeler Farms</td>
<td>Winchester</td>
<td>9/27 thru 11/9/73</td>
<td>233 111</td>
<td></td>
<td>Voluntarily depopulated before project was started.</td>
</tr>
</tbody>
</table>

1 Numerous MST reactors were disclosed from this herd during the summer of 1973. B. suis isolations made from them and from the one reactor on the herd tests.
2 Depopulated on basis of professional judgment. Located between two badly infected premises.
3 B. suis isolation made from reactors. No further testing conducted.
4 Had additional herds that were not tested prior to depopulation by reason of B. suis isolation and farm locations.
5 Small herd, voluntarily depopulated when MST reactors were revealed in summer of 1974.
6 Located in Scott County at time of test but had been in Montezuma township during previous summer.
7 Herds had been voluntarily depopulated on two prior occasions due to severe infection.
REFERENCES

People often ask, "Are we making progress in the brucellosis eradication campaign?" In fact, they are asking, "Do we recognize the problems deterring us from this goal, and, if so, have we initiated appropriate corrective actions?" If we neither recognize the problems nor initiate appropriate action, then brucellosis eradication will never be realized.

In reports to this association in 1973 and 1974, pleas were voiced that all members of the U.S. Animal Health Association upon whose shoulders rest the major burden for identifying and resolving program problems, rise up and accept the challenge of making the final goal of eradication a reality. Program data, although crude benchmarks in determining the current position relative to the eradication goal, is of some help in measuring how well we have carried this responsibility.

**BLOOD TESTING CATTLE**
(Figure 1)

The total number of cattle blood tested in FY 1975 was over 17.7 million. This is a 21 percent increase over the previous year, and includes 6.5 million (an additional 924,000) cattle tested on farms or ranches and 11.2 million (2.25 million increase) under the MCI Program. However, this increase was offset by a 21 percent rise in brucellosis reactors to 250,000. This total includes 170,000 reactors (28% increase) disclosed on the farm and 80,000 MCI reactors (27% increase). The upward trend in the brucellosis reactor rate continued in FY 1975 with 1.46 reactors per 100 blood tests compared to 1.34 in 1974 and 1.16 in 1973. The rate of spread of brucellosis continues to exceed the effect of program activities to detect and contain the disease.

**MARKET CATTLE IDENTIFICATION PROGRAM**
(Figure 2)

The 11.2 million tests conducted under the MCI surveillance program in FY 1975 is a 29% increase and continues the upward trend since 1970. The 2,519,000 increase includes an additional 2,386,000 animals tested at packing plants and a 133,000 increase at livestock markets. Again, however, this 25 percent increase was accompanied by a sharper rise in the number of MCI reactors. The 80,000 MCI reactors is 27 percent greater than the previous year. The upward trend in the reactor rate established in 1973 continued with an increase from 0.63 in 1973, 0.70 in 1974, and 0.71 in 1975.

More than 19,950 herds of origin were identified and tested from
tracing MCI reactors. Infection was disclosed in 7,477 of these herds (37%). The animal infection rate in infected herds was 15 percent—1% higher than the previous year.

MILK RING TEST RESULTS
(Figure 3)

Surveillance data on dairy herds indicates that progress toward eradication has slowed in this segment of the susceptible livestock population. The previous downward slope of annual brucellosis data reported on dairies has leveled to the point where eradication efforts are not exceeding reinfection factors.

The 2,450 dairy herds which reacted the BRT in FY 1975 represents 0.29 percent of the herds sampled. Of the 2,012 herds blood tested as a result of a BRT positive test, infection was disclosed in 641 (32 percent). In those herds where brucellosis was detected, 3.4 percent of the animals tested were reactors on the initial herd test compared to 4.0 percent the previous year.

BRUCELLOSIS INFECTED HERDS
(Figures 4 & 5)

The 16,401 (without P.R. and V.I.) infected herds identified in the 50 States in FY 1975 represent a 15 percent increase over the previous year. Six hundred and forty-six (646) infected herds were identified in the 29 certified-free States compared to 532 in the same States the previous year. There were 15,755 infected herds in the remaining 21 modified certified States, an increase of some 2,080 in these same States in 1975.

More than 37.5 percent of the infected herds (6,151) were identified in Texas. Although this is an increase of approximately 1,198 over 1974, continuing increases in the number of infected herds should be expected in Texas in the near future. Twenty-seven and nine tenths (27.9) percent of the Nation's infection was identified in Louisiana, Mississippi, and Oklahoma. Each of these three States has reported a relatively constant number of infected herds (between 1,000 and 1,900) each year during the previous five-year period. Seven States reported infected herds within the 300 to 1,000 range and collectively account for 24.7 percent of the total. These include Alabama (1975-793; 1971-466), Arkansas (1975-710; 1971-233), Florida (1975-334; 1971-808), Georgia (1975-505; 1971-265), Kentucky (1975-365; 1971-302), Missouri (1975-403; 1971-328), and Tennessee (1975-938; 1971-416).

Only one of the 11 States which collectively reported 90 percent of the infected herds in FY 1975 has significantly reduced the level of infection during the past three years. Kentucky had 472 infected herds in 1973, 409 in 1974, and 365 in 1975.
Fifteen (15) States had 8.6 percent of the infected herds and are in the range of 30-300 infected herds reported. Twenty-four States reported less than 30 infected herds. No infection was detected in four States and the Virgin Islands.

CERTIFICATION STATUS — JUNE 30, 1975
(Figure 6)

Only 60 counties achieved Certified Brucellosis-Free status during the year. The total of 1,990 such counties represents 63 percent of the Nation's counties and includes 53 percent of the adult cows. No States qualified for statewide-free status. One thousand one hundred thirty-eight (1,138) counties in 21 States and Puerto Rico held Modified Certified Area status at the end of FY 1975. One county in Arkansas, one county in Illinois, one county in Missouri, one county in Oklahoma, and twenty-one counties in Texas were listed as non-certified areas because of program deficiencies. Action to remove certification status from 69 additional counties for similar program deficiencies was required in 1975.

Calfhood Vaccination
(Figure 7)

The previous downward trend in the number of calves vaccinated annually was halted in FY 1974 with 3.8 million vaccinations—the same number as reported in 1973. However, there were 3.7 million calves vaccinated in FY 1975, a decrease of 100,000 from the previous year. Fifty percent of the calves vaccinated were in the 29 Certified Brucellosis-Free States. This is approximately 27 percent of the eligible calves in these free areas. Fourteen and six tenths (14.6) percent of the eligible calves in the 21 modified certified States received Strain 19.

Swine Brucellosis

The swine brucellosis program made progress during FY 1975, but at a slower rate than during the preceding 2 years. This slowdown was due largely to budgetary problems which made it necessary to withdraw program funds in December from States that were not at that time fully engaged in a validation program. As a consequence, market swine blood collection was terminated in five of the eleven States that had received money for this purpose at the onset of the fiscal year.

Despite this, the number of swine blood tested during the year increased to 2.4 million, up 6 percent from FY 74 (Figure 8). This total includes 316,000 tested on farms and 2 million sows and boars tested at livestock markets and at slaughter plants under the Market Swine Testing (MST) program.
The animal infection rate on all tests was 0.07 percent compared to 0.13 in FY 74. The MST reactor rate in FY 75 was 0.04, down from the 0.08 recorded a year earlier. These low rates are due in part to the unusually high sell-off of breeding swine that occurred during the year and to the fact that over half of the testing occurred in States that were either validated free or nearing validation.

Four States—Maine, Colorado, Washington, and Minnesota—were validated as brucellosis-free areas during the year joining Arizona, Arkansas, Montana, Oregon, Nevada, Utah, Wisconsin, and Wyoming who had previously attained this status. Dropped from the list of validated areas were California and the Virgin Islands each of which failed to qualify 3 counties for revalidation.

The number of Validated Brucellosis-Free counties increased from 496 to 694 during the year (Figure 9). In addition to all counties in the validated States, there were free counties in California-55, Hawaii-3, Massachusetts-8, Michigan-13, New Mexico-1, South Dakota-12, and Puerto Rico-61.

Validated Brucellosis-Free herds decreased from 4,335 in FY 74 to 3,095 in FY 75 (Figure 10). This decrease is attributed to the fact that many owners whose herds are automatically validated when they are tested during the process of validating a State or county choose not to maintain this status by retesting their herds at the end of the initial 12-month validation period.

One obvious conclusion from the previous data is the presence of many obstacles blocking our view of an eradication horizon. However, progress has been made within the past year in identifying major weaknesses in our current program efforts with the initiation of actions to correct such deficiencies. Many examples can be cited to strengthen the program by: acquiring or improving basic authorities, increasing resources, initiating research, improving surveillance techniques, effecting containment and disease elimination methods, preventing reintroduction of disease, stimulating vaccination, improving diagnosis, improving communication and education of the livestock producer, and applying program standards within each State. Likewise, many deficiencies in these categories of activity remain unresolved.

The central point to consider is that progress is measured by how well we identify problems and how effectively we resolve them. Within the past year, the livestock industry has worked with regulatory agencies to build momentum toward facing up to our program deficiencies and initiating corrective action. Such momentum must be sustained in the future so that the guilt of complacency which this program experienced several years ago will not be repeated. Only when this momentum is built to the point where the rate of disease detection and elimination greatly exceeds the rate of spread, will we see the eradication horizon.

I shall welcome with you the opportunity for that vision.
Brucellosis Eradication

**Milk Ring Test Results (BRT)**

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Suspicious BRT Tests</th>
<th>Follow-up Herd Blood Tests</th>
<th>Infected Herds Found</th>
</tr>
</thead>
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<tr>
<td>1967</td>
<td>8,179</td>
<td>1,632</td>
<td>8,053</td>
</tr>
<tr>
<td>1969</td>
<td>4,795</td>
<td>1,964</td>
<td>2,831</td>
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<tr>
<td>1971</td>
<td>3,010</td>
<td>2,640</td>
<td>1,370</td>
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<td>1973</td>
<td>2,179</td>
<td>2,080</td>
<td>1,099</td>
</tr>
<tr>
<td>1974</td>
<td>2,577</td>
<td>2,192</td>
<td>1,385</td>
</tr>
<tr>
<td>1975</td>
<td>2,450</td>
<td>2,012</td>
<td>1,438</td>
</tr>
<tr>
<td>1976</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1977</td>
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</table>

Cooperative State-Federal

**Brucellosis Eradication Program**

- Certified: Brucellosis Free
  - Counties 1 800
  - States 20 and V 1
- Modified Certified Areas
  - Counties 1 120
  - States 21 and P 1
- Non-Certified Areas
  - Counties 74
  - States 5

**Date:** June 30, 1975
BRUCELLOSIS INFECTED HERDS FOUND
In Noncertified, Modified Certified, and Certified-Free States

NUMBER INFECTED HERDS
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0

STATES WHERE INFECTED HERDS FOUND
Certified-Free
Modified Certified
Noncertified

FISCAL YEAR
1968
1970
1972
1974
1976

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE

DISTRIBUTION OF BRUCELLOSIS REACTOR HERDS
Percent of Total Reactor Herds Found

24 STATES, <30 HERDS
8.5%

15 STATES, 30 < 300 HERDS
37.5%

7 STATES, 300 < 1,000 HERDS
28.0%

3 STATES, 1,000 < 3,000 HERDS
20.7%

FISCAL YEAR 1975

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Brucellosis Eradication

CALVES VACCINATED

MILLION CALVES VACCINATED

FISCAL YEAR

1953 '55 '57 '59 '61 '63 '65 '67 '69 '71 '73 '75 '77

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SWINE BRUCELLOSIS

Animals Blood Tested

THOUS. ANIMALS

FISCAL YEAR


U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
REPORT OF THE COMMITTEE ON BRUCELLOSIS

Chairman: Bob Laramore

Co-Chairman: G. J. Fichtner


The Brucellosis Committee met in open session on Monday and Tuesday afternoons, November 3 and 4. Approximately 300 persons attended each open meeting. All persons wishing to present proposals, recommendations, or resolutions to the Brucellosis Committee were given an opportunity to do so. The Brucellosis Committee subsequently met in closed session for two days to consider all subjects previously submitted for consideration.

Proposals and recommendations were presented by:

- Holstein-Friesian Association
- National Milk Producers Federation
- American Farm Bureau Federation
- Pennsylvania Department of Agriculture
- T. A. Kincaid, Jr.

1. The Brucellosis Committee supports continued emphasis on brucellosis research in the specific areas of vaccination, diagnosis, and disease transmission.

2. The "5-Year Plan for Brucellosis Eradication" is not approved due to the inadvisability of securing the requested necessary funds for implementation at this time, and due to the request by this committee for an unbiased study to be completed, while fully realizing many of the advantages of such a plan.

3. In response to Senate Resolution 216, the Brucellosis Committee requests that USDA include a statement favoring continued emphasis on the use of Strain 19 vaccine.

4. That USDA assist Texas in resolving the conflict with Happy Hereford Ranch regarding "S" branding brucellosis exposed animals for interstate and intrastate movement. The proposal to amend the existing program standards concerning "S" branding was not approved.

5. The Brucellosis Committee endorses the proposed regulations on the interstate movement of swine including identification as requested in past years by this Committee.
6. That USDA seek latitude in 9 CFR 51 for the payment of indemnity upon proof of sale of brucellosis reactors. Current regulations requiring proof of slaughter of such animals as a condition for indemnity payment results in unnecessary delays in the payment of indemnity and is not in the best interest of the eradication program since the control of such reactor animals between the initial point of sale and the point of slaughter is the responsibility or regulatory officials. Further, if such latitude is determined to be not available, then the USAHA and the livestock industry involved shall assume the responsibility to assist in securing the necessary amendments to the law to permit this revision in the regulation.

7. The Brucellosis Committee expresses its sorrow of the death of Dr. Chester Manthei, a long-time committee member. Dr. Manthei will be remembered for his contributions to brucellosis research and for his dedicated efforts as a member of this Association.

8. The following recommended amendments to the UMR were approved. Significant amendments include the definitions of a herd test, quarantined feedlot, approved brucella vaccine, and identification of vaccinates. Recommended amendments to UMR standard procedures involve the identification and movement of exposed animals, herd depopulations, diagnostic reagents, livestock dealers, contact herd testing, and epidemiology.


The Chairman appreciates the interest expressed in the brucellosis meetings and thanks each member of the Brucellosis Committee for their long and dedicated efforts in obtaining this report.

Brucellosis Eradication Uniform Methods and Rules

CHAPTER 1 — BOVINE BRUCELLOSIS

Part I — Definitions

C. NEGATIVE—Official vaccinates of dairy breeds 20 months of age or over; official vaccinates of beef breeds 24 months of age and over as evidenced by the presence of the first pair of permanent incisor teeth; or official vaccinates under these ages that are parturient (springers) or postparturient that disclose SPT or STT reactions of not more than complete agglutination in the 1:50 dilution and are negative to the brucellosis card test if performed; or are negative to the brucellosis card test if it is the only test procedure used.

All other cattle more than 6 months of age that disclose an agglutination reaction of less than incomplete agglutination in the 1:50 dilution and are negative to the brucellosis card test if performed; or
are negative to the brucellosis card test if it is the only test procedure used.

D. HERD TEST—The herd test must include all cattle over 12 months of age except steers, sprayed heifers, official vaccinates of dairy breeds under 20 months of age, and official vaccinates of beef breeds under 24 months of age as evidenced by the presence of the first pair of permanent incisor teeth which are not parturient (springers) or postparturient. Bulls, steers, and female cattle being held in feedlots which meet the conditions defined in Part I, E (QUARANTINED FEEDLOT), are exempted from herd test requirements. Breeding cattle tested shall be identified with an official eartag, tattoo, or other permanent identification.

A herd is a group of cattle maintained on common grounds for any purpose, or two or more groups of animals under common ownership or supervision geographically separated but which have an interchange or movement of animals without regard to health status. Herd test decisions under this definition must be based on sound epidemiological evidence.

The herd test conducted for the recertification of Certified Brucellosis-Free Herds needs to include only those breeding animals that are postparturient or are 24 months of age or older.

E. QUARANTINED FEEDLOT—A quarantined feedlot shall be a confined area approved by State and Federal animal health officials who shall establish procedures for accounting of all animals entering or leaving such quarantined feedlot. Such quarantined feedlots shall be approved annually. The quarantined feedlot shall be maintained for finish feeding of animals in drylot with no provision for pasturing or grazing. All animals except steers and spayed heifers must be permanently identified on the left jaw by a hot "S" brand upon entering such feedlots.

All animals leaving such feedlot must move only for immediate slaughter in accordance with established procedures for handling quarantined animals including issuance of permits prior to movement, segregation from other livestock sold for purposes other than slaughter at all points en route to slaughter, cleaning and disinfection of pens before reuse, and record of proof of slaughter.

G. MARKET TEST ANIMALS—Test-eligible animals moving in trade through auction markets, stockyards, or to slaughtering establishments; also those animals assembled at farms or ranches that are being readied for immediate movement to markets, stockyards, or slaughtering establishments or other sales. Such animals shall be identified by eartag or backtag to the herd of origin prior to or at the first point of concentration in marketing channels.

J. IDENTIFICATION OF VACCINATES—Vaccinated animals are to be permanently identified as vaccinates by tattoo and by official vaccination eartag. Brands may be used in addition to tattoo
and eartag. Vaccination tattoos must be applied in the right ear. The tattoo will include the U. S. Registered Shield and V, which will be preceded by a number indicating the quarter of the year and will be followed by a number corresponding to the last digit of the year in which the vaccination was done. Official vaccination eartags shall be applied to the right ear. The eartag will include the State prefix and a V, followed by a number which individually identifies each vaccinated animal. Registered tattoos may be substituted for the official eartags. Brands, if used, must be applied to the right jaw. The V will be applied in one of four different positions—the open end facing up, forward, down, or toward the back. The brand indicates in which year the vaccination was conducted. The fifth year will repeat the first year, and so on indefinitely. In 1974, the V should be placed with the open end facing up and in succeeding years, should proceed clockwise.

K. SUCCESSFUL TRACEBACK OF REACTORS—A successful traceback occurs when the premises of herd of origin of a market test reactor, including those animals identified as reactors on laboratory confirmation of market tests, is located and the herd is tested as is deemed appropriate. If all animals at the premises of origin were sold for slaughter, a successful traceback can also be claimed. Tracing to dealers, feedlots, commission firms, and such are not considered successful tracebacks of reactors.

Part II — Standard Procedures

B. REPORTING—Activities conducted privately or as part of the official brucellosis eradication program, such as results of agglutination tests or vaccination, must be reported immediately to State-Federal cooperating agencies. A complete epidemiological report shall be prepared and filed on each infected herd.

D. QUARANTINES—All cattle, except steers and spayed heifers but including officially vaccinated heifers and calves in infected herds, must be confined to the premises until the herd is freed of brucellosis or sold for slaughter under permit. Calves 8 months and under from negative cows may move to any destination within 10 days after a negative brucellosis test of the dam. Two consecutive negative herd tests are required for release of quarantine with the first test occurring not less than 30 days after the removal and slaughter of all reactors and the second test to occur not less than 90 days following the first negative test. In Certified Brucellosis-Free Areas, a third negative herd test is recommended 90 days after the second negative test for quarantine release. Exceptions on the interval between tests required for the release of quarantine are allowable only when *Brucella abortus* Strain 19 organisms have been isolated from the reactor animal or all epidemiologic evidence is consistent with a *Brucella abortus* Strain 19 infection and not with virulent in-
fection. A retest of the entire herd not less than 30 days after removal of the reactor or after the balance of herd test in case of a market test reactor will be required for the early release from quarantine.

E. RETESTS OF INFECTED HERDS—Retest of infected herds are to be conducted at approximately 30-day intervals until the herd has passed at least one negative herd test following the removal and sale for slaughter of the last reactor. Additional consecutive negative herd tests are required to qualify herds for release of quarantine as indicated in Part II-D. Continuation of testing is strongly recommended at 30-day intervals between the first and the second negative herd tests which qualifies the herd for quarantine release.

G. CLASSIFICATION OF CATTLE

4. Reclassification of Reactors

Animals initially classified Reactors under Part I, A, may be eligible for recategorization provided a complete epidemiological investigation of the herd is conducted including a serological test of the herd if necessary, and there is no evidence of brucella infection or exposure thereto; and, provided final classification is determined by a trained brucellosis epidemiologist or an appropriate livestock official. The herd must remain under quarantine pending final classification.

I. IDENTIFICATION OF EXPOSED CATTLE—All exposed cattle moving from a premises of origin or from a livestock market to a quarantined feedlot or to slaughter shall be identified by branding with heat the letter S (at least 2 x 2 inches) placed on the left jaw prior to movement or; exposed cattle may be identified and permitted to a livestock market where they shall be identified by “S” brand upon arrival. Exposed cattle returned from the livestock market to the herd of origin under quarantine pending further testing are exempt from this requirement. Exposed cattle may move directly from a herd of origin to slaughter in an officially sealed truck without permanent identification of “S” brand.

M. SERVICES TO OWNERS—Services shall be rendered without expense to the livestock owner as long as funds for such purposes are available; however, owners are to provide for handling of their cattle. Contracts with accredited veterinarians, nonprofessionals, other State and Federal agencies, or with the management of privately owned firms are to be used as needed to assist State and Federal animal health personnel accomplish necessary sample collections, animal identifications, and other activities. Within 3 days of an initial herd test, an owner may request an additional test on reactors at his expense provided such request is based on sound epidemiological evidence and provided all animals remain under herd quarantine.

N. HERD DEPOPULATION—Herds with high animal infection rate or chronic infection of long duration shall be reviewed for pos-
sible depopulation. All infected herds shall be reviewed for possible depopulation in States which previously had been free of brucellosis.

O. DIAGNOSTIC REAGENTS FOR BRUCELLOSIS—Brucella antigens used for the diagnosis or detection of brucellosis in domestic livestock will be restricted to regulatory personnel and approved accredited veterinarians.

P. LIVESTOCK DEALERS

1. All livestock dealers as defined by P & S and/or by state requirement must maintain complete records available upon demand of all livestock transactions which identify the animal with the buyers and sellers.

Part III — Participation in Herd or Area Certification Plans

B. MAINTAINING CERTIFICATION STATUS—State and Federal officials in charge of the program activities in each State are responsible for conducting a continuous evaluation on the efficiency of local procedures for locating and eliminating infected cattle. The minimum standards as described for each plan in Parts IV, V, and VI, Brucellosis Eradication Recommended Uniform Methods and Rules, must be met or exceeded throughout the certification period to maintain continuous status. An annual report showing the degree of uniformity and adequacy of the surveillance, suspicious and infected herds disclosed, infection rates and adequacy of program standards within the area, is required to maintain certification status of an area larger than a county.

C. TERMINATING CERTIFICATION STATUS—Herd or area certification status may be terminated at any time during the period with 10 days notice. If the herd or area does not maintain adequate surveillance measures or fails to comply with quarantine, testing schedule, initial testing schedules for suspicious herds detected by the surveillance measures, excessive herd infection rates due to repeated failure to maintain program standards, or proper disposal of reactors or exposed animals unless legal action against the livestock owner has been initiated by States’ attorneys and is in progress.

Part V — Modified Certified Brucellosis areas

A. QUALIFYING METHODS—(An area may qualify by one or more of the following methods.)

1. Milk Ring Test—The milk ring test shall be conducted at least three times per year at approximately equal intervals, and all herds with positive milk ring test results must be tested within 30 days from date of laboratory tests. To qualify individual herds for initial Modified Certified Brucellosis status, the four most recent consecutive tests for each herd during the testing period must be negative. In new or recently assembled dairy herds, one or more consecutive
negative ring tests will qualify the herd for area certification purposes provided each milk ring test on samples from such herds are negative. The milk ring test procedure will be adjusted in accordance with herd size. The quality of the samples used for the milk ring test will be monitored at the time of collection.

2. Market Cattle Identification Program
   a. Coverage

   Recertification: At least 30 percent (10 percent per year during the 3-year testing period) of the breeding cows in the area over 2 years of age from herds not covered by the milk ring test. Modified certified states with high infection rates shall require the test of all eligible cattle in a consignment where one or more cattle are sold for breeding purposes.

   b. Reactors—90 percent of the reactors must be successfully traced to the herd of origin and a herd test conducted. Herd tests shall be conducted within 30 days of disclosure of market cattle test reactors or the herds shall be confined to the premises under quarantine. All market cattle test reactor herds shall be tested within 6 months of disclosure of the market reactor.

3. Complete Herd Test
   a. Initial Certification

   Complete herd test of all eligible cattle in each herd which has not qualified for initial certification under Part V, A-1 or A-2.

   Recertification: Complete herd test of at least 20 percent of the representative herds in the area. A different 20 percent will be selected for each recertification. When less than 15 percent of the breeding cows in the area over 2 years of age from herds not covered by a milk ring test are tested under MCI during the 3-year testing period, then a complete herd test of at least 20 percent of the representative herds in the area that have not qualified by MCI or BRT coverage is needed.

   A complete herd test of herds with direct animal contact with an infected herd must be conducted during the period the infected herd is under test and quarantine. A second test at the time of release of quarantine of the infected herd is recommended. This provision applies equally for initial certification and recertification of areas.

4. Suspects
   a. Initial Certification

   Animals classified as suspects shall be retested at 30-60 day intervals one or more times until the animals' status can be reasonably determined. The various supplemental tests (ME, RIV, CF, and APA at pH 3.50, pH 3.25, and/or pH 3.0, etc.) shall be conducted on blood samples, and the results evaluated by a brucellosis epidemiologist and a determination made that an infection does not exist before the surveillance on suspects is dropped. If the suspects
requiring retest are not available, then a complete herd test is necessary.

7. Area Status, if Excessive Infection Rates Occur—If an area does not qualify for recertification because of excessive herd infection rates, even after herd retest results are used, it shall become necessary to make a complete area retest. If the area completes the necessary testing to qualify for recertification within 6 months after the due date, consideration will be made for reinstating such an area.

8. Other Considerations—A concerted effort through effective screening programs and extensive epidemiologic investigations to locate infection and to eradicate the disease is required.

MCI samples showing a standard agglutination titer at 1:25 or above or that are positive to the card test, shall be tested further by use of the supplemental tests. All of the results are to be furnished to the State of origin. The test results and herd investigation will be reviewed by a brucellosis epidemiologist in arriving at a diagnosis. Those animals requiring a followup herd blood test must be successfully traced to the herd of origin as indicated in A-2, b, above. Herds which do not progress favorably under routine testing procedures may be considered for enrollment in a brucellosis problem herd program under the direct supervision of a trained brucellosis epidemiologist. Such enrollment must be for the eradication of brucellosis from the herd and may include altered testing schedules, management practices, supplemental testing, etc., as approved by the owner and officials in charge of program activities in the State.

Part VI — Certified Brucellosis-Free Areas

A. QUALIFYING METHODS—An area may qualify by one or more of the following methods:

1. Milk Ring Test—The milk ring test shall be conducted at least three times per year at approximately equal intervals, and all herds with suspicious milk ring test results must be tested within 30 days, based on date of laboratory test. In new or recently assembled dairy herds, one or more consecutive negative ring tests will qualify the herd for area certification purposes provided each milk ring test on samples from such herds are negative. The milk ring test procedure will be adjusted in accordance with herd size. The quality of the samples used for the milk ring test will be monitored at the time of collection.

3. Complete Herd Test.

   Initial Certification: Complete herd test of all eligible cattle in each herd which has not qualified for initial certification under Part VI, A-1 or A-2.

   Recertification: Complete herd test of all eligible cattle in each herd qualifying under this method. It is strongly recommended that
all herds known to have been infected during the current certification period should qualify by a complete herd retest at least 90 days following the test for quarantine release.

A complete herd test of all herds with direct animal to animal contact with an infected herd must be conducted at least once and preferably twice during the period the infected herd is under test and quarantine. The first test should be conducted at the earliest opportunity and the second test at the time of release of quarantine of the infected herd.

B. QUALIFYING STANDARDS

5. This section omitted from UMR.

6. Suspects—Animals classified as suspects shall be tested at 30-60 day intervals one or more times until the animals' status can be reasonably determined. Various supplemental tests (ME, RIV, CF, and APA at pH 3.50, pH 3.25, and/or 3.0, etc.) shall be conducted and the results evaluated by a brucellosis epidemiologist and a determination made that an infection does not exist before the surveillance on suspects is dropped. If the suspects requiring retests are not available, then a complete herd test is necessary.

7. Area Status, if Excessive Infection Rates Occur—If an area does not qualify for initial certification because of excessive herd or animal infection rates, then a new testing period must be established. Areas failing to qualify for recertification because of excessive herd or animal infection rates will revert to modified certified status. If the necessary testing can be accomplished to qualify the area for recertification within 6 months after the due date, consideration will be made for reinstating such an area. For both initial and recertification, all cattle herds in which brucellosis has been known to exist must be legally released from quarantine prior to certification. In addition, all herds of other species of domestic livestock in which brucellosis has been found or suspected must be tested negative, slaughtered, or quarantined leaving no known foci of infection in any species uncontrolled at the time of certification. If the annual review required to maintain Statewide certified Brucellosis-Free Status indicates excessive herd infection rates within individual counties, the Certified Brucellosis-Free Status of those counties will be removed with 10 days notice.

Recommendation No. 9

All proposals that were presented were given thorough consideration. The concepts included in the Competitive Livestock Marketing Association proposal were unanimously rejected.
RECENT DEVELOPMENTS IN BOVINE LEUKEMIA RESEARCH AND REGULATORY PROGRAMS

M. J. Van Der Maaten* and Janice M. Miller*

The observation that cases of the adult, or enzootic, form of bovine leukosis (lymphosarcoma) were not randomly distributed among the cattle population provided the first clue that the disease might be associated with an infectious agent. It was subsequently recognized that this clustering of cases did not occur with the thymic or skin form of the disease or with generalized lymphoid tumors in calves less than 6 months old. On the basis of these observations the thymic, skin and calf cases were designated as sporadic or juvenile leukosis and were not considered to be associated with an infectious etiologic agent.

Continued epidemiologic investigations of adult or enzootic bovine leukosis provided additional evidence that a transmissible agent was involved and the familial aggregations of cases indicated that genetic factors might also be involved in tumor development. It was extremely difficult, however, to reach any firm conclusions regarding probable causes of the disease from tumor data alone. Transmission studies conducted during this time provided additional evidence of the existence of an infectious agent but the infrequency of tumor development, the long (2-10 year) incubation periods and the use of crude inocula made it difficult to interpret the results of such experiments.

A major advance in our knowledge of enzootic bovine leukosis resulted from the recognition that persistent lymphocytosis was a frequent precursor of tumor development. It was subsequently determined that the incidence of persistent lymphocytosis far exceeded that of tumor formation and it was assumed that the lymphocytotic state was due to the presence of an underlying virus infection. Lymphocyte counts thus provided a much more sensitive detection method for conducting epidemiological studies. Results of such studies provided sufficient evidence for the existence of an infectious agent that a number of European nations developed control or eradication programs based on various lymphocytosis indexes or “keys”. This approach was most widely used in Denmark where there is, at present, a nation-wide mandatory eradication program based on the detection of persistent lymphocytosis. Lymphocytosis also became an additional criterion for use in the evaluation of results of experimental transmission studies and provided further evidence that an infectious agent had, in fact, been transmitted to the experimentally inoculated

calves. Tumor formation was still only rarely observed, however, and the inability to identify any specific infectious agent in the crude inocula used in many calf experiments, or to obtain proof of the biological activity of virus particles observed by electron microscopy, made it impossible to identify the etiologic agent.

It was the identification of C-type virus particles in short-term lymphocyte suspension cultures in 1969 that ushered in a new era in bovine leukosis research. Using the suspension culture technique, it was determined that cattle were infected with a virus morphologically similar to the leukemia viruses of other species. Because virus recognition was based on electron microscopic examination, only limited virological and epidemiological investigations could be completed, but the correlation of these results with the incidence of tumors and persistent lymphocytosis provided a basis for further studies. These results were subsequently confirmed in other laboratories and information obtained using the lymphocyte culture technique formed the basis of all of our more recent knowledge of bovine leukosis. Similar C-type virus particles were subsequently found in continuous suspension cultures established from cattle with lymphosarcoma and these cultures have provided an additional source of materials for virological, biochemical and serological studies. Monolayer cell cultures have now also been developed and serve as the most satisfactory source of virus for characterization studies and antigens for serological tests. The virus, originally referred to as bovine C-type virus because of its morphological characteristics, is now more commonly identified as bovine leukemia virus (BLV). It is, with certain reservations, gaining widespread acceptance as the etiologic agent responsible for enzootic bovine leukosis.

The recent observations that BLV infection evokes specific antibody responses have resulted in the development of a number of serological tests. These include immunodiffusion, complement fixation, and indirect immunofluorescent techniques. These tests are considered to be specific and sensitive detectors of antibodies to BLV and it is commonly accepted that the presence of antibody is indicative of the existence of a persistent virus infection. Limited studies indicate that there is a general correlation between serological test results and the existence of persistent lymphocytosis but that lymphocytosis test results underestimate the number of infected animals within a given herd. It is thus evident that a new, and more precise, level of sensitivity in the detection of BLV infections has been achieved. It therefore seems likely that the serological tests will be widely applied, not only in this country, but also as an additional diagnostic method in the programs which have already been established in a number of European nations.

Results of limited seroepidemiological studies of cattle in this country indicate that the virus is widely distributed in our cattle
population, particularly among dairy cattle. Studies of a few beef herds with multiple tumor cases clearly indicate, however, that beef breeds are not inately less susceptible to infection. It must therefore be assumed that beef cattle are exposed to less virus, perhaps due to management practices.

The application of serological techniques has shown that virtually all adult animals with lymphoid tumors are infected with BLV. A fairly large proportion of the cattle in herds where multiple tumor cases have been diagnosed are also infected and a considerable number of animals in herds where there is no history of tumor cases may also be infected. It is thus apparent that the virus is widely distributed in the cattle population but of quite low oncogenicity so that only infrequently does infection lead to tumor development. The oncogenicity of the virus is, however, readily demonstrable in experimentally inoculated sheep. Tumor formation in sheep has been demonstrated as a result of the inoculation of blood and tissues from cattle with lymphoid tumors, cultured lymphocytes from infected cattle, and more recently with filtered fluids from BLV infected cell cultures.

From a comparative virological standpoint, BLV shares the C-type morphology previously described for leukemia viruses of other mammalian species but is quite different in other characteristics. The major internal polypeptide does not contain the gs-3 or interspecies specific antigen which was originally thought to be a marker of all mammalian leukemia viruses. The reverse transcriptase associated with BLV is also different from that of the feline and murine leukemia viruses in that it requires Mg$^{++}$ rather Mn$^{++}$ ions for maximum activity. These differences have resulted in some hesitancy to classify BLV as a typical C-type virus and indicate that it may represent a new oncornavirus group. These differences have, however, in no way detracted from the apparent importance of the virus in bovine neoplasia. The lack of positive evidence of oncogenicity in experimentally inoculated calves would thus seem to remain as the single factor preventing universal acceptance of the virus as the causative agent of bovine lymphosarcoma. For the reasons pointed out previously, it seems unlikely that good evidence of oncogenicity in experimentally inoculated cattle will be available in the near future. It now seems likely that programs to control or eradicate BLV will be developed in some countries and results of these programs may establish the etiological significance of the virus in the cattle population at large long before the requisite laboratory experiments can be successfully completed.

REFERENCES


EVALUATION OF ACETYLETHYLENEIMINE-KILLED BOVINE VIRAL DIARRHEA—MUCOSAL DISEASE VIRUS (BVD) VACCINE FOR PREVENTION OF BVD INFECTION OF THE FETUS

A. W. McClurkin, M. F. Coria and R. L. Smith

SUMMARY

Pregnant cows vaccinated at different stages of gestation with a killed bovine viral diarrhea—mucosal disease virus (BVD) vaccine and experimentally exposed to live virus 4 or 8 weeks later produced healthy calves generally free of antibody to BVD at birth. Non-vaccinated pregnant cows which were exposed to BVD virus at different stages of gestation produced abnormal calves or calves that were born with serum antibody against BVD, indicating an intrauterine infection. The stage of gestation at which the cows were exposed appeared to have a marked influence on the well being and general health of the calf at birth.

INTRODUCTION

Effective commercial vaccines for the bovine viral diarrhea (BVD)—mucosal disease (MD) complex are available. However, under certain conditions, reactions after vaccination may be severe. Furthermore, BVD virus can pass the placenta and infect the fetus at all stages of gestation. Abortion, cerebellar hypoplasia, ocular lesions, stillbirths, weakness, and diarrhea occurred, particularly with infections in the first half of gestation. For these reasons, vaccination of susceptible pregnant cattle with live modified virus vaccines is usually not recommended.

Experimental inactivated BVD vaccines were prepared and tested because a product is needed that can be safely used in cow-calf operations of both the beef and dairy industries, where cows might be in all stages of pregnancy. Fernelius et al. compared the effectiveness of beta-propiolactone and chloroform as inactivating agents. Kolner et al. used formalin as the inactivating agent.

Graves and Arlinghaus report the use of acetylethyleneimine (AEI) as an inactivating agent for a killed foot-and-mouth disease vaccine. The advantages cited for AEI as an inactivating agent for foot-and-mouth disease virus prompted its use in these experiments. Acetylethyleneimine is a carcinogen and we recommend following the procedures for working with this chemical that are outlined in the manual “Safety Standards for Laboratory Operations Involving

EVALUATION OF ACETYLETHYLENEIMINE

Chemical Carcinogens,” Department of Health, Education and Welfare.

The first objective of this study was to test the effectiveness and safety of AEI as an inactivating agent for BVD-MD. The second was to determine whether killed BVD virus could be sufficiently concentrated so that a single injection could stimulate an antibody response in pregnant cattle which would protect them and their fetuses from BVD infection after an intranasal exposure of the dam.

MATERIALS AND METHODS

Preparation of the Vaccine—For the first experiment approximately 30 x 10⁵ infectious units of a cytopathogenic strain (CPE), NADL-BVD virus¹⁰ was inoculated onto cultures of bovine turbinate (BT) cells¹⁵ grown in Blake bottles (100 ml media per bottle). In 3½ to 4 days, the cell sheet was showing CPE, and 0.2 ml of AEI was added to each bottle. Incubation was continued for 4 hours to complete inactivation. The inactivated virus-cell culture suspension was transferred to cellulose dialyzing tubing and concentrated 10-fold by dialysis against polyethylene glycol M.W. 20,000. Dialysis apparently removed the AEI, and the vaccine was tested for the presence of live virus by inoculation onto BT cells. Each of three blind passages was observed for the presence of CPE virus and tested for the presence of interfering virus according to the method described by Gillespie et al.⁸ Suspension of Rehsorptara in sterile distilled water was added to the concentrated virus-cell culture suspension at a rate of 10% by volume, and 5 ml, representing approximately 50 ml of the original virus-cell culture suspension, was injected intramuscularly as one dose.

For the second experiment BT cell monolayers were grown in roller bottles with 690 CM² surface. The bottles were seeded with 100 ml of cell culture suspension. When the cell sheet was confluent, 50 ml of media was removed to reduce the volume of the vaccine in relation to virus-infected cells.

Approximately 30 x 10⁴ infectious units of a CPE strain, Singer BVD virus,¹⁵ was inoculated into each bottle. After 2 days, the cell sheet was showing CPE, and 0.15 ml of AEI was added to each bottle. Incubation was continued for 4 hours to complete inactivation.

The inactivated virus-cell suspension was pooled and kept in suspension by magnetic stirring. A Brewer automatic pipetting machine was used to transfer 35-ml aliquots of vaccine to 100 ml serum bottles. The vaccine was lyophilized in a Virtis model 41 SUB freeze-dryer. The AEI was removed by the lyophilization process and

⁸Rehsorptara - aluminum hydroxide gel sold by Rehis Chemical Co., Division of Armour Pharmaceutical Co., Kankakee, IL 60901.
reconstituted virus was checked for live virus on BT cells, as was done for the vaccine prepared in the first experiment. The contents of each bottle, one dose, was reconstituted in 5 ml of 10% solution of Rehsorptar in sterile distilled water and administered intramuscularly.

Isolation Facilities for Experimental Animals—In each experiment cows were housed in a temperature-controlled isolation barn from the time they were put on experiment until 1 month after their immunity was challenged by exposure to live BVD virus. To give the cows as natural an environment as possible we turned them outside in an isolated exercise paddock surrounding the barn. Weather permitting, they were kept outside as much as possible. In the first experiment, the cows calved in May and June, and some were allowed to calve outside. In the second experiment, most of the cows calved in March and April, and because of cold rainy weather they were kept inside as calving became imminent.

Animals Used—Nine BVD-negative cows 2 to 4 months pregnant were used in the first experiment. Serum samples were taken from each animal at the beginning of the experiment. Five animals were vaccinated intramuscularly with 5 ml of vaccine, and 4 were kept as non-vaccinated controls. One month after vaccination, serum was taken and each animal was given 10 ml of the NADL strain of virus by intranasal instillation. A serum sample was taken from each animal 2 weeks after exposure.

Rectal temperatures and heparinized blood samples were taken from the 3rd through the 7th day after exposure. Total white blood cells were counted by means of a Sanborn-Frommer cell counter model 75. The blood samples were then centrifuged; and the buffy coat was removed and washed twice in phosphate buffered saline, frozen and thawed once and then inoculated onto BT cells for attempted virus isolation.

Nineteen BVD-negative cows from 3½ to 6½ months pregnant were used in the second experiment. Serum samples were taken from each animal at the beginning of the experiment. Fourteen animals were vaccinated intramuscularly with 5 ml of vaccine, and 5 were kept as non-vaccinated controls. Two months after vaccination serum was taken and each animal was given 10 ml of the Singer strain of BVD by intranasal instillation. Serum was taken again 2 weeks after exposure.

Rectal temperatures, heparinized blood for total leucocyte counts, nasal swabs, and rectal swabs were taken from each animal from the 4th through 7th day after exposure. The swabs were placed in tubes containing 3 ml of cell culture media with 300 mgm kanomycin, 1 million units of penicillin and 1 gram of streptomycin added per liter. The tubes were frozen and thawed once. The fluid from nasal swabs was inoculated directly onto BT cells, but fluid from fecal
swabs was filtered through 0.45-um Millipore filter, then inoculated onto BT cells. Each of three passages was observed for CPE and tested for interfering virus before the specimen was considered negative.

In the second experiment, serum samples were also taken at parturition, along with a colostrum sample before the calf had nursed. In the first experiment, serum neutralizing antibody was measured; approximately 1000 TCID$_{50}$ Singer-BVD was added to equal parts serum dilutions of 1:2 and higher to make the first dilution 1:4. After 30 minutes at 37°C, the virus-serum mixture was inoculated onto two 2- to 3-day old BT roller tube cultures. Results were read 5 days later; the highest dilution of serum that would prevent CPE in both tubes was taken as the end point.

In the second experiment, antibodies were determined by the microtiter method as described by Rossi and Kiesel.¹⁸

Assay for Immune Globulins—The paired pre- and post-colostrum serum samples of 2 unthrifty calves of the 2nd experiment were subjected to immune electrophoresis and diffused overnight against goat origin anti-bovine IgG.

RESULTS

No live virus could be demonstrated in either vaccine, and there was no clinical evidence that any of the vaccinated animals had any adverse reaction after vaccination. There was no serological evidence that BVD had spread to the non-vaccinated control cows before challenge-exposure, and the vaccines were considered safe to use on pregnant cattle.

Neither the vaccinated nor non-vaccinated cows in either experiment developed in appetence, nasal exudate, or diarrhea after exposure to BVD virus. In the first experiment, the highest temperature recorded was 102.8°F in one control cow. The total leucocyte counts were moderately depressed in all animals, one as low as 4,500/cmm in the control group and one as low as 5,000/cmm in the vaccinated group.

The highest rectal temperatures recorded in the second experiment were 104°F and 103.4°F, both from vaccinated cows. All other temperature recordings were within the normal range. Total leucocyte counts were moderately depressed in all animals, one of the control group went as low as 6,000/cmm and one of the vaccinated animals as low as 5,500/cmm. There was no correlation between the higher rectal temperatures and leucopenia.

In the first experiment, BVD virus was not reisolated from the buffy coat cells of vaccinated animals from the 4th through 7th day after exposure to NADL virus. However non-cytopathogenic BVD virus was isolated from the buffy coat cells of each of 4 non-vaccinated control animals from the 4th through 7th day (last day tested) after exposure.
In the second experiment, isolation of the challenge virus from the nasal secretions and feces of the vaccinated and control cattle was undertaken because this information would be of more practical value than demonstrating the virus in the buffy coat after challenge. Therefore, nasal swabs and fecal swabs, rather than buffy coat cells, were examined for virus after challenge. Cytopathogenic BVD virus was isolated from the nasal swab of vaccinated cow 28, 5 days after exposure. Virus was not isolated from the other nasal swabs or fecal swabs of this cow nor from nasal swabs or fecal swabs of any of the other cows.

The serum antibody titer in response to vaccination of pregnant cows with killed NADL BVD virus is shown in Table 1. The vaccinated cows, along with the non-vaccinated control cows were exposed to homologous live virus one month after vaccination. The antibody response 2 weeks after exposure, along with the clinical condition of the calf at birth is also recorded in Table 1. Exact breeding dates were not known for the cattle used in the first experiment, and the frequent observations at calving time that are necessary to get precolostral serum samples from each calf were not possible. However, such samples were taken from 3 calves. All calves were observed for 3 months, then transferred to other experiments. The clinical evaluation of the calves at time of transfer is recorded in the right hand column of the table.

All of the vaccinated cows appeared to develop adequate serum antibody levels to protect their calves from an in utero infection when the dams were exposed to live virus during pregnancy because all calves from the vaccinated cows were healthy at birth and remained in good health for the duration of this experiment. In contrast, only 1 of the 4 non-vaccinated cows delivered a healthy calf at parturition. Although the calf of non-vaccinated cow 17 scoured for 2 days after birth, BVD virus could not be isolated from the buffy coat, fecal material, or synovial fluid of the hock joint. The rectal temperature remained within normal range. The calves of non-vaccinated cows 6547 and 14144 had precolostral antibody titer in their serum, and BVD virus was isolated from synovial fluid of the hock joint of both calves. The calf of 6547 was weak at birth and could not stand, and required hand feeding of colostrum and help in standing to nurse for 2 days. Scours developed the 2nd day, and after a week the calf appeared unthrifty but began to gain weight.

The calf of 14144 had marked cerebellar hypoplasia and was incoordinated (Fig. 1). The calf required assistance in nursing for the first few days but was able to nurse without aid after the 5th day. However, the calf developed scours and became moribund and died at 10 days of age.

Non-vaccinated Cow 14146 aborted twins 1 month after exposure; however, BVD virus could not be isolated from the fetal tissues.
In the second experiment, all vaccinated cows developed antibodies to BVD virus after vaccination; however, vaccinated cows 24 and 35 failed to protect their calves from an in utero infection, as indicated by the precolostral serum titers of their calves. Precolostral serum antibody of the calves from control cows indicated an in utero infection in each calf. However, in contrast to the condition of 3 calves from the control cows of the first experiment all calves infected in utero in the second experiment were in excellent health.

All except 2 calves in the second experiment remained in excellent health and grew well through the 5 months from birth to the time of this writing. The calves from vaccinated cows 1785 and 2121 appeared to be all right when they were born, but they developed a mild intermittent diarrhea in the first week and remained unthrifty until 2 months of age when they began to grow and appear normal. The reason for this condition was not determined. Immunoelectrophoresis of the precolostral serum samples showed that there was no immune globulins, suggesting there had been no in utero infection with any agent. BVD virus was not isolated from the buffy coat, synovial fluid of the hock joint or fecal material; and judging from the BVD antibody titers of the colostrum that was available to them, the quality of the colostrum was probably as good as that available to the other calves on the experiment.

The antibody titers recorded in Table 2 for the serum and colostrum at time of parturition indicate considerable variation of titer between animals. There appears to be no definite correlation between serum antibody and colostral antibody. In general, titers of the vaccinated animals were lower at parturition than they were 2 weeks after challenge, whereas the titer of the non-vaccinated control cows rose during gestation (Table 2).

DISCUSSION

The results reported in these two experiments indicate that killed BVD virus vaccines can safely be used in pregnant cattle, and that they can produce adequate antibody to protect the fetus from an intrauterine infection to BVD virus.

There was a marked contrast in the condition of the calves that were infected in utero in the first experiment and the condition of those calves of the second experiment. Two factors could have had a bearing on this difference. First, the NADL strain of BVD virus used to expose the control cows in the first experiment might be more pathogenic for the developing fetus than the Singer strain of BVD virus. Second, the cattle of the first experiment were in the first half of gestation at the time they were exposed whereas the cattle in the second experiment were in the second half of gestation.

The work of Casero and Kendrick and Ward et al. indicate that the fetus is most susceptible to injury from BVD virus at 2 to 3
months, whereas the fetus is likely to respond to infection with antibody production at 5 months or older. Bognar recommends modified live virus vaccination of pregnant cows in the second half of gestation. Such calves born to vaccinated cows had an active immunity to BVD virus and had no problem with scours.

Two weeks after exposure, the controls that had been exposed to the Singer virus had higher serum titers than the control that had been exposed to the NADL virus. However, the serum antibody titers varied between each cow within each experiment and between the cows of the two experiments. These results may reflect the normal individual variation between animals that have been exposed to antigenic stimulus.

Fig. 1. Cerebellar hypoplasia. Calf of cow 14144.
TABLE 1. USE OF KILLED NADL-BVD VACCINE ON PREGNANT COWS. RESULTS OF NADL-BVD CHALLENGE-EXPOSURE OF PREGNANT VACCINATED AND NONVACCINATED COWS.

<table>
<thead>
<tr>
<th>Cow Number</th>
<th>Gestation When Challenged (Month)</th>
<th>Serotiter at Time of Challenge</th>
<th>Serotiter 2 Weeks Postchallenge</th>
<th>Condition of Calve at Birth</th>
<th>Precoital Serotiter of Calve</th>
<th>Postcoital Serotiter of Calve</th>
<th>Progress of Calve</th>
<th>Titer of Celostrum</th>
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</table>

All animals were seronegative to BVD at the beginning of the experiment.

*Number represents the reciprocal of the highest dilution of serum which would prevent CPE.

*Calves could not stand without help for 2 days. Began to scour 3rd day.

*Cerebellar hypoplasia. Calves required help to nurse for several days, then became weaker and died at 10 days of age.

*Aborted twins one month after exposure. BVD not isolated from fetal tissues.

N.D. = not done.

TABLE II. USE OF KILLED SINGER-BVD VACCINE ON PREGNANT COWS. RESULTS OF SINGER-BVD CHALLENGE-EXPOSURE OF PREGNANT VACCINATED AND NONVACCINATED COWS.

<table>
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<th>Cow Number</th>
<th>Gestation When Challenged (Month)</th>
<th>Serotiter at Time of Challenge</th>
<th>Serotiter 2 Weeks Postchallenge</th>
<th>Condition of Calve at Birth</th>
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</table>

*All animals were seronegative to BVD at the beginning of the experiment.

*Number represents the reciprocal of the highest dilution of serum which would prevent CPE.

*Unthriftv Calves began to gain after a month and grew normally, all other calves remained in good health.

*Serum taken 2 weeks after birth.

N.D. = not done.
REFERENCES


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

Chairman: G. D. Gurss, Topeka, Kansas
Co-Chairman: V. A. Seaton, Ames, Iowa


The Committee on Infectious Diseases of Cattle met on November 4, 1975, at 1:30 p.m. in Parlor E, Portland Hilton Hotel. Approximately 40 persons were in attendance and several speakers and members presented reports and papers related to infectious diseases of cattle.

Dr. Joe Bearden presented the following Sub-Committee on Artificial Insemination report.

Report of Subcommittee on A. I.
November 4, 1975

During the past year activities of this subcommittee have primarily involved communications with USDA and Industry people. As an example, two members met with Dr. Frank Mulhern, administrator of APHIS, and Dr. W. H. Dreher, chairman of NAAB Sire Health Committee in September.

The subcommittee suggests that during the coming year emphasis be directed toward informing Dairy and Beef Breed Registry Associations, their constituents and related organizations that compose the livestock industry of the need for a Federal regulation in the form of the current version under consideration and the benefits that will accrue from it, and to enlist their support. This support will be needed before the proposed regulation is republished in the Federal Registry. This report was prepared by the following subcommittee members:

Fred Hanson
Dave Bartlett
Harry Anthony
Bill Brown
Joe Bearden, Chairman

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The parent committee accepts and endorses the report of the subcommittee on Artificial Insemination.

Several members and guests made requests for reports and presentations on the following subjects.

Dr. Mahlon Vorhies, South Dakota Veterinary Diagnostic Laboratory, presented a review of the research in progress on baby calf enteritis. He indicated the procedures followed in field cases included bacterial isolation, viral isolation, fluorescent antibody procedures, electron-microscopy and sera typing. He concluded corona virus was of major significance with \textit{E. coli} next in importance.

Dr. Marlin Van der Martin, National Animal Disease Center, discussed research in bovine leukemia. He discussed the adult form of the disease and expressed the opinion that due to export requirements in the future, he felt it advantageous to think about standardization of necessary procedures and to initiate its use in breaking infection cycles within a herd as a control measure.

Dr. Arlan McClurkin, National Animal Disease Center, discussed a BVD infected male bovine as a case history. The animal did not carry antibodies, yet was positive for BVD virus isolation from buffy coat, nasal swabs, eye swabs and synovial fluid. The animal is now being used in breeding experiments.

Dr. George Lambert, National Animal Disease Center, discussed infectious keratitis research including carrier state, vaccine research and therapeutic research. He indicated a lack of correlation between circulating antibody to \textit{Moraxella bovis} and susceptibility to infection.

Dr. E. A. Butler and Dr. Fred Wertman from Iowa discussed the preconditioning program for calves in Iowa.

They indicated the program includes certain immunization procedures coupled with management practices that make pre-conditioned calves desirable for feeders to buy. These calves sell at a premium. The program was initiated by the Iowa Veterinary Medical Association.

Dr. Jim Dunn, Amarillo, Texas Diagnostic Laboratory discussed a new "rat-tail" syndrome in feeder cattle. It is characterized by diarrhea, loss of tail hair, lesions on tongue and almost complete anorexia.

The meeting adjourned at 3:30 p.m.

This Committee respectfully submits their report and asks for its adoption by the Executive Committee.
VARIABLE FACTORS INFLUENCING THE ISOLATION OF LEPTOSPIRES INVOLVING CULTURE INGREDIENTS AND TESTING

Herman C. Ellinghausen, Jr., Ph.D.*

Introduction

The ability of leptospiral mediums to grow out diluted cell inocula has been frequently overlooked. The commitment of a medium to isolation attempts without its vigorous pretesting is wasteful in time and effort of the investigator. In addition if unknowingly such medium of an inferior quality was used, the negativity of attempted isolation trials could be misleading to the epidemiologist.

Serological evidence might clearly indicate the probable presence of a serotype in an area, yet the percentage of isolations may be low. The difficulties of having an optimal specimen for culture are known and there is frequently a tendency to blame this aspect for our low recovery rate of isolates.

The medium used in this study was a bovine albumin polysorbate 80 (BA-P-80) medium which has been previously described (Ellinghausen, 1973). Since first reported, a great deal of evidence now points to the albumin supplement being the most critical ingredient in formulation procedures.

The choice of various strains for testing has been found an important one. Nutritionally fastidious strains such as serotype hardjo are extremely demanding of propagative mediums. The choice of such testing strains may be decided upon by the evidence that specific serotypes are prevalent in the geographical area served by the respective laboratory.

The objectives of this study were: (1) to document that albumin sources determine the ability of diluted cell inocula to grow in albumin polysorbate 80 medium, (2) to emphasize the value of viability testing of negative cultures which have not grown where limited inocula were employed, (3) to suggest the use of field isolates for pretesting of medium before use, (4) to demonstrate the extended shelf life of medium, (5) to evaluate the protective effect of bovine albumin upon serotype hardjo, (6) to measure the pH of various semisolid mediums stored at room temperature, (7) to develop data showing the role of agar in medium, (8) to emphasize the deleterious effect of filtration procedures upon medium and the ensuing reduction upon the efficiency of cultural growth, and (9) to develop nephelometric turbidity standards useful in standardizing leptospiral antigens, heat killed bacterins, and in various growth studies.


No endorsements are implied herein.
Materials and Methods

The liquid, semisolid, and solid mediums used in this study have been previously described\(^1\). Various albumin types (bovine, rabbit, and porcine) and production modified albumins were obtained for this study from Miles Laboratory of Kankakee, Illinois. A bacterin producer's medium (unknown albumin and sterilization procedures) was submitted to our laboratory for testing its ability to grow out diluted cell inocula.

Liquid medium was prepared, stored in 20 x 125-mm screw-capped tubes in 10-ml volumes at 25°C and tested for its ability to support the growth of 2 cells/ml of serotype *pomona* over a 36 month period.

Semisolid mediums of 3 types: bovine albumin polysorbate 80 medium with various commercial brands of agar; mediums where polysorbate 60, 40, and 20 were substituted for polysorbate 80, and semisolid mediums where polysorbate 80, ammonium chloride, thiamine, and vitamin \(\text{B}_1\) had been singly deleted from the formula; and minimal agar mediums containing only albumin were compounded and stored in screw capped tubes in 10-ml amounts at 25°C. The pH values of these mediums were determined after various periods of storage.

Solid 1% agar bovine albumin polysorbate 80 medium with different brands of agar were formulated. The ability to grow out diluted cell inocula was tested by delivering 0.2 ml volumes of decimal dilutions of culture to the dry surface of the agar plates. A non-fastidious leptospire *illini* (3055) and a pathogenic serotype *pomona* (DM, H) were employed as test organisms. Inoculum suspensions were standardized to a nephelometer reading of 25 and the cell count of both *illini* and *pomona* found to be approximately 200 x 10^6 cells/ml. Either a Coleman 7 nephelometer or Coleman 9 nephelocolorimeter were used and standardized with a titanium dioxide (Dow 276-V9) resin standard. Nephelometric turbidity standards were prepared using Kimble or Bellco 18 x 150 mm disposable test tubes. The tubes so used were screened for their percent transmittance values with a solution of 5% copper sulfate at 590 millimicrons. Titanium dioxide, Zopaque R-55, 0.25 to 0.30 microns from the Glidden Co., Baltimore, MD, was suspended in an alpha-methyl styrene polymer (DOW 276-V9) from the Dow Chemical Co. It was necessary that the resin be at 80°C and the titanium dioxide thoroughly mixed in the resin at this temperature. Dilutions of an appropriate stock solution were then made in clear resin. The standards so produced were assigned turbidity values when compared with Roessler & Brewer primary standards. The standards were then sealed by a conventional torch and subsequently stored at 25°C and periodically tested for their stability.
Bovine albumin polysorbate 80 liquid medium (100% of its volume as completed medium) was filtered in 2000 ml volumes with asbestos and membrane filters. The asbestos filters were of the serum sterilizing type, 0.025 microns in porosity, and 14 cm in diameter. Membrane filters were of 0.22 micron porosity and the same diameter.

In efforts to eliminate the water well cuvette holder an aluminum well was fabricated to fit either a Coleman 7 or Coleman 9 nephelometer and the interior of the well darkened with DoAll steel ink available from the DoAll Co. of Des Plaines, Illinois.

Results

**Liquid medium.** Data related to the ability of *pomona* to grow from diluted cell inocula in various lots of bovine, rabbit, porcine, and production modified albumins when used in polysorbate 80 medium appears in Table 1. When pretested before commitment to use in isolation medium, various lots of bovine albumin as a key medium ingredient can be shown to vary in efficiency and such a pretest procedure is to be encouraged. Additionally, it would be shown that special products SP-99 and 111 derived from a specific lot of plasma differed in their ability to support the growth of inocula in the range of $2 \times 10^3$ to 2 cells/ml. Rabbit albumin was superior to porcine albumin in similar titrations of diluted inoculum. Research modified albumin products (bovine RAM 1 and 10) varied greatly in their ability to support maximum growth.

The value of viability testing for leptospires in polysorbate 80 medium made with different albumin lots is shown in Table 2. After 21 days incubation at 29 C, 1 ml amounts of the various series of tube cultures were inoculated into semisolid isolation medium. The viability patterns give another insight into the quality of albumin. Careful pretesting of an albumin source would be important where albumin was used as a protective diluent (transport medium) in the dilution of blood, urine, kidney, liver, and brain homogenates. It can be seen that though the diluted cell inocula remained viable in EXP #1 BSA, vigorous replication did not take place while another albumin, EXP #10, rendered low cell numbers non-viable.

The difference in growth response between a lab adapted versus a field strain of *hardjo* is seen in Figure 1; this is one of the most demanding serotypes to grow in liquid medium in our current state of limited knowledge of this *hebdomadis* serogroup organism.

With properly pretested albumin, optimal in its nutrient value, a medium can be prepared well in advance of its use and thereby give assurance of its propagative ability even though it has been in storage for as long as 36 months (Table 3). The choice of serotype test strain poses a difficult one to make since there is no universally acceptable leptospiral strain to satisfy all contingencies. As can be
seen at the level of 2 to $2 \times 10^2$ cells/ml incubation periods of 28 days are needed to document the conversion of minimal turbidities to measurable values. In addition an added procedure is to subculture low turbidity cultures 5, 0, 0, 6, and 0 (Table 3) at 21 days on semisolid medium and establish their viability, thus correlating with the final turbidity at 28 days.

**Protective value of albumin.** The use of 1% bovine albumin diluent for the viability of diluted leptospiral cell numbers has been previously reported. In addition, diluted blood and kidney suspension have been found to yield viable leptospiral cultures after storage at 25°C for as long as 40 to 60 days. In an attempt to further study this preservative effect of albumin diluent in practical use; studies were made by inoculating a virulent bovine isolate of *hardjo* (New Zealand #12) into sterile bovine urine and bovine albumin diluent. The distinct superiority of albumin to protect the viability of *hardjo* against the toxic effect of bovine urine can be seen from the data of Table 4. Twenty-four hours after exposure, a minimal number of cells could be counted in the bovine urine while cell count was maximal in albumin diluent. The cells/ml was progressively diminished in urine while if anything there was a modest suggestion that cell replication had occurred in albumin. Motility had disappeared in sterile urine but motility was maintained in albumin. All suspensions of *hardjo* exposed to urine become nonviable yet viability persisted in albumin diluent.

**pH of semisolid mediums.** The pH of a wide range of semisolid bovine albumin mediums averaged pH $7.1 \pm 0.1$ when measured soon after preparation. In semisolid medium made from a variety of agar sources and stored as long as 4 years, pH was not significantly changed (Table 5). The same was true of mediums where polysorbate 60, 40, or 20 were substituted for polysorbate 80 or where single nutrient deletions of polysorbate 80, NH₄Cl, thiamine, or vitamin B₁₂ were made (Table 6.) Mediums made only with 0.2% agar in phosphate buffer were slightly more alkaline (pH 7.6) after storage for 538 days.

Solid agar mediums provided a contrast (Table 7 and 8) between growth of a non-fastidious leptospire *illini* and *pomona*. Serotype *illini* grows vigorously on solid agar mediums, whereas *pomona* was severely restricted in its growth as a function of the agar source used. Where evaluating agar as a critical medium component once a satisfactory albumin has been selected, both growth tests in semisolid and solid medium might be considered if the individual laboratory has these capabilities.

**Filter sterilizing effects.** Since 1965 when bovine albumin polysorbate 80 medium was first described, one method of sterilization has been adhered to. The complete medium as buffer, salts, trace metals, vitamins, and polysorbate has been autoclaved. To this has been added
bovine albumin which has been membrane filter sterilized. The results of various filtration procedures (Table 9) upon the growth of diluted cell inocula of *pomona* emphasizes the reduced ability of asbestos and membrane filtered mediums to support growth. Slight improvement was noted where a prefilter was not employed with a sterilizing membrane (Millipore). Only dilutions of $2 \times 10^4$ and $2 \times 10^6$ result in growth when GA type (starch treated, Gelman filters) were used. The same was true for the TCM (tissue culture Gelman membrane) with or without prefilter.

**Nephelometric turbidity standards.** When two types of $18 \times 150$ mm disposable tubes were used to prepare turbidity standards, they were found to have excellent light transmittance qualities: 61.0% T-Kimble and 64% T-Bellco when tested with a 5% CuSO₄ at 590 millimicrons (Table 10).

In a plot of turbidity units versus milligrams of titanium dioxide per gram of 276-V9 resin, the extracted curve from the Roessler and Brewer⁴ publication results in a straight line (Fig. 2). The same was true of the recently prepared NADC standards. Although further studies will be required, it was felt that the particle size of the more recently acquired titanium dioxide could account for the slope of the line along with the use of 276-V9 resin which was sparingly used in the Roessler studies.

Three Roessler primary turbidity standards were available to our laboratory during the course of these studies, a 77.5, 69, and 29 set of standards.

Since the Roessler 77.5 standard had been found, the most useful in a variety of studies since 1965 it was decided to assign values to the new standards made at NADC by adjusting the nephelometer to a dial reading of 77.5 and rating a series of new standards (Table 12): 115.5 to 41. After these values were determined, 3 nephelometers were used in further determinations. With these instruments adjusted with the NADC standards (115.5 to 41), the 77.5 standard was placed in the instruments and measurements made; column 2, Table 11. Excellent correlation was obtained.

The instruments were then adjusted with the 77.5, 69, and 29 Roessler primary standards. Triplicate determinations with NADC standards were made and again excellent correlations found.

When the 10 year old 29 Roessler standard was used to adjust the nephelometer, the 77.5 Roessler standard read 76.2 and the 64 standard read 64.

The growth curve of *pomona* performed using a new aluminum well, not encasing the culture growth tube in a water well, is shown in Figure 3. Such a device makes for greater safety in performing readings and obviously increased speed of determinations although the safety aspect is by far the more important.
Discussion

The fabrication of a leptospiral culture medium such as albumin polysorbate 80 involves ingredients which should be pretested before use in formulation. Using a combination of nephelometric turbidity, adjustment of inoculum and Petrof Hauser counting chamber techniques various leptospiral strains can be tested in albumin medium intended for use. Although data presented in this study identifies albumin lots that can be judged inferior; 95% of albumin lots tested since 1965 have been satisfactory and meet the most stringent of requirements to grow out diluted inoculum of 1 to 10 cells/ml. Viability testing of negative growth cultures in a decimal dilution series also can identify the properties of an albumin lot that protects the viability of leptospires.

Far more investigation than the studies by Kirschner and Maguire on such a hardy serotype as pomona will have to be conducted on the deleterious effect of urine upon viability of leptospires. Seitz asbestos filter sterilization of leptospiral medium has been shown to lyse leptospires and asbestos filters where used in the Kirschner-Maguire studies. In addition, our studies with hardjo were done in the absence of the competing bacterial flora of bovine urine. It could well be that as proximal convoluted tubule fluid becomes distal convoluted tubule fluid and thence urine that toxicity is on the increase. Various serotypes and virulent strains of these serotypes need to be studied in a wide variety of animal urine in order to better appraise the shedder state in leptospirosis. Aside from urine toxicity little is known about the role that competing bacteria or other agents might play in urine that harbors the leptospirae.

Diagnostic laboratories should employ a variety of test cultures both lab adapted and field isolate in nature to evaluate their propagative mediums both liquid and semisolid. Liquid mediums should at least be tested with inoculums of $2 \times 10^6$ to $20 \times 10^6$ cells/ml, preferably out to 2 cells/ml. Where antigens are involved serial subculture should never fail and growth should be of a predictable turbidity within a given incubation period. Semisolid medium should be tested with the most difficult to grow strains since success in this regard should suggest that the capabilities of isolating will be rewarding.

It can not be too strongly emphasized that laboratories committed to serious leptospiral diagnostic efforts should consider the acquisition of instrumentation practically suited for the measurement of leptospiral cell turbidity. No longer can the reason for not attempting cultural isolations be used because of the non-availability of medium. The shelf life of semisolid isolation medium has been found to be at least 36 months. With the prevention of evaporation it can be deduced that the medium has a shelf life at 25 C of five years. This storage advantage allows for thorough testing of medium sterility and per-
mits sufficient volume of medium to be formulated well in advance of its need and pretested for effectiveness.

Agar medium in the form of semisolid (0.2% agar) media has occupied a prominent position as a medium of choice for isolation attempts. Still at this juncture in our knowledge there is little if any evidence that can explain why semisolid medium is thought to be superior to liquid medium for isolation purposes. One major difficulty is that mechanically one cannot quantitate the amount of subsurface growth that occurs in a semisolid medium. Diluted cell inocula, a method of simplicity, offers to those laboratories making vigorous efforts to isolate leptospires a readily achievable technique to pretest their semisolid isolation medium. In recent years at NADC agar has been found to vary in its medium value from source to source and from lot to lot. Exactly what role this has played in the success of cultivating leptospires in the past can never be known. Now, slightly more alert to the significance of agar and its availability, it is hoped awareness of its role in medium will no longer be neglected. A secondary use of agar in leptosiral studies involves attempts to clone cultures in order to study individual colony populations in a multiplicity of studies involving particularly the stability of virulence and to a lesser extent immunogenicity and antigenicity.

The widespread use of filtration techniques related to the production of tissue culture mediums frequently prompts a laboratory to filter leptospiral culture medium as a method of sterilization. This of course is aimed primarily at liquid medium production. Yet there are reports that certain laboratories elect to aseptically add sterile agar to achieve formulation of their semisolid isolation medium. Ten years since the description of bovine albumin polysorbate 80 medium the method of choice still remains one of autoclaving the basal medium with or without agar and then aseptically adding membrane filtered albumin as a lipid detoxifier. Although the phenomenon cannot be explained, saprophytic leptospires are completely resistant to the deliterious effect of asbestos filtered medium in contrast to pathogenic leptospires. Although the ultimate characterization of the pathogenicity of these organisms is their demonstrated ability to infect and cause death in specific animals; of 17 leptospiral isolates submitted to NADC for characterization, all 17 have proven saprophytic as judged by their ability to grow diluted cell inocula in asbestos filtered medium and not infect animals.

The universal solvent—water should not be neglected in the fabrication of mediums once the establishment of the optimal quality of albumin and agar has been arrived at. Caution in the use of deionized water combined with sterile filtration of whole medium (basal + albumin) or filtration of albumin solution made with deionized water has resulted in two cases of free-living leptospiral saprophytes contaminating media used in attempts to isolate pathogenic serotypes.
The non-availability of Roessler turbidity standards found useful in our previous studies prompted the effort to fabricate standards within the last year. Since fabrication in January 1975, these standards have shown no change in their turbidity values when periodically checked against Roessler primary standards. In this instance no cavitation within the sealed tube has taken place in the resin titanium dioxide mixture and neither has the percent light transmittance characteristics changed. Such standards at present are in laboratories in New Zealand, Canada, France, and the United States. In the United States they have been found useful by commercial firms producing leptospiral bacterins and research laboratories doing a variety of studies where measurement of cell mass is most important. As a final aid to increased quality control of leptospiral medium production an investment in instrumentation for growth measurement, standardization of antigens, and measure of media quality can be clearly recommended to any laboratory that seriously wants to upgrade its potential to improve upon the number of isolations of leptospires and the reproducibility of their serological procedures.
TABLE 1  RESULTANT GROWTH OF L. POMONA WHEN USED TO ASSAY DIFFERENT LOTS, TYPES, AND PRODUCTION MODIFIED ALBUMINS USED IN POLYSORBATE 80 MEDIUM

<table>
<thead>
<tr>
<th>Albumin</th>
<th>$2 \times 10^6$</th>
<th>$2 \times 10^5$</th>
<th>$2 \times 10^4$</th>
<th>$2 \times 10^3$</th>
<th>$2 \times 10^2$</th>
<th>20</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine 231</td>
<td>75**</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>60</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Bovine 163</td>
<td>60</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bovine 251</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bovine 254</td>
<td>69</td>
<td>73</td>
<td>78</td>
<td>78</td>
<td>46</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Bovine SP-99</td>
<td>62</td>
<td>66</td>
<td>70</td>
<td>75</td>
<td>79</td>
<td>74</td>
<td>51</td>
</tr>
<tr>
<td>254 Plasma:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine Sp 111</td>
<td>65</td>
<td>73</td>
<td>77</td>
<td>79</td>
<td>84</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>254 Plasma:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 Rabbit</td>
<td>67</td>
<td>65</td>
<td>56</td>
<td>60</td>
<td>52</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>4 Porcine</td>
<td>67</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bovine Ram 1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bovine Ram 10</td>
<td>82</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Cells/ml inoculum.
**Turbidity reading, 21 days, 29 C, static growth, tube cultures, 19 x 150 mm.

TABLE 2  THE VALUE OF VIABILITY TESTING FOR L. POMONA IN POLYSORBATE 80 MEDIUM MADE WITH VARIOUS BOVINE ALBUMIN PREPARATIONS

<table>
<thead>
<tr>
<th>Albumin</th>
<th>$2 \times 10^6$</th>
<th>$2 \times 10^5$</th>
<th>$2 \times 10^4$</th>
<th>$2 \times 10^3$</th>
<th>$2 \times 10^2$</th>
<th>20</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine RAM 1</td>
<td>5**</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Bovine RAM 10</td>
<td>82</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>V</td>
<td>NV</td>
<td>NV</td>
<td>NV</td>
<td>NV</td>
<td>NV</td>
</tr>
<tr>
<td>Bacterin</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Company's</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>NV</td>
<td>NV</td>
</tr>
<tr>
<td>Control</td>
<td>78</td>
<td>78</td>
<td>83</td>
<td>81</td>
<td>78</td>
<td>58</td>
<td>43</td>
</tr>
</tbody>
</table>

*Cells/ml inoculum.
**Turbidity reading, 21 days, 29 C, static growth, tube cultures, 19 x 150 mm.
V = Viable; NV = Nonviable.
### TABLE 3  ABILITY OF 36 MONTH STORED (25°C) BOVINE ALBUMIN POLYSORBATE 80 LIQUID MEDIUM TO SUPPORT MINIMAL INOCULA OF LEPTOSPIRA INTERROGANS SEROTYPE POMONA

<table>
<thead>
<tr>
<th>Medium Age</th>
<th>Days Incuba.</th>
<th>Nephelometer Reading*</th>
<th>Cells/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>49 58 60 51 51 30 5 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>49 49 52 59 58 52 54 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 mths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>49 53 55 58 56 40 28 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>44 42 43 46 56 54 54 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 mths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>47 45 46 60 40 13 6 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>41 39 36 52 40 41 41 15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Turbidity Readings at 21 and 28 days respectively, static culture, 19 x 150 mm tube, steel closure.
Underlined values represent increase in growth turbidity with additional 7 days incubation.

### TABLE 4  THE VIABILITY OF LEPTOSPIRA INTERROGANS SEROTYPE HARDJO (N.Z. #12) IN STERILE BOVINE URINE AND 1% BOVINE ALBUMIN DILUENT

<table>
<thead>
<tr>
<th>Day</th>
<th>Cells Counted</th>
<th>Cells/ml</th>
<th>% Reduction</th>
<th>% Motility</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urine Suspension:</td>
</tr>
<tr>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>NV</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>88</td>
<td>2.2 x 10^7</td>
<td>64</td>
<td>0</td>
<td>NV</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>5.5 x 10^6</td>
<td>91</td>
<td>0</td>
<td>NV</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2.5 x 10^5</td>
<td>99.6</td>
<td>0</td>
<td>NV</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2.5 x 10^5</td>
<td>99.6</td>
<td>0</td>
<td>NV</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>NV</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bovine Albumin:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

*Motile cells counted Petroff Hauser.
Inoculum = 6.08 x 10^7 cells/ml of urine or albumin.
### Table 5
**pH Values of Semisolid* BSA P-80 Medium Formulated From Various Agar Sources After Storage (25 C)**

<table>
<thead>
<tr>
<th>Agar</th>
<th>Lot Number</th>
<th>Days Stored</th>
<th>Medium pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBL</td>
<td>011630</td>
<td>45</td>
<td>7.30</td>
</tr>
<tr>
<td>BBL</td>
<td>011630</td>
<td>90</td>
<td>7.12</td>
</tr>
<tr>
<td>BBL</td>
<td>011630</td>
<td>1068</td>
<td>7.02</td>
</tr>
<tr>
<td>BBL</td>
<td>011630</td>
<td>1424</td>
<td>7.02</td>
</tr>
<tr>
<td>DIFCO</td>
<td>504509 Purif.</td>
<td>70</td>
<td>7.08</td>
</tr>
<tr>
<td>DIFCO</td>
<td>609894 Bacto.</td>
<td>70</td>
<td>7.10</td>
</tr>
<tr>
<td>DIFCO</td>
<td>504380 Techni.</td>
<td>70</td>
<td>7.08</td>
</tr>
<tr>
<td>DIFCO</td>
<td>609714 Flake</td>
<td>10</td>
<td>7.00</td>
</tr>
<tr>
<td>DIFCO</td>
<td>609714 Flake</td>
<td>0.5</td>
<td>7.12</td>
</tr>
<tr>
<td>NBC</td>
<td>4332</td>
<td>70</td>
<td>7.20</td>
</tr>
<tr>
<td>INOLEX</td>
<td>2607</td>
<td>70</td>
<td>7.10</td>
</tr>
<tr>
<td>AGAROSE</td>
<td>(0.1%)</td>
<td>10</td>
<td>7.10</td>
</tr>
</tbody>
</table>

*0.2% agar.

### Table 6
**pH Values of Differential and Simplified Semisolid* BSA Mediums**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Medium pH</th>
<th>Days Stored**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysorbate 80</td>
<td>7.02</td>
<td>1068</td>
</tr>
<tr>
<td>Polysorbate 60</td>
<td>7.02</td>
<td>1068</td>
</tr>
<tr>
<td>Polysorbate 40</td>
<td>7.05</td>
<td>1068</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>7.00</td>
<td>1068</td>
</tr>
<tr>
<td>P-80 Deleted</td>
<td>7.02</td>
<td>1068</td>
</tr>
<tr>
<td>NH₄Cl Deleted</td>
<td>7.10</td>
<td>1068</td>
</tr>
<tr>
<td>Thiamine Deleted</td>
<td>7.10</td>
<td>1068</td>
</tr>
<tr>
<td>Vit. B₁₂ Deleted</td>
<td>7.10</td>
<td>1068</td>
</tr>
<tr>
<td>Agar</td>
<td>7.62</td>
<td>536</td>
</tr>
<tr>
<td>Agar Alb.</td>
<td>7.40</td>
<td>536</td>
</tr>
<tr>
<td>Agar Alb. B₁₂</td>
<td>7.28</td>
<td>536</td>
</tr>
<tr>
<td>Agar Alb. B₁</td>
<td>7.10</td>
<td>536</td>
</tr>
<tr>
<td>Agar Alb. 2 vitamins</td>
<td>7.30</td>
<td>536</td>
</tr>
</tbody>
</table>

*0.2% BBL #011630.
**Stored at 25 C.
## ISOLATION OF LEPTOSPIRES

### TABLE 7 COMPARISON OF VARIOUS SOLID AGAR MEDIUMS USING LEPTOSPIRA ILLINI (3055)

<table>
<thead>
<tr>
<th>Agar</th>
<th>Cells/Plate</th>
<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 x 10⁶</td>
<td>4 x 10⁶</td>
<td>4 x 10⁵</td>
<td>4 x 10⁴</td>
<td>4 x 10³</td>
<td>4 x 10²</td>
<td>40</td>
</tr>
<tr>
<td>BBL Purified</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>13*</td>
</tr>
<tr>
<td>412601</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco Noble</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>VB</td>
<td>16</td>
</tr>
<tr>
<td>612277</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco Noble</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>TNTC</td>
<td>11</td>
</tr>
<tr>
<td>504461</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BBL J6DC HR</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>VB</td>
<td>TNTC</td>
<td>9</td>
</tr>
<tr>
<td>BBL 307609</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>VB</td>
<td>TNTC</td>
<td>11</td>
</tr>
<tr>
<td>Meer MK-60</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>7</td>
</tr>
</tbody>
</table>

V = Confluent heavy veil of subsurface growth.
* = Numbers of distinct colonies.
VB = Broken veil.
TNTC = Distinct colonies but too numerous to count.
- = Negative growth.

### TABLE 8 COMPARISON OF VARIOUS SOLID AGAR MEDIUMS USING LEPTOSPIRA POMONA (DM₂H)

<table>
<thead>
<tr>
<th>Agar</th>
<th>Cells/Plate</th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>40 x 10⁶</td>
<td>4 x 10⁶</td>
<td>4 x 10⁵</td>
<td>4 x 10⁴</td>
<td>4 x 10³</td>
<td>4 x 10²</td>
<td>40</td>
</tr>
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<td>BBL Purified</td>
<td>V</td>
<td>VB</td>
<td>TNTC</td>
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<td>71*</td>
<td>-</td>
<td>-</td>
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<td>412601</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco Noble</td>
<td>V</td>
<td>VB</td>
<td>TNTC</td>
<td>52</td>
<td>9</td>
<td>1</td>
<td>-</td>
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<td>612277</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco Noble</td>
<td>V</td>
<td>TNTC</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>504461</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BBL J6DC HR</td>
<td>TNTC</td>
<td>58</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BBL 307609</td>
<td>TNTC</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meer MK-60</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

V = Confluent heavy veil of subsurface growth.
VB = Broken veil.
TNTC = Distinct colonies but too numerous to count.
* = Numbers of distinct colonies.
- = Negative growth, + = Very light veil.
### TABLE 9  REDUCED EFFICIENCY OF BOVINE ALBUMIN POLYSORBATE 80 MEDIUM WHEN STERILIZED BY VARIOUS FILTRATION PROCEDURES TO SUPPORT GROWTH OF LEPTOSPIRA INTERROGANS SEROTYPE POMONA

<table>
<thead>
<tr>
<th>Medium</th>
<th>Nephelometer Reading Cells/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$2 \times 10^6$</td>
</tr>
<tr>
<td>Control:</td>
<td></td>
</tr>
<tr>
<td>Autoclav.</td>
<td>69</td>
</tr>
<tr>
<td>Basal and memb. filtered BSA.</td>
<td></td>
</tr>
<tr>
<td>Asbestos:</td>
<td>66</td>
</tr>
<tr>
<td>filtered.</td>
<td></td>
</tr>
<tr>
<td>Membrane* sterilizing filtered.</td>
<td>43</td>
</tr>
<tr>
<td>Membrane* sterilizing with prefilter.</td>
<td>43</td>
</tr>
<tr>
<td>GA type**, without or with prefilter.</td>
<td>61</td>
</tr>
<tr>
<td>TCM type; with or without prefilter.</td>
<td>61</td>
</tr>
</tbody>
</table>

0 = nonviable.
* = Millipore.
** = Gelman

### TABLE 10  NEPHELOMETRIC TURBIDITY STANDARDS (NADC JAN. 1975) USED IN MEASURING LIGHT SCATTERING OF LEPTOSPIRAL GROWTH, ANTIGENS, AND HEAT KILLED BACTERINS; WITH COLEMAN 7 OR 9 NEPHELOMETERS

<table>
<thead>
<tr>
<th>Material</th>
<th>Use</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 18 x 150 mm; Kimble, Disposable</td>
<td>Standard Cuvette</td>
<td>61.0% T ± 0.5% CuSO$_4$ $\times 5$ $\times 100$</td>
</tr>
<tr>
<td>Tube 18 x 150 mm; Bellco, Disposable</td>
<td>Standard Cuvette</td>
<td>64% T ± 1</td>
</tr>
<tr>
<td>Titanium Dioxide Zofiaque R-55</td>
<td>Whitening Agent</td>
<td>0.25 to 0.30 Microns</td>
</tr>
<tr>
<td>Glidden Co.</td>
<td>Experimental Zopaque LDC</td>
<td>0.18</td>
</tr>
<tr>
<td>Resin DOW 276-V9</td>
<td>Suspending Agent for Titanium</td>
<td>Liquid at 80 C</td>
</tr>
<tr>
<td>Alpha-methyl Styrene polymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roessler Calibration Standard #77.5 1967</td>
<td>Primary MU and ZZ Reference</td>
<td>400 47.5</td>
</tr>
<tr>
<td></td>
<td>Appl. Micro. 1967</td>
<td>500 49.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600 55.3</td>
</tr>
</tbody>
</table>
TABLE 11  NADC NEPHELOMETRIC 276-V9 RESIN TITANIUM DIOXIDE STANDARDS CALIBRATED AGAINST W. G. ROESSLER STANDARDS OF 77.5, 69, and 29 (1967, Appl. Microbiol.)

<table>
<thead>
<tr>
<th>NADL Standards Used to Adjust Instrument</th>
<th>Subsequent Reading of Roessler 77.5 Std</th>
<th>Reading of NADL Stds against Various Roessler Stds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R#77.5 R#69 R#29</td>
</tr>
<tr>
<td>115.5</td>
<td>76.0</td>
<td>26.0 26.2 26.0</td>
</tr>
<tr>
<td>108.0</td>
<td>75.6</td>
<td>26.0 26.2 25.6</td>
</tr>
<tr>
<td>97.0</td>
<td>76.0</td>
<td>28.0 28.2 27.6</td>
</tr>
<tr>
<td>96.0</td>
<td>78.2</td>
<td>31.5 31.5 30.8</td>
</tr>
<tr>
<td></td>
<td>75.2</td>
<td>32.5 32.5 32.0</td>
</tr>
<tr>
<td>52.5</td>
<td>75.5</td>
<td>35.5 35.8 35.4</td>
</tr>
<tr>
<td>52.0</td>
<td>78.0</td>
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<td>77.5</td>
<td>42.0 41.5 41.2</td>
</tr>
<tr>
<td>51.5</td>
<td>78.0</td>
<td>49.0 48.5 48.5</td>
</tr>
<tr>
<td>51.5</td>
<td>77.5</td>
<td>51.5 49.5 49.5</td>
</tr>
<tr>
<td>51.5</td>
<td>76.0</td>
<td>82.0 80.0 79.6</td>
</tr>
<tr>
<td>51.5</td>
<td>75.5</td>
<td>93.0 93.8 94.2</td>
</tr>
<tr>
<td>41.0</td>
<td>77.5</td>
<td>96.5 93.8 94.2</td>
</tr>
<tr>
<td>41.0</td>
<td>77.5</td>
<td></td>
</tr>
</tbody>
</table>

Instrument Set With Reading of Roessler Standards

<table>
<thead>
<tr>
<th>Roessler #29</th>
<th>#77.5</th>
<th>#64</th>
<th>#29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reads</td>
<td>76.2</td>
<td>Reads 64</td>
<td>...</td>
</tr>
<tr>
<td>Roessler #64</td>
<td></td>
<td>Reads 77.5</td>
<td>...</td>
</tr>
</tbody>
</table>

Fig. 1 Effect of Laboratory Adaption upon the Rapid Growth Response of *Leptospira interrogans* serotype *hardjo* (DOWN-EY.)
Fig. 2 Relationship between Turbidity Units and Milligrams of Titanium Dioxide per Gram of 276-V9 Resin.

Fig. 3 Comparison between Growth Curves of Leptospira interrogans serotype pomona (DM,H); Where the Nephelometer is Equipped with Dry Type Cuvette Well.
REFERENCES


REPORT ON THE COMMITTEE ON LEPTOSPIROSIS 1975

Chairman: H. G. Stoenner, Hamilton, Montana
Co-Chairman: J. W. Glosser, Helena, Montana

H. Stewart Powell, Nashville, Tenn.; S. L. Diesch, St. Paul, Minn.;
G. B. Smith, Kansas City, Mo.; O. H. Stalheim, Ames, Iowa;
H. C. Ellinghausen, Ames, Iowa; Charles S. Roberts, Auburn,
Ala.; J. R. Ragan, Nashville, Tenn.; Lewis P. Thomas, Charleston,
W. Va.; L. E. Hanson, Urbana, Ill.; W. E. Lyle, Madison,
Wisc.; L. A. Rosner, Jefferson City, Mo.; R. L. Larson, Fruitdale,
S.D.; Larry Schaffer, O'Neil, Nebr.; R. L. Morter, Lafayette,
Ind.; R. E. Smith, Amherst, Mass.; H. R. Smith, Cincinnati,
Ohio; Bruce Walker, Jacksonville, Tex.

The committee has completed the revision of the Information Re-
lease on Leptospirosis, which will be distributed by the U. S. Depart-
ment of Agriculture. It has been submitted to the Department for
final review and publication.

Reports were received on problems concerning prevalence in live-
stock, occurrence of outbreaks of disease, and diagnosis and control
of leptospirosis.

The committee solicited results of serologic tests from 48 state
diagnostic laboratories and received valuable reports from 34. Sincere
appreciation is expressed to these laboratories for their cooperation.
A more detailed report will appear in the proceedings, but a summary
is appropriate for this report. Analysis of these data indicate that
leptospirosis is still widespread in the United States. The North-
eastern states had the lowest seropositive rate among cattle and
swine, but not necessarily so among horses. Highest rates with the
pomona serotype were reported from the Western states; the com-
bined infection rate with hardjo and pomona serotypes among cattle
was higher in Southeastern and Western states than in the Midwest.
As based on results of all tests performed, seropositive rates for
pomona and hardjo among cattle were 4.8 percent and 6.8 percent,
respectively. However, laboratories using the microscopic agglutina-
tion test reported respective rates of 5.4 percent and 10.7 percent
among cattle. In all areas of the United States, the principle sero-
type in swine was pomona. Antibodies to grippotyphosa were found
in cattle, swine, and horses, but rates were much lower. Some un-
usually high seropositive rates with canicola and/or icterohemorrh-
agiae were reported in horses and swine, chiefly from states in the
Midwest. A brief report was received from Dr. R. E. Smith, Uni-
versity of Massachusetts, on the prevalence of leptospirosis in horses
in the Northeastern United States.

Several participants reported that infertility is a prominent
feature of some outbreaks of hardjo leptospirosis in beef cattle. In
four large herds in Montana, vibriosis was originally suspected as
LEPTOSPIROSIS

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the cause, but leptospirosis was subsequently diagnosed as the basis of the problem. In some of these herds, over 50 percent became sero-positive to hardjo and as high as 42 percent failed to conceive during the usual breeding season.

Several problems relating to the serologic diagnosis of leptospirosis were discussed. The subdued humoral response of many cattle to hardjo infection has been consistently observed, and results of our serologic survey suggest that some antibodies to this serotype are not detected at the 1:40 dilution with plate antigen. Therefore, the committee recommends that a reaction in the 1:10 serum dilution be considered as significantly positive with hardjo plate antigen. No change in the minimum positive titer of other serotype plate antigens is suggested.

The committee re-emphasizes the importance of adequate sampling for confirming a clinical diagnosis of leptospirosis. Serums should be submitted from at least ten animals, of if greater, from ten percent of the herd and the sample should be representative of normal and affected animals. The practice of submitting acute- and convalescent-phase serums, as required for the diagnosis of viral diseases, is not necessarily helpful and delays antibiotic treatment that can be used to avert further losses from abortion in some outbreaks.

The committee considered some problems relating to the use of bacterins for controlling leptospirosis. In view of the report received that hardjo bacterin does not cross protect against infection with mini-szwajizak, the committee is concerned about the true prevalence and distribution of this serotype in the United States. This organism was recovered from diseased cattle in Oregon several years ago and infections with this serotype cannot be distinguished serologically from those caused by hardjo. Some commented that owners of beef cattle were reluctant to use the more expensive polyvalent bacterins for controlling outbreaks known to be caused by a single serotype, such as hardjo. The committee weighed advantages and disadvantages of both types and recommends that both be made available to the livestock industry.

The committee discussed those aspects dealing with the control of leptospirosis in a U. S. Department of Agriculture proposal to update a notice of rule making published in the Federal Register, September 30, 1975 (36 FR 19169 - 19170), which was a proposal to control the interstate shipment of bovine semen. Some members of the industry are concerned about the problem and have requested means of certifying that their bulls are free of leptospirosis. The committee supported the proposal in principle, but emphasized that further research is necessary to define the basis for a valid certification.

The committee continues to emphasize the public health aspects of leptospirosis in livestock. Although hardjo is the most prevalent
serotype in cattle in the United States, few human cases have been recognized. In contrast, human infection with *hardjo* exceeds that of *pomona* among dairy farmers in New Zealand (N. Z. Med. J. 1974, 79: 904-906).

Among research problems relating to the control of leptospirosis the following are of primary concern to the committee:

1. developing serologic means of differentiating *hardjo* from *mini-szwajizak* infections in cattle,

2. determining the prevalence and distribution of the *mini-szwajizak* serotype in the United States,

3. further investigations on leptospirosis in horses to identify organisms responsible for the high incidence of antibodies to *canicola* and *icterohemorrhagiae* in some areas of the country and,

4. more extensive epidemiologic investigations of human cases to establish the magnitude of the public health problem.

Finally, the committee is concerned about continued maintenance of national competence for typing isolates of leptospiras. Since the termination of the typing program supported by the Walter Reed center, only one major typing facility remains in the United States. Immediate measures should be taken at some government level to assure continued support of a typing center available to research workers in leptospirosis of livestock and to diagnostic laboratories.
SUMMARY REPORT ON THE 1975 SURVEY FOR LEPTOSPIROSIS IN THE UNITED STATES

Herbert G. Stoenner

The response to the committee's request for results of serologic tests for leptospirosis performed in diagnostic laboratories was excellent. Of 48 state laboratories contacted, 34 provided useful data for this report.

To reflect the general magnitude of the problem in various sections of the country, I have assembled the data according to the following state groups—northeast, southeast, midwest, and west. Because of the geographic separation of Hawaii from the mainland, their results were not tabulated with the west group. They reported 4% of 101 cattle sera positive for *pomona* and 2.6% of 38 dog sera positive for *icterohemorrhagiae*. Differences in the distribution and prevalences of the 5 serotypes among these state groups should be viewed only as trends or suggestive evidence, because of the variations in sampling, criteria for positive titers, and testing procedures. The wide range in seropositive rates among livestock in state groups suggests that these and other variables, such as practitioner awareness of the disease, influenced survey results. Data from laboratories using the microscopic agglutination (MA) test have been compiled separately from those using plate tests, because the temporal relationship between disease and the presence of antibodies detectable by these tests differs. Those reactive in the MA test persist much longer after infection. I had asked respondents to indicate the number of sera that reacted with more than one antigen so that the true percentage of positive sera could be calculated. Because so few provided this information, the total serotype positive rate is not included in the tabular summaries.

Twenty-one laboratories used the plate test exclusively, and one had used the plate test and MA test during the past year. Three laboratories did not consider titers below 1:160 significant. So that data from these could be compared with results that included 1:40 positive titers, the number of positives recorded by these three was doubled. In routine diagnostic serology, the number of sera that react at 1:40 dilution is roughly equal to the number that react at 1:160 and higher.

Twelve laboratories used the MA test exclusively. In 8, titers of 1:100 or greater were considered positive. One laboratory considered reactions positive at 1:50 dilution, one at 1:200 dilution, one at 1:1,000 and one did not indicate the criterion.

Several general conclusions can be drawn from the results of this survey. Obviously, leptospirosis is still widespread among the livestock population in this country. A comparison of results obtained by plate tests conducted throughout the United States sug-
gests that the northeastern states have the lowest infection rates among cattle and swine, but not necessarily so among horses. Highest infection rates with the *pomona* serotype among cattle were reported from the western states. A comparison of results obtained by MA tests suggests that leptospirosis among cattle due to both *pomona* and *hardjo* serotypes is more prevalent in the southeastern and western states than in the midwest.

Among 13 state laboratories using the MA test, 11 reported higher infection rates with *hardjo* than with *pomona* among cattle. Of 56,203 serums tested in these 13 laboratories, 5.4% had antibodies for the *pomona* serotype and 10.7% for the *hardjo* serotype. In contrast, all 22 laboratories using the plate test reported higher infection rates with the *pomona* serotype than with *hardjo* serotype. Of 77,664 serums tested against *pomona* antigen, 4.7% were positive; of 50,709 serums tested against *hardjo* antigen, 2.4% were positive. The relative prevalence of *hardjo* and *pomona* serotypes among cattle probably varies in different states, but this observation suggests that the MA test may be more effective than the plate test in detecting antibodies to the *hardjo* serotype. Compared with the humoral response to most leptospiral serotypes, that to the *hardjo* serotype is subdued. Many infected animals do not develop maximum titers exceeding 1:100, and many of these would not react at the 1:40 dilution on the plate test.

Antibodies to *grippotyphosa* serotype were detected in cattle, swine, and horses in all sections of the country, but this serotype is not as prevalent as *pomona* and *hardjo*. *Grippotyphosa* appears to be prevalent in horses in Missouri, where 15 of 79 (18.7%) equine serums tested by plate or MA tests were positive.

The survey results suggest that further inquiries should be made on the role of *canicola* and *icterohemorrhagiae* in leptospirosis of livestock. It is known that animals infected with *pomona* produce cross-reacting antibodies to these serotypes. However, in Montana, Texas, Tennessee, Pennsylvania, Vermont, Ohio, Illinois, Indiana, and Missouri unusually high infection rates with *icterohemorrhagiae* were reported in horses. In Iowa, Missouri, and Illinois, the infection rates with *canicola* or *icterohemorrhagiae* exceeded that of *pomona* in swine. Some effort should be made to isolate and classify leptospiries from these infected herds.

Because of the limited number of serums of sheep, goats, and dogs tested, tabular summaries were not prepared. Laboratories using the MA test reported that 11.6% and 10.8% of 749 dogs were seropositive for *canicola* and *icterohemorrhagiae*, respectively. Those that used plate tests reported respective infection rates of 8.5% and 5.8% among 241 dogs examined. Only 208 sheep serums were tested in all reporting laboratories; 3% of these were positive for *pomona* and 3% were positive for *icterohemorrhagiae*. Of 342 goat serums
tested, 6.1% were positive for *icterohemorrhagiae* and 1.4% for *pomona*.

The following comments are made in the interest of promoting more uniformity in testing and reporting procedures. As stated before, most of the 23 laboratories using the MA test consider a titer of 1:100 as the lowest considered positive. Most infected animals develop higher titers, but unquestionably, some animals, especially those infected with *hardjo*, may not develop sufficient antibody to reach a titer of 1:100. Nevertheless, the criterion appears reasonable because most infections are identified and few nonspecific reactions should occur at this dilution. Likewise, most laboratories using Ft. Dodge plate antigen consider a titer of 1:40 on the plate test as the lowest considered positive. A comparison of results obtained by plate and MA tests suggests that this is a valid criterion, except in the use of the *hardjo* antigen. With this antigen, a titer of 1:10 (at least 2 plus reaction) should be considered positive. This antigen is highly specific, and I have rarely encountered serums positive at this dilution that did not contain agglutinins detectable also by MA test. Equally important is the interpretation of serologic findings. The presence of antibodies merely indicates past experience with leptospires and it cannot be correlated with shedding of organisms in the urine. Because of the variable humoral response of animals and the long persistence of antibodies in some animals, the diagnosis of the disease should be based on tests of an adequate representative sample of a herd, and not on results obtained from a few affected animals. The adoption of these criteria by all diagnostic laboratories would help reduce the amount of confusion now surrounding leptospiral serology.
### Serologic Tests for Leptospirosis in Northeastern U.S.A.

Results of plate tests performed in:
Maine, Vermont, Massachusetts, Pennsylvania, Connecticut, and New York
July 1, 1974 - June 30, 1975

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of sera tested</th>
<th>pomona</th>
<th>hardjo</th>
<th>grippotyphosa</th>
<th>canicola</th>
<th>icterohaemorrhagiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>34,577</td>
<td>708</td>
<td>23 (10,797)</td>
<td>64 (10,797)</td>
<td>16 (4,392)</td>
<td>22 (4,392)</td>
</tr>
<tr>
<td></td>
<td>1.0 - 2.0 **- 6.5</td>
<td>0 - 0.6 - 1.7</td>
<td>0 - 0.4 - 1.0</td>
<td>0 - 0 - 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>4,505</td>
<td>92</td>
<td>(234)</td>
<td>(234)</td>
<td>(234)</td>
<td>(234)</td>
</tr>
<tr>
<td></td>
<td>0 - 2.0 - 6.2</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Horses</td>
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<td>33</td>
<td>(121)</td>
<td>6 (121)</td>
<td>5 (121)</td>
<td>16 (121)</td>
</tr>
<tr>
<td></td>
<td>4.0 - 17.0 - 28.0</td>
<td>0</td>
<td>0 - 4.9 - 37.3</td>
<td>0 - 4.1 - 25.0</td>
<td>0 - 13.2 - 25.0</td>
<td></td>
</tr>
</tbody>
</table>

* = Number of sera tested with this serotype antigen.
** = Average percent positive. Minimum and maximum percent in small type.

### Serologic Tests for Leptospirosis in Southeastern U.S.A.

Results of plate tests performed in:
West Virginia, Tennessee, North Carolina, Mississippi, and Florida
July 1, 1974 - June 30, 1975

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of sera tested</th>
<th>pomona</th>
<th>hardjo</th>
<th>grippotyphosa</th>
<th>canicola</th>
<th>icterohaemorrhagiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>15,065</td>
<td>977</td>
<td>624</td>
<td>146</td>
<td>199</td>
<td>16 (1.1 - 3.9)</td>
</tr>
<tr>
<td></td>
<td>0.6 - 6.4 **- 17.5</td>
<td>0.1 - 4.1 - 12.4</td>
<td>0.1 - 0 - 9 - 3.2</td>
<td>0 - 1.3 - 8.9</td>
<td>0 - 1.1 - 3.9</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>252</td>
<td>29</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0 - 11.5 - 14.3</td>
<td>0 - 11.1 - 4.8</td>
<td>0 - 11.1 - 4.8</td>
<td>0 - 11.1 - 4.8</td>
<td>0 - 11.1 - 4.8</td>
<td></td>
</tr>
<tr>
<td>Horses</td>
<td>197</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0 - 2.0 - 6.3</td>
<td>0 - 0.5 - 3.1</td>
<td>0</td>
<td>0 - 1.0 - 5.3</td>
<td>0 - 5 - 31.3</td>
<td></td>
</tr>
</tbody>
</table>

Results of MA Tests performed in Georgia and Alabama

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of sera tested</th>
<th>pomona</th>
<th>hardjo</th>
<th>grippotyphosa</th>
<th>canicola</th>
<th>icterohaemorrhagiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>11,775</td>
<td>1,183</td>
<td>2,700</td>
<td>134</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6.3 - 10.0 - 11.6</td>
<td>22.0 - 22.9 - 25.2</td>
<td>1.0 - 11 - 1.5</td>
<td>0 - 0.2 - 0.4</td>
<td>0 - 0.1 - 0.1</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>965</td>
<td>32</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2.0 - 3.0 - 3.8</td>
<td>0.1 - 0.5 - 2.0</td>
<td>0.5 - 0.8 - 2.0</td>
<td>0 - 0.5 - 0.7</td>
<td>0 - 0.2 - 0.3</td>
<td></td>
</tr>
<tr>
<td>Horses</td>
<td>94</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4.5 - 5.3 - 7.0</td>
<td>0 - 10 - 6.3 - 13.6</td>
<td>0 - 2.1 - 3.0</td>
<td>0</td>
<td>0 - 2.1 - 3.0</td>
<td></td>
</tr>
</tbody>
</table>

* = Average percent positive. Minimum and maximum percent in small type.
## SURVEY FOR LEPTOSPIROSIS

**Serologic Tests for Leptospirosis in Midwestern U.S.A.**
Results of plate tests performed in:
Missouri, Iowa, and Michigan
July 1, 1974 - June 30, 1975

<table>
<thead>
<tr>
<th>Species</th>
<th>Number serums tested</th>
<th>Number and percent positive against serotype antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pomona</td>
<td>hardlo</td>
</tr>
<tr>
<td>Cattle</td>
<td>17,344</td>
<td>322</td>
</tr>
<tr>
<td>Swine</td>
<td>2,532</td>
<td>2</td>
</tr>
<tr>
<td>Horses</td>
<td>120</td>
<td>&gt;0-1</td>
</tr>
</tbody>
</table>

Results of MA tests performed in:
Iowa, Illinois, Minnesota, N. Dakota, S Dakota, Indiana, Wisconsin, Missouri, and Ohio

<table>
<thead>
<tr>
<th>Species</th>
<th>Number serums tested</th>
<th>Number and percent positive against serotype antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pomona</td>
<td>hardlo</td>
</tr>
<tr>
<td>Cattle</td>
<td>41,865</td>
<td>2,647</td>
</tr>
<tr>
<td>Swine</td>
<td>10,567</td>
<td>97</td>
</tr>
<tr>
<td>Horses</td>
<td>1,024</td>
<td>20</td>
</tr>
</tbody>
</table>

* = Average percent positive. Minimum and maximum percent in small type.

---

## Serologic Tests for Leptospirosis in Western U.S.A.
Results of plate tests performed in:
Washington, Oregon, Utah, Arizona, Nevada, and Texas
July 1, 1974 - June 30, 1975

<table>
<thead>
<tr>
<th>Species</th>
<th>Number serums tested</th>
<th>Number and percent positive against serotype antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pomona</td>
<td>hardlo</td>
</tr>
<tr>
<td>Cattle</td>
<td>10,678</td>
<td>356</td>
</tr>
<tr>
<td>Swine</td>
<td>178</td>
<td>2</td>
</tr>
<tr>
<td>Horses</td>
<td>104</td>
<td>0</td>
</tr>
</tbody>
</table>

Results of MA tests performed in Montana

<table>
<thead>
<tr>
<th>Species</th>
<th>Number serums tested</th>
<th>Number and percent positive against serotype antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pomona</td>
<td>hardlo</td>
</tr>
<tr>
<td>Cattle</td>
<td>2,563</td>
<td>263</td>
</tr>
<tr>
<td>Swine</td>
<td>69</td>
<td>14</td>
</tr>
<tr>
<td>Horses</td>
<td>148</td>
<td>12</td>
</tr>
</tbody>
</table>

* = Number serums tested with this serotype antigen.
** = Average percent positive. Minimum and maximum percent in small type.
† = Only reporting state laboratory in western U.S.A. that used the MA test.
CHARACTERIZATION OF AND BOVINE INTRAMAMMARY INFECTION BY GROUP B \textit{STREPTOCOCCUS AGALACTIAE} OF HUMAN ORIGIN

J. S. McDonald, T. J. McDonald, and A. J. Anderson

\textit{Summary}

Twenty-five strains of \textit{Streptococcus agalactiae} (SA) of human origin were injected into mammary glands of 30 dairy cows. Of 98 glands inoculated, 78.6 percent became infected. The disease was more acute in dairy cows than that produced by bovine SA strains and most infections were eliminated by the cow without the use of antibiotics.

Based on our studies, we have concluded that SA of human origin are more pathogenic than SA of bovine origin and are different biotypes. Therefore, there is no basis to suspect that bovine strains are involved in human disease.

\textit{Introduction}

In laboratories concerned with diagnosis of human disease, about 0.5\% of the bacterial recoveries are Lancefield Group B SA\textsuperscript{6}. One to 2\% of human neonates contract encephalitis or meningitis, and postpartum females develop septicemia due to SA. A number of other human diseases that can be caused by SA include abortion, abscesses, endocarditis, genitourinary infections, mastitis, meningitis, osteomyelitis, otitis, pneumonia, septicemia, sinusitis, and tonsillitis.\textsuperscript{6}

The reported reservoirs of infection in humans by SA\textsuperscript{6} are the female genitourinary tract, the upper respiratory tract, and wound infections.\textsuperscript{4} In cattle, the only reservoir is the infected mammary gland. We have not proved that man can acquire SA infection by consumption of contaminated dairy products or products that are improperly pasteurized. Some researchers\textsuperscript{4} have reported isolation of heat-resistant SA from commercial milk supplies.

The purpose of this study was to determine infectivity and pathogenicity of SA of human origin when injected into the bovine mammary gland. We were unable to find reports of these types of studies in the literature. In addition, we determined biochemical and antibiotic differences between SA of human and bovine origin.

We also carried out immunological studies where we compared human and bovine SA strains. If human SA strains originate from the bovine, specifically from infected mammary glands, SA is a public health problem and eradication procedures should be initiated.

\textsuperscript{150} From the National Animal Disease Center, North Central Region, Agricultural Research Service, US Department of Agriculture, Ames, Iowa 50010.

No endorsements are implied herein.
Materials and Methods.

Cultures—A group of 25 SA of human origin were available for study. These cultures had been isolated from Wisconsin\textsuperscript{a} and Texas\textsuperscript{b}. In addition, a group of 13 SA of bovine origin were selected for comparison with SA of human origin.

Cultures were received on blood agar slants. All cultures were purified and frozen until needed.

Biochemical tests—The following tests were carried out\textsuperscript{7} on all SA cultures: hemolysis of bovine erythrocytes, CAMP, esculin hydrolysis, hippurate hydrolysis, ammonia production from arginine, growth in 0.1\% methylene blue milk, growth in 6.5\% salt broth, growth at 45 C, and acid production in mannitol, salicin, sorbitol, lactose, inulin, trehalose, xylose, raffinose, arabinose, maltose, fructose, sucrose, and rhamnose.

Antimicrobial sensitivity tests—Standardized agar diffusion antimicrobial sensitivity tests were carried out. Sensitivity to the following antimicrobial agents was determined: ampicillin, bacitracin, carbenicillin, cephalothin, chloramphenicol, cloxacillin, erythromycin, gentamicin, lincomycin, methicillin, nafcillin, neomycin, nitrofurazone, oxacillin, penicillin, streptomycin, tetracycline, novobiocin, colistin, kanamycin, oleandomycin, vancomycin, nalidixic acid and selected sulfas.

Other tests—Four lactose-negative SA of human origin were selected to determine whether lactose utilization could be induced. Each culture was transferred daily for 7 weeks into sterile phenol red broth\textsuperscript{c} (PRB) containing 0.1\% lactose and 0.5\% glucose. Once a week, each culture was transferred into PRB containing 1.0\% lactose. All SA were tested with group B antisera;\textsuperscript{d} a double diffusion agar gel method was used.

In vivo studies—Thirty cows were available for this study. Secretions from mammary glands of all cows were culturally negative before injection of SA. SA was injected into 98 quarters.

Cultures for injection were grown for 6 hours at 37 C in brain heart-infusion broth\textsuperscript{e} (BHIB). The cultures were diluted in 0.1\% protease peptone \#3\textsuperscript{e} broth so that 1.0 ml contained from 56 to 1,020 colony-forming units (CFU). All quarters were injected via the teat canal with 1.0 ml of diluted culture immediately after the afternoon milking.

Milk samples were collected daily for 3 days before SA was injected. After SA was injected milk samples were collected just be-

\textsuperscript{a}Mr. W. A. Baker, St. Mary's Hospital, 720 South Brooks St., Madison, WI 53715.

\textsuperscript{b}Dr. T. W. Huber. University of Texas Medical School, 7703 Floyd Curl Drive, San Antonio, TX 78229.

\textsuperscript{c}Difco Laboratories, Detroit, MI.

\textsuperscript{d}The Biological Reagents Section, Communicable Disease Center, Atlanta, GA.

\textsuperscript{e}Baltimore Biological Labs., Baltimore, MD.
fore the morning and evening milking for 5 days. After 5 days, milk samples were collected once a day until sampling was stopped.

Vaginal swabs were taken from all 30 cows before SA was introduced into the mammary glands.

Results

Results of biochemical tests on human and bovine SA strains are shown in Table 1. All SA of bovine origin utilized lactose, but only 1 strain of 25 human SA strains utilized lactose. We recovered 1 lactose-negative SA from a bovine vagina which was identical to the human SA strains (Table 1). The vaginal SA carrier rate of the cows in our herd was quite low, 3.3 percent.

The antibiogram of 25 SA of human origin is shown in Table 2. Bovine SA strains have a similar antibiogram but in addition are sensitive to bacitracin and nitrofurazone.

Four lactose-negative SA strains of human origin were tested each week for development of adaptive enzymes for utilization of lactose. Over the 7 weeks, none of the 4 strains were able to utilize lactose. In addition, all recoveries from persisting infections were tested weekly for utilization of lactose. No isolate was able to utilize lactose.

All 25 human SA strains were Lancefield Group B test positive. All strains showed precipitin lines of identity with bovine SA strains.

Intramammary infection of the bovine mammary gland with SA of human origin was easy to establish. The optimum number of CFU for production of infection was between 100 and 500; 93.1% of injected quarters became infected (Table 3). When either a lower or a higher number of CFU were used, the percentage of new infection decreased. Infection was quite acute in the mammary gland after injection of SA strains of human origin. The glands were swollen, red, and painful. Abnormal secretion with clots and flakes, along with decreased milk production, resulted.

The length of human SA infection of the bovine mammary gland varied (Table 4). Infection in 66 quarters by lactose-negative human SA strains varied from 1 to 61 days and averaged 5.9 days in duration. None of these infections required treatment because they were eliminated by the cow. Infection in 3 quarters persisted for 90 days and was eliminated by treatment. Within 2 days after establishment of infection, several of the cows showed various degrees of lameness and stiffness. All cows recovered within 2 weeks. When a lactose-positive human SA strain was injected into 8 quarters of 2 cows, all quarters remained infected for 90 days until the infection was eradicated by treatment.
Discussion

There are only a few differences between human and bovine SA strains. Human SA strains are nearly always lactose-negative and we were unable to convert 4 cultures to utilize lactose. One bovine vaginal strain was identical to the human strains. Human SA strains are resistant to both bacitracin and nitrofurazone whereas bovine strains are sensitive to these two antibiotics. We cannot explain these differences in antibiotic sensitivity.

Although there are differences in biochemical tests and in antibiotic sensitivity between human and bovine isolates, serologically, all are Lancefield group B test positive. This finding is not unexpected because there are different biotypes in other Lancefield serological groups.

When 100 to 500 CFU of a SA of human origin were injected into a bovine mammary gland, most glands became infected. Infection was more acute and severe than infection by bovine SA strains. Nearly all human SA infections in the bovine mammary gland were eliminated by the cow whereas bovine SA infections nearly always have to be treated with antibiotics before they can be eliminated.

If these human SA isolates originate from the bovine and are carried in contaminated dairy products, one would expect to see more throat problems; e.g., tonsillitis and pharyngitis. Possibly, consumption of contaminated dairy products could result in tonsillitis or pharyngitis. Even though these 2 diseases do occur in humans and are related to SA, most human disease caused by SA is related to the genitourinary tract and subsequent contamination of the neonate during delivery.

From our studies and those of others, we have concluded that SA of human origin are more pathogenic than SA of bovine origin and are different biotypes. Also, in some human and bovine vaginal canals, there are lactose-negative SA strains. The carrier rate may be lower in the bovine than in the human population. However, bovine strains of SA are not likely to be involved in human disease.

Infection of the bovine mammary gland is a disease that affects the dairyman economically. The main effect of this infection is the decrease in milk production by the infected gland. Infection by SA is an eradicable disease, and the organism is sensitive to penicillin. SA should be eradicated from the national dairy herd.
### TABLE 1. Results of Biochemical Tests on Bovine and Human Strains of *Streptococcus agalactiae*

<table>
<thead>
<tr>
<th>Source of Strain</th>
<th>Hemolysis</th>
<th>CAMP</th>
<th>Esculin Hydrolysis</th>
<th>NH$_3$ Production</th>
<th>Salicin</th>
<th>Lactose</th>
<th>Growth in 6.5% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine$^A$ (13)$^B$</td>
<td>$-$</td>
<td>+</td>
<td>Neg.</td>
<td>+</td>
<td>+$^C$</td>
<td>+</td>
<td>+$^D$</td>
</tr>
<tr>
<td>Bovine$^E$ (1)</td>
<td>$-$</td>
<td>+</td>
<td>Neg.</td>
<td>+</td>
<td>+</td>
<td>Neg.</td>
<td>+</td>
</tr>
<tr>
<td>Human (25)</td>
<td>$-$</td>
<td>+</td>
<td>Neg.</td>
<td>+$^G$</td>
<td>+$^G$</td>
<td>Neg.</td>
<td>+</td>
</tr>
</tbody>
</table>

$^A$ Intranasal
$^B$ Number of strains
$^C$ were negative
$^D$ Vaginal
$^E$ $\beta$-hemolytic, 1 was non-hemolytic
$^F$ was negative
$^G$ gave trace reactions

### TABLE 2. Antibiogram of 25 Strains of *Streptococcus agalactiae* from Human Sources

<table>
<thead>
<tr>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>Ampicillin</td>
<td>Bacitracin</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>Novobiocin</td>
<td>Colistin</td>
</tr>
<tr>
<td>Cephalothin</td>
<td></td>
<td>Gentamicin</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td></td>
<td>Kanamycin</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td>Nalidixic acid</td>
</tr>
<tr>
<td>Lincomycin</td>
<td></td>
<td>Neomycin</td>
</tr>
<tr>
<td>Methicillin</td>
<td></td>
<td>Nitrofurazone</td>
</tr>
<tr>
<td>Nafcillin</td>
<td></td>
<td>Polymyxin B</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td></td>
<td>Streptomycin</td>
</tr>
<tr>
<td>Oxacillin</td>
<td></td>
<td>Sulfachlorpyridazine</td>
</tr>
<tr>
<td>Penicillin G</td>
<td></td>
<td>Sulfamethoxypyridazine</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td>Triple sulfas</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3. Effect of Number of Colony-Forming Units of Streptococcus agalactiae of Human Origin Injected on Establishment of New Bovine Intramammary Infection

<table>
<thead>
<tr>
<th>CFU/quarter</th>
<th>Quarters Injected Number</th>
<th>Infections Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>56-99</td>
<td>12</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>100-500</td>
<td>72</td>
<td>67</td>
<td>93.1</td>
</tr>
<tr>
<td>&gt; 500</td>
<td>14</td>
<td>9</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98</td>
<td>77</td>
</tr>
</tbody>
</table>

TABLE 4. Length and Outcome of Bovine Intramammary Infection with Streptococcus agalactiae of Human Origin

<table>
<thead>
<tr>
<th>Cows</th>
<th>Quarters Number</th>
<th>Infections Number</th>
<th>Infected %</th>
<th>Lactose</th>
<th>Length of Infection No. of Days</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8</td>
<td>8</td>
<td>100.0</td>
<td>+</td>
<td>90</td>
<td>Treated</td>
</tr>
<tr>
<td>28</td>
<td>87</td>
<td>66</td>
<td>75.9</td>
<td>Neg.</td>
<td>1-61*</td>
<td>Eliminated by cow</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>100.0</td>
<td>Neg.</td>
<td>90</td>
<td>Treated</td>
</tr>
</tbody>
</table>

*Average = 5.9 days
REFERENCES


REPORT OF THE MASTITIS COMMITTEE

Chairman: L. F. Williams
Co-Chairman: R. B. Bushnell


The annual open meeting of the committee convened at 1:30 PM on November 5, 1975. Twenty-six guests and seven members were present.

There were several reports presented at the meeting.

Dr. Kirkbride reported that New Zealand, an exporter of large amounts of dairy products, is presently initiating a milk quality control program. The level of somatic cells considered acceptable is 750,000/ml. This figure is an average of 3 monthly tests. The electronic method of counting cells will be the reference system while the Ruakura rolling ball viscometer will be used for routine testing.

Preliminary data indicate that 85% of herds produce bulk milk somatic cell counts of less than 500,000 cell/ml. These producers will be given incentive payments for quality milk.

Proposed is a 5 point mastitis control program including:
1. Correction of inadequate milking machine function and management practices.
2. Post milking teat dipping.
3. Dry cow therapy.
4. Treatment of clinical cases and
5. Culling of cows with chronic mastitis.

This program will be carried out through cooperative efforts of dairymen, practicing veterinarians and the Dairy Division and Animal Health Division of the Ministry of Agriculture and Fisheries.

Dr. R. F. Weidner reported on the proper use of drugs under the Food, Drug and Cosmetic Act. Some points discussed were:

When properly used according to label direction, drugs in current use do not endanger public health. However, FDA is always on the alert for misuse.

FDA’s basic regulatory objectives are simply stated: to assure that marketed products for which FDA is responsible are safe for use and perform as they are represented.

FDA engages in 4 general types of activities.
1. Setting standards for product composition, manufacture, performance and labeling;
2. Evaluation prior to marketing of safety and effectiveness of those products that must have premarket clearance;
3. Conducting inspections, surveys, and analyses to monitor
compliance with statutory requirements, administratively set standards and conditions of approval; and

4. Initiating enforcement action where necessary to effect compliance with the laws.

In addition to new animal drugs requiring premarketing safety and effectiveness, there are other categories of drugs that may be on the market that have no premarket approval. These include products that have been approved for use on food contact surfaces as food additives and are now used on animals such as teat dips.

The Food, Drug and Cosmetic Act exempts veterinarians from registering with the FDA as a drug manufacturer for drugs the veterinarian compounds and dispenses in the course of his professional practice. However, the veterinarian is responsible for the purity, safety, efficacy, identity, dosage and residue of the drug he compounds.

Dr. Bedell reported on the Georgia Mastitis control program. The milking machine serviceman, the veterinarian and the dairymen are the key personnel in the program. About 1,500 grade A dairies are on the program. 500,000 quarter milk samples were cultured during the past year. 99 percent of bulk milk samples were below 1.5 million cells/ml. Average somatic cell counts in bulk milk samples are approximately 800,000 cells/ml.

Dr. McDonald reported on studies with 25 human strains of Lancefield Group B Streptococcus agalactiae. 78.6 percent of inoculated quarters became infected, with spontaneous elimination occurring in most quarters. Based upon these studies, it was concluded that human strains were of a different biotype. There is no basis to suspect that bovine strains are involved in human disease.

Dr. Bushnell reported that studies are in progress on the effect of filtration of bulk milk on the somatic cell count and the bacterial content.

There was an extended discussion on finite as contrasted to zero tolerance for drugs in meat, milk, and eggs. The committee unanimously recommended that finite rather than zero tolerance for drugs be established. This recommendation is prompted by the ever increasing sensitivity of newer methods for detecting of drug residues. An example is the recent finding of penicillin residues in non-fat dried milk. Finite tolerances should be based on adequate toxicological data and not, as is currently being done, on the increasing sensitivity of new methods.

This constitutes the report of the Committee on Mastitis and we respectfully submit this report for approval by the Executive Committee.
STATUS REPORT ON ELECTRONIC IDENTIFICATION*

D. M. Holm, R. E. Bobbett, A. R. Koelle, J. A. Landt, W. M. Sanders, S. W. Depp, and J. C. Hensley

ABSTRACT

A significant milestone was passed in September 1975, with the successful operation of a subdermally-implanted, temperature-indicating transponder having hybrid circuitry. This transponder had no batteries and showed that an implant transponder could be powered by an external microwave beam and transmit encoded temperature information back to the receiver. The encoding of temperature was done because it was very similar to identification, but required much less circuitry. The microwave power levels at the animal skin were well within established safety limits. Additional work needs to be performed on optimizing circuitry and antenna design.

BACKGROUND

The "proof-of-principle" of Electronic Identification and Temperature Monitoring was demonstrated with a passive, external transponder at the October 1973 meeting of USAHA. Since that time, the work has been directed at developing a more efficient coding method, investigating the problems associated with subdermal implanting, and determining the needs of the various branches of the livestock industry.

The original instrument encoded three-decimal digits of identification and three-decimal digits of temperature. The code was read two times in succession and the two readings were compared. If the readings were identical, the numbers were indicated on the digital readout lights. The total time for a readout was a few tenths of a second, and the identity requirement of the two readings made it quite error-free. Since the final system is to use 15-decimal digits of identification, the old coding scheme would have taken more than one second to read the complete code. This was considered too long, so a more efficient method was designed which was also even less error prone.

There was little doubt that an external "ear-tag" type of transponder could be built, but we considered it less desirable than an im-

*This work was supported by the United States Department of Agriculture and the Energy Research and Development Administration under ERDA/USDA interagency agreement. The work was performed at the Los Alamos Scientific Laboratory. Presented at the U. S. Animal Health Association's Annual Meeting, Portland, Oregon, November 3-7, 1975.
planted transponder. Before undertaking the development of a sub-
dermal version of the transponder, it was decided to make sure that the livestock industry favored its development. A meeting was held in Oklahoma City in December 1974, with key representatives of various branches of the industry. A strong preference for a sub-
dermally implanted transponder was expressed by those attending the meeting, so top priority was given to proving the principle of a subdermal implant. While a combined temperature indication and identification transponder is desired for the final configuration, it was agreed that a temperature-only indicating transponder would prove the principle of operation and would be much simpler and less expensive to implement.

PRESENT STATUS

The first subdermally-implanted, temperature-only reading trans-
pounder was successfully tested in September 1975. The interrogating antenna requires about 20 watts of microwave power to energize the transponder to a range of two meters. This power is well within ac-
cepted health standards.

A picture of the transponder is shown in Figure 1. The major electronics portion of the circuit is in hybrid form inside an hermeti-
cally sealed container about the area of a small postage stamp, but about 3 mm (1/8th inch) thick. The antenna is a halfwave dipole in the shape of a “bow tie.” Four electronic components connect be-
tween it and the hybrid circuit. The transponder is coated with “circuit coate” (URLANE 8267) to provide a moisture barrier and then is encapsulated in silicone elastomer (Dow Corning 382) for biological compatibility and improving antenna performance. This is the same type of material used in plastic surgery prosthetic de-
vices. Essentially no developmental work has been done at the Los Alamos Scientific Laboratory (LASL) on encapsulation because of the limited budget, and the existence of commercially available ma-
terials. The problems associated with antenna design have been very serious and are discussed in considerable detail in the Appendix of Reference 2. They can be summarized as follows: (1) A large (greater than 30 cm long) antenna is desirable for supplying power to operate the transponder circuit and to give a large encoded reflection signal to the receiver. (2) A small (2 cm long) antenna is desirable for making a small implant.

A compromise was reached for the “proof-of-principle” experi-
ment by choosing a frequency (and corresponding antenna of 10 cm) which was best from a bio-electronic point of view. However, this frequency has been set aside by the FCC for a TV channel. Thus, another frequency is desired, but it will require additional develop-
ment to work out the problems associated with a different frequency.

A picture of the steer being interrogated is shown in Figure 2,
and the interrogator/receiver is shown in Figure 3. It should be emphasized that the interrogator/receiver shown is not intended for extended field application. It was assembled with commercially available components for this experiment.

There is considerable development that needs to be done to translate this "proof-of-principle" to a viable electronic identification and temperature-monitoring system for livestock. We believe that there is a high probability that it can be realized. While the present transponder is larger than desired, it is expected that the volume can be reduced four-fold without seriously affecting its response. The present hybrid-circuit electronics (exclusive of antenna) occupies approximately the same total volume as a completely integrated circuit would occupy which has 15-decimal digits of identification and 3 digits of temperature. Recent developments in integrated circuit passivation by a commercial manufacturer of integrated chips, give reason to believe that the present design of hermetic seals will not be necessary.

The absence of a computer-compatible, animal-identification system has seriously hindered the application of automatic data processing methods to livestock management and disease control. The large volume of data, and the need for greater efficiency in production, will require the large-scale application of computers and increased testing of animals for disease. LASL scientists have also been working on these aspects of the problem of increasing livestock production efficiency. Experience has been gained in the application of computers to the Brucellosis Indemnity Program in Texas. A description of the work is contained in Reference 2. From the experience which is being gained, it will be possible to make meaningful extrapolations from which the requirements of a functional system can be deduced. The work so far has demonstrated the need for computers to handle the large amount of data, to identify problem areas, and to evaluate the program.

The LASL developed serological test has considerable potential for automated, low-cost, high-speed, multiple disease screening of animals. The recently completed experiments on hog cholera demonstrated high accuracy and sensitivity of the Enzyme-Labeled Antibody test (ELA). We believe that: 1) disease detection will be improved by application of the ELA test to multiple diseases of animals, 2) electronic identification will make traceback through commerce much more effective, and 3) computer data processing of records will increase the effectiveness of paying claims in the disease eradication programs. If the livestock industries integrate these separate activities into their operations, there will be much improved disease surveillance and eradication.
FUTURE PLANS

The new encoding system for identification has been designed, and preliminary experiments indicate it will be very close to an optimum system for encoding 15-decimal digits of identification and will have acceptable error rates and speed. A three-decimal digit model will be constructed and tested subdermally. Since this model will be in hybrid circuit form, rather than an integrated chip, it will occupy a volume larger than the final design, but will provide the necessary hardware for field testing on a limited scale. These units will also have temperature indicating capabilities. Experiments are scheduled to be performed at the Veterinary Services Laboratory, Ames, Iowa, on evaluating the usefulness of subdermal temperature measurements. Some experiments of this nature are being carried out at LASL with the temperature indicating transponders and with a battery-powered, transmitter-type of temperature monitor. These experiments have not progressed far enough to establish the usefulness of the subdermal temperature measurements, but do show significant temperature variations over a period of time in a healthy animal. Figure 4 shows the ear-canal temperature obtained with a continuously transmitting temperature probe. Data are currently being collected from two locations, on a single animal, with the transmitter and transponder units.

While the experiments with the temperature-monitoring transponders are being performed, work will continue on the antenna and circuit development. We expect to produce a design which will satisfy both the communications and livestock industries. Field testing of a hybrid circuit of both identification and temperature must then be undertaken along with the many other problems which have not been addressed. The various parts of the total system must be developed and fitted together to form a viable management tool for both disease control and efficient livestock production. The technical experiments on the electronics must be done in parallel with the biological encapsulation development, and the individual animal management computer code programming. The possible benefits of individual electronic identification and temperature monitoring of animals are enormous if this system is enthusiastically applied in the livestock industry.
Fig. 1. An encapsulated transponder is at the top. Below is a view of the transponder with the hybrid electronics at the left and the bridge rectifier in the center. The bow tie antenna is on the back side of the electronics.

Fig. 2. A steer with a transponder implanted to the rear of the shoulder. The antenna for interrogating the transponder temperature is pointing at the shoulder.
Fig. 3. The interrogator/receiver electronics for activating and reading the transponder.

Fig. 4. Ten-day temperature measurements taken during the winter with a thermocouple probe in the ear canal.
REFERENCES


3. A silent moving picture of the temperature-only reading system working in a field experiment was shown with the oral presentation of this paper.

4. Dr. George C. Saunders gave an oral report to USAHA Committee on, "Transmissible Diseases of Swine" at this meeting.

REPORT OF THE COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: S. H. Flora


The Committee was very pleased with the interest and attendance at its session.

Mr. Dennis Joyce of the FBI reported on how the National Crime Information Center could assist in the problems of livestock theft. We thank the FBI for its participation and interest. A letter to this effect will be sent to the Director of the FBI by the Committee.

Dr. Fred Hanson, USDA, APHIS, VS, reported on the effectiveness and problems of MCI activities, the current swine identification program and the related change of federal regulations pertaining to swine identification. The Committee voted to recommend that the Association support the proposed changes. We commend Veterinary Services for its work in improving the MCI program, Meat and Poultry Inspection Programs for their intensified efforts, urge that the intensified effort be continued and that the states strictly enforce their supporting programs.

Mr. Paul Zillman of Livestock Conservation Incorporated, provided copies of the report emanating from their workshop on bovine identification. This report will be of great value to the Committee in its future work.

Dr. Keith Farrell reported on the increasing implementation of the freeze branding equine identification system, and his work on laser branding. We urge Dr. Farrell to continue this important work and commend him on his progress.

Dr. Calvin Campbell of USDA, APHIS, VS, described the brand card developed by the New Mexico Livestock Board and its Executive Director, Mr. Lee Garner, in cooperation with USDA, APHIS, VS. Uses and potential were presented to the audience. The Committee urges each state and those who serve the marketing needs of the livestock industry to carefully study this system and consider its use. This work is very commendable and the Committee voted to recognize it as such.

Dr. Dale Holm of the Los Alamos Scientific Laboratory, and Dr. J. Coleman Hensley, USDA, APHIS, of Los Alamos, reported on the
progress made in developing an electronic livestock identification device. A device was implanted in a steer in September 1975. This prototype is functioning successfully. The final type will meet most of the criteria established by the Committee for a total livestock identification system. It is adaptable to computerization; would trace an animal moving in commerce, strayed, etc., to herd of origin; cannot be altered; easily implanted; low enough in cost to make its use feasible; will operate for a time that will exceed the life span of an animal; and, in addition, give accurate temperature readings. To complete the work in progress, and prove the device in the field so that it can be turned over to the electronics industry for production, an additional eighteen months and two hundred twenty thousand dollars is needed. The Committee drafted a proposed resolution to accommodate this need and submitted it to the appropriate committee for its action. Dr. Hensley reported briefly on progress in the systems analysis for implementation of computer programs in electronic identification. The Texas Animal Health Commission is commended for its cooperation in this work.

This Committee recommends to the Association that time be set aside at the next annual meeting to hold a workshop for the membership to show how a total, automated, computerized, electronic livestock identification and disease detection system could work and the potential it has for other uses. The Committee suggests this so that the livestock industry can then make decisions as to what action it wants to take in implementing all or a part of the system when it is perfected and available for use.
IMMUNOLOGIC RELATIONSHIP BETWEEN RINDERPEST AND PESTE DES PETITS RUMINANTS VIRUSES

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Plum Island Animal Disease Center, Greenport, Long Island,
New York 11944

SUMMARY

Rinderpest and peste des petits ruminants viruses were (shown to be) immunologically related as demonstrated by complement fixation reaction and cross-protection tests. The two viruses, however, were differentiated by the virus neutralization tests. By ferritin tagging and electron microscopy, localization of peste des petits ruminants viral antigens in virus-infected cells treated with homologous antibody was demonstrated around the virions, virus particles at the budding stages, and in some regions of the plasma membrane of infected cells. Little, if any, tagging was seen with specimens treated with rinderpest antiserum. The possible origin of the common antigens between rinderpest and peste des petits ruminants viruses was discussed.

INTRODUCTION

Peste des Petits Ruminants (PPR) is a contagious disease of sheep and goats, clinically and pathologically similar to rinderpest (RP) in cattle. Gilbert and Monnier reported that RP and PPR viruses are antigenically identical on the basis of cross-protection tests. It was proposed that PPR virus was a strain of RP virus that had lost its pathogenicity to cattle through natural adaptation to goats. The relatedness of RP, PPR, measles and canine distemper viruses was demonstrated by the complement fixation reaction (DeBoer, Dardiri and Hamdy, 75th Annual Meeting of American Society for Microbiology, N.Y., E 115 p. 80 Abs.) This report describes the immunologic relationship between RP and PPR viruses as investigated by cross protection, cross-complement fixation, cross-virus neutralization tests and immuno-electron microscopy.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.
MATERIALS AND METHODS

Vero Cell Culture:
The Vero cell line was obtained from the American Type Culture Collection. The medium for growth and maintenance of Vero cells was Earle's balanced salt solution containing 0.05% lactalbumin hydrolysate, 0.005% yeast extract, 0.0015% phenol red, 5% bovine serum, and 100 units of penicillin and 100 µg of streptomycin per ml. The final pH was 7.3.

Viruses:
PPR virus was originally obtained from the Laboratoire National De l Elevage et des Recherches Veterinaires, Dakar-Hann (Sénégal), as a lyophilized lung extract. Buffy coat cells were collected during the peak of temperature elevation following virus injection into susceptible goats. The virus was subsequently grown in bovine embryonic spleen and Vero cell cultures.

The attenuated and virulent forms of the Kabete “O” strain of RP virus were obtained from the East African Veterinary Research Organization Laboratory, Muguga, Kenya, as a lyophilized Vero cell culture harvest. The virulent virus was passaged once in Vero cell culture.

Lapinized avianized (LA) strain of RP virus was received from the National Institute of Biological Science, Tachikawa, Tokyo, Japan. The passage history of the virus is as follows: 736 passages in rabbit, 77 passages in chick embryo, 41 passages in rabbit, 607 passages in chick embryo, 1 passage in rabbit, and 1 passage in chick embryo fibroblast followed by three successive plaque purifications in chick embryo fibroblasts.

First Experiment:
One of two groups of six goats was injected intramuscularly twice at a three week interval. The inoculum was 2 ml of inactivated spleen suspension (56°C for 30 min.) from goats infected with PPR virus. The second group was inoculated with Kabete “O” attenuated strain of RP virus. Each group received 2 doses intramuscularly of 1 ml virus suspension containing 1x10^4.8 TCID_{50} at the same schedule as with PPR virus. Goats were bled at 21 days post second inoculation (DPSI) and their sera were assayed for antibody response against RP and PPR viruses by complement fixation (CF) and virus neutralization (VN) tests. The presence of neutralizing antibody against RP virus was tested in Vero cell culture and chicken embryo systems as explained below. At 21 DPSI each group of goats was divided into two subgroups of 3 goats (Plus two untreated controls) and challenged with the homologous or heterologous virulent virus. Animals were kept under observation for development of any clinical signs or lesions.
Second Experiment:

Four goats were inoculated intramuscularly with PPR virus grown in Vero cell culture. Each goat received 1 ml of virus infected cell culture harvest containing $1 \times 10^5$ TCID$_{50}$. Two uninoculated goats were kept in contact with the inoculated ones. Animals were bled at 21 days post inoculation (DPI) and the VN and CF serum antibody responses (against PPR and RP viruses) were measured. Each of the survivors was subsequently challenged with virulent RP virus 35 DPI by intramuscular inoculation of 1 ml cell culture harvest of RP virus at a titer of $1 \times 10^{4.5}$ TCID$_{50}$. Two normal goats were similarly inoculated with the RP virus.

Third Experiment:

Four cross-bred yearling sheep were inoculated with PPR virus by the same method as in the second experiment, and two sheep were housed with the inoculates and served as uninoculated contact controls. The animals were bled 21 DPI and the sera were tested for CF and VN antibodies against PPR and RP viruses. At four weeks post exposure, the survivors were challenged intramuscularly with 1 ml of RP Kabete “O” virulent virus containing $10^{4.5}$ TCID$_{50}$; two control sheep were also injected.

Complement Fixation Tests:

Goat and sheep sera were tested against RP and PPR antigen by a method essentially the same as that of the Laboratory Branch Complement Fixation.$^5$ Complement titrations were performed in the presence of antigen and 5 C'H$_{50}$ units were used in the test proper. Infected bovine (RP) and caprine (PPR) lymph node suspensions subjected to 3 cycles of freezing and thawing, and clarified by centrifugation served as antigens and were used at their “optimal activity dilutions” as predetermined by block titrations.

Lymph nodes from animals that died in the acute stage of PPR or RP virus infection were tested for CF antigen activity against PPR and RP antisera using 4 CF antibody units as predetermined by block titration.

Virus Neutralization Tests:

VN tests in Vero cell culture were performed by two methods. In the first, the sera were tested at a 10-fold final dilution against serial ten-fold dilutions of each virus. In the second, a series of two-fold dilutions of sera were tested against 100 TCID$_{50}$ of each virus. The virus-serum mixtures and controls were incubated at 37°C for 30 min. and 0.2 ml of each mixture was then inoculated onto duplicate Vero cell monolayers grown on 50 ml Falcon plastic flasks. Antibody titers were recorded either as neutralization indices (representing the numbers of logs of virus neutralized by the test serum), or as the highest dilutions of serum capable of neutralizing 100 TCID$_{50}$ of the virus:
Chicken Embryos (CE) and LARP-VN Test:

Fertile white Leghorn eggs were used for LARP virus passage and neutralization test according to the method described by Nakamura with some modifications as follows:

Serum dilutions were mixed with equal volumes of the LA strain of RP virus suspension containing 100 CEID₅₀. The virus-serum mixtures and controls were incubated at 37°C for 30 min., chilled and then 50 ul of each mixture was inoculated intravenously in each of eight 10-12-day-old chicken embryos. The embryos were examined daily for viability and their spleens were examined on the sixth day of inoculation for reddening and congestion lesions. Spleens from each group of embryos were pooled and 10% (wt/vol) suspensions were prepared in Veronal buffer diluent. The spleen suspensions were subjected to three cycles of freezing (−70°C), and thawing (37°C), and then clarified by centrifugation at 2,000 xg for 15 min. The supernatant fluids, kept frozen until use, were tested for RP viral antigen in a microtiter CF test (5). The absence of spleen lesions and CF activity was interpreted as absence of infectivity of the inoculum and therefore as evidence of neutralization of the virus by the test serum. The highest serum dilution which neutralized the infectivity of 100 CEID₅₀ was considered to be the serum titer.

Immuno-electron Microscopy:

The indirect method of ferritin-tagged antibody was used to label PPR viral antigens in infected Vero cell cultures. The method used is summarized as follows: Control or PPR virus infected Vero cell monolayers were gently scraped by rubber policemen, and the cells were lightly centrifuged and resuspended in 0.2 ml of previously inactivated (56°C–30 min.) serum from control, PPR immune or RP immune goats, incubated at room temperatures for 30 min., washed three times with phosphate buffer saline (PBS) and treated 30 min. at room temperature with ferritin-conjugated rabbit antigoat immunoglobulin. After ferritin-conjugate treatment, the cells were washed three times in PBS and fixed in glutaraldehydeosmium, dehydrated in ethyl alcohol-propylene oxide series and embedded in Epon 812. Thin sections were cut on a Porter Blum MT-2 Ultramicrotome (Sorvall, Inc. Norwalk, Conn.) with a diamond knife and the sections were stained with a saturated solution of uranyl acetate. Electron micrographs were taken with an EMU-3G-RCA electron microscope.

RESULTS

First Experiment:

Goats immunized against PPR or RP virus developed complement fixing antibodies in their sera against both the homologous and heterologous virus. (Tables 1 & 2). These animals resisted challenge
with both virulent viruses without any clinical signs but the control
goats with no previous exposure exhibited signs and lesions\(^7\) characteristic of the challenge virus and succumbed 9-13 days post chal-

However, neutralizing antibodies were specific for only the im-
munizing virus (Tables 1 & 2). In virus neutralization tests con-
ducted in chicken embryos, infectivity of the LA strain of RP virus
was neutralized only by goat RP immune sera and not by PPR anti-
sera or normal sera. Similar results were obtained in Vero cell cul-
ture system against Kabete “O” strain of RP virus. (Tables 1 & 2)

Second Experiment:

One of the contact goats died after showing clinical signs and
lesions associated with PPR virus infection\(^9\) while the other goats
recovered. Sera from recovered animals cross reacted in the CF test
with PPR and RP viral antigens but no cross neutralization was ob-

Listed in Table 3 are the CF and VN titers of serums from PPR
recovered goats obtained 21 days after infection with PPR virus that
grew in Vero cell cultures. The median VN titer of the serum against
PPR virus was 640; against RP virus <10 which equalled that of
normal sera. Goats that survived PPR virus infection acquired suf-

Third Experiment:

Convalescent sera from sheep obtained 21 days after infection
with PPR virus had the same pattern of CF and VN antibodies
against RP and PPR viruses as that of the goats in the first and
second experiment. Table 4 shows the CF and VN antibody levels of
sera at 21 DPI, obtained before challenge with RP virus. Two sheep
(1 inoculated and 1 contact control) died at the acute stage of PPR
virus infection, before the detection of antibodies. The remaining
sheep were resistant to RP virulent virus challenge; controls ex-
hibited clinical signs and lesions characteristic of RP virus infection\(^9\) and died 10-14 days after exposure.

Results of Neutralization test using constant serum varying virus
method:

Table 5 summarizes the cross neutralization results between RP
and PPR viruses using the constant serum varying virus method. Pre-

Presented data were obtained from goat sera obtained in the first
and second experiment. A similar pattern was observed in sheep sera.
Levels of CF antigen in goat and sheep infected with PPR virus:

Goats and sheep that died from acute PPR or RP virus infection
had CF antigen titers against both RP and PPR immune sera. Anti-
gen titers were always higher in the homologous system than in the
heterologous system. (Table 6)
Immuno-electron Microscopy:

The indirect ferritin tagged antibody technique was used to investigate the reaction of the PPR virus particles with PPR and RP immune sera. Electron microscopic observations showed qualitative differences between the homologous and heterologous systems. (Figures 1 & 2). Tagging was intense with the PPR serum; little, if any, tagging was seen with the RP serum.

Ferritin tagging was observed at the surface of the virion envelopes, at the budding stages and also on some regions of modified plasma membrane of infected cells which presumably contain incorporated viral envelope proteins.

DISCUSSION

The results of the cross CF test between RP and PPR viral antigens suggest that PPR and RP viruses are immunologically related but not immunologically identical. Their non-identity was readily detected in the VN test which showed obvious dissimilarity in neutralizing activity. A differential diagnosis between the two viruses was achieved in a constant virus-serum dilution method that utilized 100 TCID$_{50}$ of virus and an initial ten-fold dilutions of the serum. The neutralization of the virus used for immunization by serum antibody was always at a level of significance to the homologous virus, but the heterologous virus was not significantly neutralized.

There was cross neutralization between the LA and Kabete “O” strains of RP virus, two strains having very different passage histories. The results of neutralization by the two strains were of similar magnitude suggesting that RP virus is antigenically stable. On the other hand, the lack of cross neutralization between PPR and RP viruses would suggest that they are distinct viruses.

It was generally believed that differentiation between PPR and RP viruses depended on a virulence test in bovines, in which only RP virus is virulent and usually has fatal consequences. Our finding that the two viruses can be differentiated by the VN test could be a useful inexpensive tool in studies to clarify the relationship between RP and PPR viruses and their relationship to the other paramyxoviruses, such as canine distemper, measles and subacute sclerotic panencephalitis.

The observations that CF antigen titers were consistently higher in the homologous system than in the heterologous system and that CF antibody titers was higher against RP antigen than PPR antigen in both systems suggested that (1) RP antigen is more efficient than PPR as an inducer of CF antibody and (2) CF antibody has a broad spectrum of activity.

The cross reaction between RP and PPR viruses detected by the CF test, could be the result of shared antigens of nucleocapsid origin and not of the viral envelope proteins because cross neutralization was
lacking. Neutralizing antibodies are generally antibodies to surface components of the virions. Also, ferritin tagging and electron microscopic examinations revealed specific tagging in the homologous system but no significant tagging in the heterologous systems.

The mechanism of resistance of immunized goats and sheep to subsequent heterologous virus challenge was not clear; specific neutralizing antibodies were not demonstrable against the challenge virus. Cell mediated immunity, biologic antagonism, or other unknown mechanisms may provide hypothetical explanations.

The differentiation between the two viruses by cross neutralization tests and ferritin tagging and the morphologic differences (size differences) on virus population basis suggest RP and PPR viruses are different but related entities.

ACKNOWLEDGMENT

The authors would like to thank Messrs. T. Franke, J. Giovanelli and S. Pyne for their technical assistance.
Table 1. Virus Neutralizing and Complement Fixing Antibody Levels in Sera of Goats Immunized Against Peste des Petits Ruminants Virus.

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Antibody Levels Against RP Virus</th>
<th>Antibody Levels Against PPR Virus</th>
<th>Response to PPR Virus Challenge</th>
<th>Response to RP Virus Challenge</th>
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</thead>
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<tr>
<td></td>
<td>CF Titera</td>
<td>VN Titer Against KO Strainb</td>
<td>CF Titera</td>
<td>VN Titerb</td>
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<tr>
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<tr>
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<td></td>
<td>Death 13 DPI</td>
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<td></td>
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<td></td>
<td></td>
<td>NA</td>
<td>Death 10 DPI</td>
</tr>
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</table>

CF: Complement Fixation; VN: Virus Neutralization; KO: Kabete 0; LA: Lapinized Avianized; PPR: Peste des Petits Ruminants; RP: Rinderpest; NA: not applicable.

*Animals were virus challenge controls.
aNumber represent the highest serum dilutions that fix complement by ≥70%.
b“<” “<” “<” “<” neutralize 100 TCID<sub>50</sub> of the virus tested.
cby absence of lesions and lack of RP virus specific CF antigens in chicken embryo spleen pools.

Table 2. Virus Neutralizing and Complement Fixing Antibody Levels in Sera of Goats Immunized Against Rinderpest Virus.

<table>
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<tr>
<th>Identification Number</th>
<th>Antibody Levels Against RP Virus</th>
<th>Antibody Levels Against PPR Virus</th>
<th>Response to RP Virus Challenge</th>
<th>Response to PPR Virus Challenge</th>
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<td>VN Titer Against KO Strainb</td>
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</tr>
<tr>
<td>2401*</td>
<td></td>
<td></td>
<td>NA</td>
<td>Death 10 DPI</td>
</tr>
</tbody>
</table>

CF=Complement fixation; VN: Virus Neutralization; KO: Kabete 0; LA: Lapinized Avianized; PPR: Peste des petits ruminants; RP=Rinderpest; NA=not applicable.

*Animals were virus challenge controls.
aNumbers represent the highest serum dilutions that fix complement by ≥70%.
b“<” “<” “<” “<” neutralize 100 TCID<sub>50</sub> of the virus tested.
cby absence of lesions and lack of RP virus specific CF antigens in chicken embryo spleen pools.
Table 3. Virus Neutralizing and Complement Fixing Antibody Levels in Sera of Goats Infected with Peste des Petits Ruminants Virus grown in Vero Cell Culture.

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Route of Infection</th>
<th>Antibody Levels Against PPR Virus</th>
<th>Antibody Levels Against RF Virus</th>
<th>Response to RF Virus Challenge 35 days Post PPR Virus Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CF Titer(^a)</td>
<td>VN Titer(^b)</td>
<td>CF Titer(^a)</td>
</tr>
<tr>
<td>3543</td>
<td>Contact with inoculates</td>
<td>20</td>
<td>2560</td>
<td>80</td>
</tr>
<tr>
<td>3544</td>
<td>IM Inoc.</td>
<td>20</td>
<td>640</td>
<td>40</td>
</tr>
<tr>
<td>3545</td>
<td>IM Inoc.</td>
<td>20</td>
<td>640</td>
<td>160</td>
</tr>
<tr>
<td>3546</td>
<td>IM Inoc.</td>
<td>10</td>
<td>640</td>
<td>40</td>
</tr>
<tr>
<td>3547</td>
<td>IM Inoc.</td>
<td>20</td>
<td>2560</td>
<td>160</td>
</tr>
<tr>
<td>3543(^*)</td>
<td>Contact with inoculates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3705(^**)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3708(^**)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Numbers represent the highest serum dilutions that fix complement by >70%.  
\(^b\)Numbers represent the highest serum dilutions that neutralize 100 TCID\(_{50}\) of the virus tested.  
\(^*\) Animals died at the acute stage of PPR virus infection before detection of antibodies.  
\(^**\) Animals were RF virus challenge control.

IN Inoc.: Intramuscular inoculation.  
PPR=Peste des Petits ruminants; RF=rinderpest; CF=complement fixation; VN=Virus neutralization.  
All sera were collected at 21 days post exposure.

Table 4. Virus Neutralizing and Complement Fixing Antibody Levels in Sera of Sheep Infected with Peste des Petits Ruminants Virus grown in Vero Cell Culture.

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Route of Infection</th>
<th>Antibody Levels Against PPR Virus</th>
<th>Antibody Levels Against RF Virus</th>
<th>Response to RF Virus Challenge 28 days Post PPR Virus Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CF Titer(^a)</td>
<td>VN Titer(^b)</td>
<td>CF Titer(^a)</td>
</tr>
<tr>
<td>3721</td>
<td>IM Inoc.</td>
<td>20</td>
<td>640</td>
<td>20</td>
</tr>
<tr>
<td>3722</td>
<td>IM Inoc.</td>
<td>20</td>
<td>640</td>
<td>20</td>
</tr>
<tr>
<td>3709</td>
<td>IM Inoc.</td>
<td>10</td>
<td>640</td>
<td>20</td>
</tr>
<tr>
<td>3724(^*)</td>
<td>IM Inoc.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3725(^*)</td>
<td>Contact with inoculates</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3869</td>
<td>Contact with inoculates</td>
<td>20</td>
<td>1280</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^a\)Numbers represent the highest serum dilutions that fix complement by >70%.  
\(^b\)Numbers represent the highest serum dilutions that neutralize 100 TCID\(_{50}\) of the virus tested.  
\(^*\) Animals died at the acute stage of PPR virus infection before detection of antibodies.  

IN Inoc.: Intramuscular inoculation.  
PPR=Peste des Petits ruminants; RF=rinderpest; CF=complement fixation; VN=Virus neutralization.  
All sera were collected at 21 days post exposure.
Table 5. Results of Cross Neutralization between Rinderpest and Peste des Petits Ruminants Viruses.

<table>
<thead>
<tr>
<th>Sera</th>
<th>Virus</th>
<th>PPR</th>
<th>RP</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPR</td>
<td>5.3</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>&lt;1</td>
<td>5.5</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

PPR = Peste des Petits Ruminants; RP = Rinderpest

Numbers represent logs of virus neutralized by sera at 10-fold dilution and they constitute the mean of 12 sera tested.

Table 6. Complement Fixing Antigen Levels in Goats and Sheep Mesenteric Lymph Nodes After Death from Peste des Petits Ruminants or Rinderpest.

<table>
<thead>
<tr>
<th>Species</th>
<th>Identification Number</th>
<th>Treatment</th>
<th>Route of Infection</th>
<th>CF Titer Against PPR Viral Serum Antibody</th>
<th>CF Titer Against RP Viral Serum Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>3343</td>
<td>PPR Virus</td>
<td>By contact</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3221</td>
<td>&quot;</td>
<td>IM Inoc.</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3322</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3223</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>3224</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>3307</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>320</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3508</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>320</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3724</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3725</td>
<td>&quot;</td>
<td>By contact</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Goat</td>
<td>2400</td>
<td>RP Virus</td>
<td>IM Inoc.</td>
<td>80</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td>2401</td>
<td>&quot;</td>
<td>&quot;</td>
<td>160</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td>2402</td>
<td>&quot;</td>
<td>&quot;</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>2403</td>
<td>&quot;</td>
<td>&quot;</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>3761</td>
<td>&quot;</td>
<td>&quot;</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>3762</td>
<td>&quot;</td>
<td>&quot;</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>3726</td>
<td>&quot;</td>
<td>&quot;</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Sheep</td>
<td>3727</td>
<td>&quot;</td>
<td>&quot;</td>
<td>40</td>
<td>160</td>
</tr>
</tbody>
</table>

CF: complement fixation; PPR: Peste des Petits Ruminants; RP: Rinderpest; IM Inoc.: Intramuscular inoculation.
Titers represent the highest lymph node antigen dilution that fix complement by ≥ 1X.
Figure 1.

Figure 2.
REFERENCES

Jet injection affords the veterinarians an excellent means to control infectious diseases and contain diseases of zoonotic importance. There is a good body of data available on the efficacy, use and economy of jet injection in human medicine. This information has been accumulated over the past several years and is based on the extensive use of the jet injection equipment at all levels of government.

This technique has not been used in animal medicine except on an experimental basis. The reasons are not known; however, equipment design has perhaps played a major role in the under-utilization of this procedure.

Jet injections, simply stated, can be defined as the intradermal, subcutaneous, intramuscular or intranasal deposition of an inoculum. So far as we know, all fluid vehicles which serve as carriers for biological products can be injected readily with the jet injector. This includes such diverse substances as bacterins, fluid vaccines, and a number of different adjuvants with oil or water in oil bases.

The historical development, utilization and applicability of jet injection to the field of medicine was reviewed recently by Whitford and McConnell. The purpose of this presentation is to present current data on the jet injector.

What is jet injection and what does it offer the veterinary profession? This question is frequently asked and can be answered simply. Jet injection is the application of biologicals or drugs using a mechanical device operated on a pressure drive basis. It is a needleless, semiautomatic, multiple dose inoculator which is available as one of 3 basic models. It has broad applicability, is very reliable, flexible and offers an economical advantage in many instances over the more conventional methods of needle and syringe inoculation.

The technique is well suited for field application of a number of vaccines and should prove beneficial in at least three areas: (1) feedlot immunization and treatment programs, (2) control of epizootic diseases and (3) immunization of pets and domestic animals on a large scale basis to contain disease.

The jet injector is available in 3 models: a foot operated pump suitcase injector, an electrically operated injector and a hand operated back pack design. For purposes of illustration, the suitcase model is shown in Figure 1. The unit consists of a power source and an injector gun. The power source serves to compress a coiled spring

*S. McConnell, Department of Veterinary Microbiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas. Supported by U. S. Army Contract #C-1087 and Texas Agricultural Experiment Station Project #H-1679
which automatically fills the syringe with a pre-set dose. Release of the compressed spring forces the plunger forward driving the fluid through the orifice and into the animal.

Key differences between the unit used in man and the unit designed for domestic animals are illustrated in Figures 2 and 3. Design changes are crowned head, enlarged diameter of the jeweled orifice, and a barrel with a 2 ml capacity. For use in man, an orifice diameter of 0.005 inches is optimal. This diameter is not suitable for the larger domestic animal species. A series of orifice diameter sizes were developed and tested. The optimum size ranges are 0.009, 0.010 and 0.011 inches in diameter.

Depth of penetration and dose delivery can be altered by changing the physical configuration of the injector. Both 1 cc and 2 cc stainless steel barrels are available and the dose delivered can be pre-set by adjusting the settings with a knurled knob on the rear of the gun. Depth of penetration is dependent on the spring rating used. Three spring sizes ranging from 300 to 600 psi can be used either alone or in tandem by adding the intensifier unit to the gun. Depth of penetration is illustrated in Figures 4 and 5, which shows the penetration of a radiopaque dye delivered by needle and by jet.

Both the electrically operated and the back pack units offer the veterinarian distinct advantages over the foot-operated unit under select conditions. The electrically driven unit can be used principally in static situations such as examination and treatment rooms in a clinic or hydraulic chute areas in feedlot operations.

The hand-pump back pack unit was designed as a portable unit for use in the field. Any situation in which large numbers of animals are to be processed will be ideal for this unit. The back pack design and its component parts are shown in Figure 6.

The jet injector should prove ideal for (1) rabies clinics in urban areas, large cities or any area where large numbers of animals can be accumulated i.e. dogs in cities or cattle in vampire bat ecosystems, (2) under intense management conditions such as with feedlot cattle, feedlot sheep or large swine herds, (3) for the mass immunization of any species of animal during an epizootic where containment of the disease is paramount and (4) for disease prevention of large populations such as for brucellosis control, clostridial disease, foot-and-mouth disease or other such conditions.

Extensive studies have been conducted using jet injection in a variety of animal species. Comparisons between jet injection and needle and syringe were made and information on animal responses documented. Our studies show no significant differences in host response nor have we encountered any untoward reactions in any of the animals we have injected.

A number of potential advantages can be derived from jet injection: (1) no transmission of the infectious agents of disease, (2) a reduced incidence of sterile abscesses, (3) rapid, effective vaccina-
tion of animals ie. over 400 equine per hour and (4) economical savings over the present one needle for 1 animal required with syringe injections.

In addition, at least one more conceptual advantage can be mentioned. Since the deposition of vaccine by the jet injector is partially intradermal, partially subcutaneous and partially intramuscular, it is conceivable that better immunity may be obtained with bacterins and jet injection. I feel that the intradermal deposition of vaccine may serve to accentuate the immune response because of the slower release of antigen from the dermis. In operations where only a single injection of killed vaccines are used because of handling costs, an improved host response would be very beneficial.

Figure 1. Jet injector in carrying case.
Figure 2. Comparison of head designs. Note crown design for use in animals.

Figure 3. Component parts of jet injector.
Figure 4. Deposition of 2 cc diodrast.

Figure 5. Deposition of 1 cc diodrast.
Figure 6. Prototype back pack jet injector.

REFERENCES

FOREIGN ANIMAL DISEASE COMMITTEE REPORT

Committee Members:
Chairman: Col. T. G. Murnane
Co-Chairman: H. Q. Sibley

This year marked the 22nd annual meeting of the Committee on Foreign Animal Diseases. When established in 1953, this Committee was referred to as the Committee on Exotic Animal Diseases. The Committee was formed at a time of great national concern for the threat, accidental or intentional, posed by exotic animal disease on the livestock of this nation. The United States had then successfully concluded a program in collaboration with the Government of Mexico to eradicate FMD from the North American Continent. At the same time the U.S. was placing increasing emphasis on national defense against radiological, chemical, and microbiological warfare that might be employed against this nation—its people, livestock, and crops. Veterinary medical research had been directed against such exotic diseases as rinderpest, fowl plague, newcastle disease, and other exotic animal diseases would be investigated, i.e. African horse sickness and African swine fever.

The Foreign Animal Disease Committee published the first handbook on exotic animal diseases, actually a text on tropical veterinary medicine, in 1954, which was revised in 1964. The publication of this handbook superceded the publication of the British text, Tropical Veterinary Medicine, by eight years. The Committee is completing the third, or 1975, revision of this text, Foreign Animal Diseases, Their Prevention, Diagnosis and Control, and will submit it to the USAHA for approval and publication.

Over the years, the Committee membership has encompassed many veterinarians who have distinguished themselves by their contributions to the research and investigation of exotic animal diseases and as well to national and international programs for eradication of animal diseases. Because of this international involvement, the Committee on Foreign Animal Diseases has become concerned not only with exotic diseases of food and work animals as they may affect the livestock health and economy of this nation, but also with animal health as a matter of international interest.
The United States extends financial and technical assistance to international agencies and nations for programs to improve human health and livestock economy by controlling and eradicating important animal diseases such as trypanosomiasis, FMD, vampire bat transmitted rabies in livestock, rinderpest, contagious bovine pleuropneumonia and others. These programs indirectly benefit U.S. livestock by minimizing or eliminating the occurrence of these diseases. These are all diseases which are regarded as foreign animal disease threats to U.S. livestock. The Committee has previously advocated greater international involvement as an additional means of reducing the threat of foreign animal disease and to bring U.S. veterinary medical expertise to bear on the critical problem of animal protein shortages in areas of the world in which they occur.

The Committee encourages and supports international animal health programs; however, the Committee is concerned for the lack of veterinary staff officers within the U.S. Department of State to exercise the necessary professional supervision of these programs. The Committee will continue to maintain, and hopefully increase, its surveillance over such international animal health programs.

The Committee wishes to pay special recognition to the U.S. Senate for approval of the United States to become a member of the International Office of Epizootics. U.S. membership in this important international agency is attributable in a major part to the efforts of this Association.

Recent Developments in International Agricultural Research and Technology

Programs for Agricultural research and technical assistance have in recent years been expanded by the Agency for International Development, Department of State (AID). Expansion of these programs recognizes the key role which agriculture plays in the economic improvement of developing nations and the need for a coordinated multidisciplinary attack on the many deep rooted agricultural problems in these areas.

AID involvement through the office of Agriculture, Technical Assistance Bureau (TA/AGR), includes financial support to international assistance agencies; central funding for agricultural research project that have widespread application and technical assistance projects in developing countries as requested.

In 1972, two centers of international research were added to the four operating centers. More recently, two more international research institutes were improved. Of the eight centers, three are concerned with animal health and exotic diseases of interest to the Committee on Foreign Animal Diseases. These are the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya, and the International Livestock Center for Africa.
The CIAT which started in 1969 represents a departure from the crop-centered approach to agricultural improvement. Its primary focus is on a region, the lowland tropics of the Western Hemisphere and the factors that influence development of a productive farm sector. Programs may be commodity centered, or on systems of farming. CIAT's program for beef cattle concentrates on providing adequate feed supply through improved pastures, supplementation, control of disease and parasitism and development of beef production systems. The Center works closely with the Colombian Institute of Agriculture (CIA). Among the research activities are those concerned with reproductive performance and hemoparasitic diseases. CIAT's program on swine is directed to identification of cheap local sources of protein and energy, improved management practices feasible on the small farm and major swine diseases.

The ILRAD is the result of a memorandum of Agreement concluded in 1973 between the Consultative Group on International Agricultural Research and the Government of Kenya. ILRAD's primary and most urgent purpose is the development of immunological procedures for the prevention of trypanosomiasis and East Coast fever. These two diseases affect livestock in over four million square mile belt spanning the African continent, about 30 developing countries being wholly or partially in the infected area. Control of these two diseases could make a radical difference to their economies and improve the lives of the inhabitants. Through the Africa Bureau, AID is expected to provide up to 25% of the operating and capital budget requirements of the ILRAD. The TA/AGR centrally funded research projects with Texas A & M University (hemoproteozoal parasites of livestock) and with the UDDA (Tsetse fly technique for control of trypanosomiasis) will be closely linked with the laboratory. Dr. James B. Henson assumed the Directorship of the ILRAD in January 1975. The Institute is now progressing toward full administrative functional capability.

In 1974, the Ethiopian Government and the World Bank acting on behalf of the Consultative Group, signed an agreement establishing the ILCA. The Institute's purpose is to assist national efforts which aim to effect a change in production and marketing systems in tropical Africa so as to increase the yield and output of livestock products and improve the quality of life of people in the region. The Institute's three main areas of activity are research, training and documentation. Much of the information collected in African research centers has never been published. ILCA will retrieve, store and classify this information so that it will be readily available to meet specific developments and research purposes. The Institute will work cooperatively with existing organizations on important livestock marketing and production systems in Africa. Work will include
disease control marketing, dry season nutrition, forages and grasses improvement in reproductive performance and parasite control. AID expects to provide up to 25% of the operating and capital budget requirements of ILCA. TA/AGR centrally funded livestock research projects will be linked with the Center.

Livestock production research projects currently supported by TA/AGR include research on hemoprotozoal disease of food producing animals (Texas A & M University), research on sterility method of tsetse fly control (ARS, USDA), control of vertebrate pests — rats, vampire bats and noxious birds (Bureau of Sports Fisheries & Wildlife, US Department of Interior) and research on mineral adequacies, deficiencies and toxicities of grazing ruminants in Latin America.

The project for control of the vampire bat has met with considerable success and is to be phased out as the project has attained its objective.

PAN AMERICAN HIGHWAY

The Pan American Highway is designed to connect North and South America by a land route. However, as reported previously by this Committee, connecting FMD-infected Colombia with Foot-and-Mouth Disease-free Panama constitutes a major threat of spread of FMD first into Panama and subsequently into Central America, Mexico, and the United States.

Highway planning and construction in Panama is proceeding although construction on the section near the Panama-Colombia border has not been initiated. In Colombia, only one short segment of the road has been built and all other construction of the road has been postponed pending establishment of a satisfactory program to prevent spread of FMD from Colombia into the free areas north and west of Colombia.

As reported to this Association last year, USDA has established a cooperative program with animal disease control authorities in Colombia, aimed at providing the FMD protection required for road construction and operation. To date this program has failed to accomplish the objectives agreed upon between the two countries.

We also reported to you last year that administrative difficulties in Colombia had slowed purchases of equipment, building of quarantine facilities, movement of cattle herds located near the border and other similar activities. This situation has not improved. The program is moving very slowly and unless it is speeded up, will not provide the FMD protection necessary.

It is the opinion of this Committee that Pan American Highway construction in Colombia should not proceed until an adequate FMD program is fully operational in Colombia and until there is assurance that such a program will be continued as long as there is a threat of FMD from that country.
Tuxtla Gutierrez is the capital of the State of Chiapas and is located in southern Mexico. Near this town, of about 100,000 population, the Governments of Mexico and the United States are cooperating in the construction of a screwworm fly production plant. This plant will be the first in the world designed and constructed from the ground up for screwworm fly production. Two previous plants, one at Sebring, Florida, and one near Mission, Texas, were constructed by modification of existing buildings.

At Tuxtla Gutierrez, the main plant building is 90 x 210 meters or 295 x 689 feet and is designed for production of 300 million screwworms per week, but will have room to expand production if required. A companion warehouse is 60 x 90 meters or 196 x 295 feet and will provide storage for more than six months' supply of materials required for fly production.

In addition to distributing sterile flies from Tuxtla Gutierrez, sterile pupae will be flown in bulk shipments in DC-6 aircraft to primary Distribution Centers located at Guadalajara, Jalisco, and Tampico, Tamaulipas. From each of these points, sterile flies will be distributed using DC-3 aircraft.

Although the factory will not be ready for sterile-fly production until some time in 1976, field work has already started. One hundred and fifty Mexican inspectors have been hired. These inspectors are located strategically throughout Mexico where they can contact livestock owners to enlist their cooperation in screwworm eradication activities. Livestock owners are requested to examine their animals frequently for evidence of infestation, and when larvae infestations are found, to collect specimens for laboratory identification, and to treat wounds to kill any remaining larvae.

Initiation of fly production at Tuxtla Gutierrez has been delayed more than a year, primarily for budget reasons. There are now sufficient funds available to complete construction. Production should begin sometime during the second quarter of calendar year 1976.

FOREIGN ANIMAL DISEASES SURVEILLANCE

During FY 1975, 52 investigations of suspicious cases of foreign animal diseases involving 30 trained foreign animal disease diagnosticians were conducted. These 52 investigations, which do not include those conducted for suspicious reports of exotic Newcastle disease and Venezuelan equine encephalitis, were conducted in 49 counties of 22 states. There were 48 of these investigations suspicious for vesicular conditions. All 52 investigations were negative for exotic diseases.

Only two bovine in a herd consisting of two bovine and 43 swine
which were located in Columbus County, North Carolina, were positive for New Jersey vesicular stomatitis.

In contrast, during FY 1974, foreign animal disease surveillance activities resulted in 90 investigations which excludes those conducted for exotic Newcastle disease and Venezuelan equine encephalitis. Of these 90 investigations, 82 of them were suspicious for vesicular conditions. All were negative for exotic diseases. Five cases, all in bovine, were positive for New Jersey vesicular stomatitis. Two cases occurred in Concordia Parish, Louisiana, and single cases were confirmed in Washington and Evangeline Parishes, Louisiana, and Warren County, Mississippi.

During FY 1975, in cooperation with the Plum Island Animal Disease Center, Agricultural Research Service, 31 veterinarians were trained as foreign animal disease diagnosticians. Participants include veterinarians from the military as well as state and Federal regulatory agencies. In addition, veterinarians from three other countries participated.

The foreign animal disease diagnosticians have the responsibility for conducting surveillance for foreign animal diseases and making investigations of suspicious cases. As of October 1, 1975, 159 veterinarians had completed the course and were available to make investigations.

Currently, two foreign animal diseases courses consisting of approximately 12 participants each are held annually in cooperation with the Plum Island Animal Disease Center, Agricultural Research Service. Beginning in FY 1977, plans provide for increasing this training to three courses annually.

The development of 10 foreign animal diseases films was initiated in cooperation with the Plum Island Animal Disease Center. These films are directed primarily toward professional audiences but will be useful in increasing public awareness of foreign animal diseases.

Preparedness for Dealing with Emergency Diseases

The five Regional Emergency Animal Disease Eradication Organizations (READEO's) were fully staffed and maintained to rapidly respond to emergency disease outbreaks. During FY 1975, the Northern and South Central READEO's were activated to combat outbreaks of exotic Newcastle disease at Bay Shore, Long Island, New York, and Pharr, Texas, respectively. These outbreaks were rapidly and effectively eliminated. Three test exercises were conducted by the North Central, Northern, and Western READEO's.

In addition, a formal request by the Secretary of Agriculture to the Secretary of Defense for extending contingency planning and support of Regional Emergency Animal Disease Eradication Organization activities to include Puerto Rico, Hawaii, Alaska, Panama
Canal Zone and the Trust Territories of the Pacific Islands was approved.

A list of disease specialists who will be available to serve as advisors and consultants for the five READEO's was established.

The Animal Protein Conservation work group which was established to determine safe methods and procedures for salvaging meat and animal products from noninfected exposed animals involved in an outbreak of an exotic disease completed its study and a final report was issued. The findings of this group could be beneficial in conserving animal protein in certain outbreaks of exotic diseases.

The county profiles designating animal populations at risk, slaughtering establishments, cold storage warehouses, milk processing plants, and rendering plants were established and maintained.

THE WORLD EPIZOOTIOLOGY OF VESICULAR DISEASES

Foot-and-Mouth Disease (FMD)—Russia reported 49 outbreaks in the first 3 months of 1975. No cases were reported in April and May. The viruses involved were 0, and A22.

Western Europe again reported a favorable disease situation with sporadic outbreaks only recorded in the Federal Republic of Germany, Austria, Italy, Spain, Yugoslavia and Greece. The continued use of periodic vaccination was instrumental in containing the type C virus epizootic in France and the type O outbreak in Belgium.

Vaccination on a large scale continues in South America with apparent improvement in the disease situation. The countries of Chile, Peru, Ecuador, Colombia and Venezuela remain free of type C infections. In the same area, however, types A, O, and C were identified in Bolivia. Chile reported that 20 of their 25 provinces had no outbreaks of FMD. Only type A virus was identified in outbreaks in the other 5 provinces. The remaining countries reported overall improvement from the previous year; however, a relative increase in the prevalence of type C outbreaks was reported in Brazil, Argentina, Paraguay, and Uruguay.

After 20 years absence, type SAT-3 was the cause of outbreaks of FMD in cattle and game in Rhodesia. A few sporadic outbreaks caused by the same virus type were recorded in Mozambique, Liberia and the Ivory Coast reported SAT-2 for the first time. The incidence of outbreak due to this virus is increasing in the countries of Zaire and Zambia. Type SAT-1 and SAT-2 were the cause of outbreaks in wildlife and cattle in South Africa. These viruses were also identified in Angola.

Asia-I was the cause of an outbreak in Lebanon which was the first incidence of this virus in that country since the 1959-60 near east epizootic. The remainder of the countries of the near east remained free of this type. Israel remained free of FMD throughout the year.

For the first time in 10 years, type Asia-1 virus was identified
from an outbreak in Hong Kong, but no disease spread occurred.

An outbreak of FMD occurred in the Philippines in the spring of 1975. Type O virus was identified and vaccination was instituted as a control measure.

Swine Vesicular Disease (SVD)—This disease continues to be identified and has been reported from Great Britain, Federal Republic of Germany, Austria, Italy and Hong Kong. The eradication campaign in Great Britain seems to be achieving success.

No significant changes in Vesicular Stomatitis or Vesicular Exanthema of swine have occurred since the previous report.

RINDERPEST

The most alarming situation is the continuing recrudescence of this disease in the countries of West Africa which were covered in the early phase of the vaccination campaign. In fact, Mauritania reported that losses in 1974 were as heavy as in 1967, i.e. prior to the campaign. Much of this may be attributed to the disruption to the cattle industry caused by the severe drought that has affected this area, but lack of resources and plans for a continuing vaccination campaign is undoubtedly a major contributing factor.

CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

CBPP continues to be a major disease problem in much of Africa and coordinated efforts for its control have not been instituted. Areas in Gambia free of CBPP since 1971 have reported recent outbreaks. Outbreaks have also occurred in Liberia and Kuwait. In most of the endemic African countries, vaccination is now being applied jointly with the periodical rinderpest vaccination, but as discussed under rinderpest, this vaccination program is having severe difficulties.

AFRICAN SWINE FEVER (ASF)

During 1974, outbreaks of ASF were reported from Spain (221), Portugal (126), Angola (8), South Africa (4), Malawi (3), France (10) and Mozambique (2). Portugal reported outbreaks of ASF on the Island of Madiera for the first time in 6 years. Incomplete reports from 1975 indicate AFS continues with increasing frequency in Spain. The Committee is concerned for the persistence of ASF in Europe, particularly the Iberian Peninsula, where the disease has, in addition to the classical form, assumed a chronic, less lethal form in domestic swine.

RESEARCH ON VESICULAR DISEASES

The following brief review notes some of the highlights that have taken place in the field of vesicular disease research over the past
year. The vesicular group consists of the viruses of vesicular stomatitis (VS), foot-and-mouth disease (FMD), vesicular exanthema of swine (VES), and the newer member of the group, swine vesicular disease (SVD).

**VESICULAR STOMATITIS VIRUS (VSV)**

A large volume of research has been done with VSV, however, it has essentially all been done in "molecular biology". The fact that this viral agent is capable of causing a disease has almost been lost to the research worker's mind.

A survey of Georgia wildlife carried out by Jenney, et al., showed that 4 out of 105 raccoons, 2 out of 115 opossum and 1 out of 9 gray squirrels had antibody to VSV New Jersey type. In the same survey foxes, skunks, bobcats and coyotes were all negative in the serological tests done.

One study done by Schloemer and Wagner may have some significance to the actual disease. They found that VSV harvested from tissue cultures of mosquito cells was deficient in sialic acid. This resulted in markedly reduced hemagglutinating activity and infectivity. In vitro addition of the acid resulted in a 100 fold increase in the infectivity of the harvested virus. They postulated this as a possible explanation of why VSV infection is limited in spread in nature.

An extensive bibliography has been developed for vesicular stomatitis and its virus by Uskavit. He has now added the fifth supplement to it which covers the period from October 1973 to September 1974.

This is essentially all of the literature on vesicular stomatitis that may have some meaning and significance to those concerned with disease as more than a laboratory entity.

**VESICULAR EXANTHEMA OF SWINE VIRUS (VESV)**

At the time of the writing of this report, VES is still an extinct disease of domestic swine. However, the continuing study and isolation of the closely related San Miguel sea lion virus (SMSV) from sea mammals is being closely followed. Antibody to this virus has been reported in serum samples from the California gray whale and the stellar sea lions. The same study also found antibody in the serum from the northern elephant seal. Smith, et al., who has been studying the problem of abortion in the California sea lion, which led to the first isolations of the San Miguel virus has now implicated Leptospira as another infectious agent that can lead to abortion and newborn death.

Pritulin of the Soviet Union, has reported VES and SVD as the same disease. This is contrary to the world literature. Apparently neither of these diseases has occurred in the Soviet Union.
SWINE VESICULAR DISEASE VIRUS (SVDV)

This disease is the newest member of the four in the vesicular disease group and has demanded considerable research effort in the past year.

Delagneau, et. al.\(^7\) have confirmed what others have already reported, that SVDV and FMDV are different viruses. They did their work by analysis of the structural proteins by polyacrilimide gel electrophoresis. Brown and Wild,\(^8\) in their study on the comparison of the early isolate of Coxsackie B-5, the Faulkner strain, and recent isolates from man, found that the newer virus differed from the older to the same degree that the Faulkner differs from SVDV. They postulate that if man was the origin of the original SVD outbreak, then the virus probably was of a more recent vintage.

An extensive study of the SVDV that caused the outbreak in France in 1973 has been published. Its physico-chemical properties and immunology showed it to be an enterovirus.\(^9\) These workers as well as another group found they could prepare a vaccine from Beta propiolactone inactivated virus that would protect against exposure to the virulent virus.\(^10\)

Pigs from 7 of 369 premises sampled in 35 counties of England were found to have serum positive to SVDV antibody.\(^10\) Virus was isolated from pigs on one of these 7 farms. The original serum samples were collected in pigs in slaughterhouses in different parts of the country.

Swine vesicular disease virus inoculated into newborn mice caused a diffuse encephalitis of a degenerative-inflammatory nature.\(^12\) These workers claim this can be used to distinguish this disease from FMD in samples submitted from the field for diagnosis. One report showed that SVDV disappeared from pig tissues about the eighth day after infection but that it could be found in body secretions and excretions for up to 3 months after initial infection had occurred.\(^13\)

De Simone, et. al.,\(^14\) reported on a rapid complement fixation test for use in the diagnosis of SVD. They found that to work well, the test had to be conducted with specific serums prepared against the test virus in guinea pigs.

Swine vesicular disease was reported for the first time in Japan in December, 1973.\(^15\) An immediate campaign of eradication was instituted with apparent success.

The first supplement covering the literature for 1974 has been added to the bibliography on this disease by Uskavitch.\(^16\) A review of this disease and its clinical features was presented as part of a training course given in Kansas.\(^17\) Another review with illustrations of the clinical features of experimentally infected pigs was published by Terpstra.\(^18\)
Foot-and-mouth disease continues to be the subject of considerable study. It is interesting to note that there seems to be more attention focused on the virus than on the disease it causes.

Attempts to determine the genetics of foot-and-mouth disease virus (FMDV) have been studied. Much of this work involves the development of temperature sensitive mutants to be used as markers for further growth characteristic determinations. Lake, et al, working at Pirbright, developed a genetic recombination map for Type O FMDV. They used temperature sensitive mutants to study the influence of guanidine on the virus. The sensitive site of mutation was found near the center of their map.

Virus growth in animals and tissue culture is studied to gain insight on fundamental principles of its growth. It was found that sheep slaughtered during the viremic stages harbour the virus in the lymph nodes in various amounts. Casings prepared from such sheep were infectious after cleaning and storage in salt for 14 days but not after treatment with lactic or citric acid.

Transmission of FMDV via the placenta in guinea pigs was studied. It was also found that secondary tissue cultures prepared from bovine kidney cells were more sensitive to infection with FMDV when they were 5 to 7 days old than when they were less than 5 days old.

Ahl found that when cells were infected with FMD and then suspended in a culture medium containing 1 mm of EDTA, a single cycle infection of the cells was induced.

Serum neutralizing antibody and resistance to infection were enhanced by administration of divinyl ether-maleic anhydride. This occurred with the virus either in aqueous or oil-emulsified preparations.

There is great interest in the ability of FMDV to survive either in the environment or the recovered animal. Neither Open Air Factor or daylight had a significant effect on the survival of FMDV. These studies were done by suspending the virus on micro threads from spider webs. Wittmann found that FMDV stored at -20°C for 18 weeks was unsuitable for use as vaccine antigen. Storage at - 65°C, however, was not detrimental to the antigen.

The serology and immunology of FMD is of considerable importance particularly as it relates to diagnosis of the disease and vaccine prophylaxis. Antibody forming cells from the spleen and lymph nodes were studied by the Local Hemolysis in Gel test after infection of the donor animals with FMDV. There was no correlation between the number of plaque forming cells from spleen and lymph nodes and the neutralizing, precipitating and complement fixing antibodies in the serum.

Black and Pay did extensive hypersensitivity tests on cattle after
FMDV vaccination. Reagenic antibodies were demonstrated with increasing frequency as the number of vaccinations increased. None of the animals had shown allergic responses.

Guinea pig protection tests, kinetic neutralization and micro-neutralization all showed strain differentiation of FMDV viruses. These tests were all measuring the same antibody as the currently used subtype test done by complement fixation. The hemagglutinating activity of two strains of SAT-2 FMDV were found to be type specific in the inhibition test and as sensitive as serum neutralization in detecting antibody. Forman concluded that the use of a reference strain within each subtype of FMDV would be the best method of classifying the different FMD viruses.

Protection of pigs occurred as early as 1 day after vaccination when they were exposed to virulent virus by donor infected pigs. The vaccine used was absorbed on aluminum hydroxide gel. Comparison of FMDV vaccines in Spain prepared with gel and DEAE-Dextran showed the latter adjuvant to be far superior in the protection of vaccinated pigs. These same workers found that the vaccine antigen was not absorbed to the Dextran at the isotonicity of the body and concluded that the adjuvant effect was due to something other than delayed absorption.

Bahnemann inactivated FMDV for vaccine purposes with binary ethylenimine, this latter compound could be formed apart or directly in the vaccine suspension to be inactivated. Forming the compound in the suspension was an added safety factor for the persons preparing the vaccine.

A technique for growing FMDV in layers of the bovine omasum has been developed. Titers near 10⁷ TCID₅₀ were produced in tissues from this organ agitated in culture medium. Strobbe et al developed a method of testing FMDV vaccine antigen by using the scanning analytical ultracentrifuge. Antigen concentration in the vaccine was expressed as weight and was measured by comparing the centrifuge data to complement fixation results. These same workers studied various fractions of saponin adjuvant to determine the ones responsible for increased antibody response. They associated this increased activity with the fraction that also produced the most irritation.

Breese and Bachrach determined the molecular weight of RNA fragments from FMDV by electron microscopic determinations. Rowlands et al studied naturally occurring empty capsids of FMD as well as those artificially produced which were found to be different from each other. The N-terminal amino acids of the three major FMDV polypeptides have been identified. At least 13 proteins were synthesised in FMDV infected BHK cells. Only a small proportion of these virus-induced proteins were incorporated into the final virus particle; trypsin treatment of purified FMDV destroyed the immunogenicity and resulted in displacement of one of the bands observed by polyacrylamide gel electrophoresis.
REFERENCES


SURVEY FINDINGS OF EQUINE INFECTIOUS ANEMIA
POSITIVE HORSES IN NEW YORK STATE

S. R. Nusbaum*

INTRODUCTION

Identification of an antigen to detect animals with antibodies against equine infectious anemia virus (AGID-EIA test)¹ and evidence that the test was accurate for the diagnosis of the disease,² made possible for the first time, rapid, inexpensive testing of large numbers of animals.

On April 1, 1973 the New York State Department of Agriculture and Markets initiated a program to control and eradicate equine infectious anemia. The design of a control program should take into consideration all clinical and laboratory aspects of the condition, cost (direct and indirect) to the animal owner, the industry and the taxpayer, available manpower and probable duration of the program. In this instance there were many omissions from this list. Part of the unique character of this project was rooted in the fact that except for the piroplasmosis eradication campaign, and the 1971-72 emergency generated effort against Venezuelan equine encephalomyelitis, there had not been any continuing equine disease control program in the United States for a generation. The program did enjoy the enthusiastic support and assistance of the industry which had participated in its formulation.

In April 1974 the Diagnostic Laboratory of the New York State College of Veterinary Medicine undertook to study the characteristics of the infected population to help evaluate the existing program and to determine whether there was a basis for modification.

HISTORY

Although 75,000 animals had been tested at the College before the start of the program there was no sound information regarding the incidence of infection. As testing had no official status prior to April 1973, identification of animals was less than precise, submission of split samples was thought to be common, the sale of infected animals was not inhibited, and a representative population was not being sampled. Taking these factors into consideration it was thought that the true rate of infection might have been about 3%.

As early as 1971 publicity about the disease and the newly developed test caused New York harness tracks to adopt a requirement that animals have a negative test before being granted entry to a track. Some horse shows adopted a similar stance, as did many

*Ithaca, New York
breeding farms which required that mares have a negative test before being accepted for breeding. These attempts at self regulation by the industry had an effect on the number of positive animals which were found once official testing began. Duncan lists the chronology of the program as follows:

1. July 1, 1972—Law allowing freeze branding of reactors became effective.
5. April 1, 1973—N.Y.S. began its official program by issuing quarantines, retesting and freeze branding horses reported positive to the AGID test.
6. July 1, 1973—Regulations requiring negative AGID test for admittance to county fairs became effective.
8. January 1, 1974—N.Y.S. regulations for negative AGID test within 12 months prior to importation became effective.
9. July 1, 1974—N.Y.S. law and regulations requiring negative AGID test within 90 days prior to sale became effective. N.Y.S. law and regulations requiring negative AGID test performed in current or previous calendar year for transportation on the highway became effective”.

During the first year of the program (April 1, 1973-March 31, 1974) 45,062 animals were tested, 669 (1.5%) were infected; the second year, (April 1, 1974-March 31, 1975) 78,238 were tested, 707 (0.9%) were infected.

PROCEDURE

A questionnaire, to be completed and returned by owners, was prepared (Fig. 1) and a list of owners was compiled by the Department of Agriculture and Markets. The laboratory sent each owner a letter which included a brief history of the control program, the purpose of the survey, and a self-addressed envelope. One survey form was provided for each infected animal up to six. Later in the course of the program the letter and survey forms were distributed to owners when state veterinarians issued quarantines on infected animals. The survey covered horses tested during the period of April 1, 1974 through July 30, 1975.
RESULTS

Results are summarized in Tables 1 through 10.

DISCUSSION

During the period covered by the survey there were seven farms which had six or more reactors. There were 178 (22% of the total) infected animals on these farms. No completed questionnaires were returned from any of the seven farms. While a detailed study of these farms is outside the scope of this paper, a brief descriptive statement is necessary because of the large proportion of the total reactors they represent. One farm, a hack and sales stable, which had 64 reactors, also sold blood to a biological supply house. The method of blood collection insured virus spread. A second, which had 34 infected animals, was a hack and sales stable in which the owner used common needles for vaccination and therapy. A third, having 38 reactors, had previously been a mare urine farm in which owners treated without regard to changing needles and syringes between horses. A fourth, with 13 reactors, was a purebred Standardbred farm in which the owner deliberately accumulated infected mares after natural infection had been found on the premises. Three others with 8, 10, and 11 reactors respectively, were owned by part-time dealers.

The distribution according to breed of infected animals (Table 2) was not remarkable. The number of infected Quarterhorses and Appaloosas appears high but as no population figures were available for the entire state their true significance cannot be known. The number of infected Standardbred and Thoroughbred animals was undoubtedly influenced by the voluntary testing which had preceded the official program.

Information concerning the origin of the reactor animals (Table 3) combined with the knowledge that 122 (44%) of the reactor animals had been purchased within 12 months (Table 4) creates a pattern illustrating one important mechanism for distribution of infected animals. This pattern should be considered in building a control program. The pattern of sales also causes one to speculate whether knowledge possessed by the previous owner played a part in the reason for the sale.

The wide distribution of the disease is illustrated by the fact that 206 animals of the 278 (Table 5) from which reports were received, were on individual owners' premises. This is important in contemplating eradication methods and suggests that eventually down-the-road testing will be necessary if the ultimate goal of eradication is to be accomplished. The fact that most of the infected animals were used for pleasure (Table 6) reinforces the concept of wide distribution and supports the observation that animals clinically ill of EIA
are most likely those associated with the stress of intense training. It is common practice for animals which first become infected and clinically ill when being used for racing, showing, and polo to be sold for less demanding purposes. Such animals may perform quite well in this less taxing environment.

It was instructive to find that 108 of 278 animals (Table 7) for which information was received, came from farms which had six or more horses. If the disease were easily transmitted the number of infected animals on these units would have been greater. Kemen4 noted the absence of extensive spread of infection in a New Jersey county although insect vectors were present; he suggested that distance between units and recognition and isolation of acute cases helped keep virus spread at a low level. Although clinically ill animals having a high viremia in the presence of large numbers of biting insects may contribute to moderate spread of infection cumulative observations lead one to believe that, at least in the northeastern United States, the improper use of hypodermic equipment is the most common method of spread. Observations in other areas where insects constitute a hazard 12 months of the year would be useful to determine whether or not this is a local situation.

Questions regarding the number of times that owners observed their animals each week (Table 8) were included in the questionnaire in an attempt to evaluate the validity of the observations (Table 9). The large number who personally cared for their horses lends weight to their observations. This personal attention helps to explain the extreme position which horse owners sometimes assumed about the program. Officials responsible for conducting a program must be aware that as a group horses constitute an industry, but as individuals they may represent attachments that approximate those for a family member.

The subjective signs, or more often their absence, noted by owners helped to delineate the most common form of the disease. All descriptions of the infection indicate that there may be subclinical or inapparent carriers, but the proportion of these to clinical cases is not known. The owners in this survey report few or none of the commonly described signs of illness. Of those who owned an infected animal for more than 12 months 7 (6%) out of 114 responding noted that the animals were in poor condition despite adequate feed, and even fewer noted the other signs listed. This is easily understood, for had the animals experienced problems of significant severity it is unlikely that the owners would have kept them. Those owning their horses 12 months or less were more prone to note problems, but even in this group less than 25% thought that the animals were in poor condition despite adequate feed; 42 (18%) thought there might be some suspicion of infection; 39 (16%) of 244 noted that
the animal did not perform satisfactorily, and lesser numbers listed complaints of animals being off feed, experiencing febrile periods, weakness, or edema. It was surprising to find that 27 out of 30 infected mares (Table 10) maintained good condition during pregnancy, for many infected mares have been known to break down near term. This observation adds a validity to other statistics and impressions which suggest that in a very large percentage of cases the infection does not affect the victim in a noticeable manner.

The seven farms which had six or more infected horses may represent one extreme of the EIA infected population which is characterized by being artificially produced and highly visible. It constitutes an important potential source of spread through sale of infected animals. The other extreme makes up the greater body of infection and is characterized by individual cases; they are used for limited work and although some signs of infection may be observed, performance will not be significantly altered and in the absence of contaminated needles there will be minimal spread to stablemates.

ACKNOWLEDGEMENTS

This work was made possible by the cooperation and assistance of Drs. Harold A. Nadler and Charles C. Duncan, Director and Assistant Director respectively of the Division of Animal Industry, New York State Department of Agriculture and Markets.
Figure I.

Questionnaire distributed to owners of EIA-infected animals.

AGE__SEX__BREED______________________________

I. A) Was the horse (1) home-raised__________, (2) purchased from dealer,_______ (3) purchased from breeding farm______, (4) purchased from private owner______, (5) other _______?

B) When did you take possession of the animal?__________________

II. A) At the time of the positive EIA test, was the horse maintained on (1) your premises______, (2) boarding stable______, (3) training farm______, (4) other__________________?

B) Was the horse used for (1) pleasure riding______, (2) show ______, (3) racing______, (4) other__________________?

C) How many other horses were maintained on the premises? 0) 1) 2) 3) 4) 5) 6-10) 10-20) 20+)

III. Did horse display any of the following signs during the twelve months previous to the positive test? During the twenty-four months previous to the test?

A) Failure to perform work satisfactorily even though no lameness or other obvious problem. 12 month 24 month

B) Periods of apparent weakness or incoordination of rear legs.

C) Poor keeper... could not keep condition despite adequate feed.

D) Periods of fever.

E) Swelling of chest, body wall, and sheath (or udder).

F) Were there any signs which made you suspicious before the test that the horse might be infected?

G) Was the horse “off feed” during this period? Occasionally... Frequently...

H) How many times a week did you personally observe the horse? 1) 2) 3) 4) 5) 6) 7)

IV. BRED MARES ONLY

A) Did she maintain good condition during pregnancy? yes) no)

B) Did she deliver a live foal this year? yes) no)
Table 1.

Survey Findings of Equine Infectious Anemia
Test Positive New York State Horses

Equines Tested, EIA-AGID, April 1, 1974-June 30, 1975

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Total</td>
<td>95,251</td>
</tr>
<tr>
<td>Infected</td>
<td>812     (0.85%)</td>
</tr>
<tr>
<td>Survey responses</td>
<td>278     (34%)</td>
</tr>
</tbody>
</table>

Table 2.

*BREED*

<table>
<thead>
<tr>
<th>Breeds</th>
<th></th>
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<tbody>
<tr>
<td>Grade</td>
<td>90</td>
</tr>
<tr>
<td>Quarterhorse</td>
<td>57</td>
</tr>
<tr>
<td>Appaloosa</td>
<td>26</td>
</tr>
<tr>
<td>Standardbred</td>
<td>13</td>
</tr>
<tr>
<td>Thoroughbred</td>
<td>13</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>52</td>
</tr>
<tr>
<td>No response</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>278</td>
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</table>

Table 3.

*ORIGIN*

<table>
<thead>
<tr>
<th>Origin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Home raised</td>
<td>16</td>
</tr>
<tr>
<td>Purchased from dealers</td>
<td>99</td>
</tr>
<tr>
<td>Purchased from breeding farm</td>
<td>8</td>
</tr>
<tr>
<td>Purchased from private owner</td>
<td>120</td>
</tr>
<tr>
<td>Other</td>
<td>29</td>
</tr>
<tr>
<td>No response</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>278</td>
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Table 4.

*PERIOD OF OWNERSHIP*

<table>
<thead>
<tr>
<th>Period of Ownership</th>
<th></th>
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<tbody>
<tr>
<td>Less than 12 months</td>
<td>122</td>
</tr>
<tr>
<td>12 to 24 months</td>
<td>18</td>
</tr>
<tr>
<td>More than 24 months</td>
<td>133</td>
</tr>
<tr>
<td>No response</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>278</td>
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Table 5.

_STABLED_

<table>
<thead>
<tr>
<th>Owner's premises</th>
<th>206</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boarding stable</td>
<td>36</td>
</tr>
<tr>
<td>Training farm</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
</tr>
<tr>
<td>No response</td>
<td>8</td>
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</tbody>
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Total 278

Table 6.

_USE*

<table>
<thead>
<tr>
<th>Pleasure</th>
<th>215</th>
</tr>
</thead>
<tbody>
<tr>
<td>Show</td>
<td>34</td>
</tr>
<tr>
<td>Racing</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>40</td>
</tr>
</tbody>
</table>

Total 297

*Some dual purpose

Table 7.

NUMBER OF OTHER HORSES ON FARM

<table>
<thead>
<tr>
<th>POSITIVE CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6-10</td>
</tr>
<tr>
<td>10-20</td>
</tr>
<tr>
<td>20+</td>
</tr>
<tr>
<td>No response</td>
</tr>
</tbody>
</table>

Total 278

Table 8.

TIMES PER WEEK OWNER OBSERVED

<table>
<thead>
<tr>
<th>POSITIVE CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x - 1</td>
</tr>
<tr>
<td>2x - 1</td>
</tr>
<tr>
<td>3x - 6</td>
</tr>
<tr>
<td>4x - 3</td>
</tr>
<tr>
<td>5x - 2</td>
</tr>
<tr>
<td>6x - 1</td>
</tr>
</tbody>
</table>
Table 9.

**SIGNS OBSERVED BY OWNER**

<table>
<thead>
<tr>
<th>(N = Affirmative/D = Total Answers)</th>
<th>Ownership 12 months</th>
<th>Ownership 12 or more months</th>
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<tbody>
<tr>
<td>Poor Condition despite adequate feed</td>
<td>54/224</td>
<td>7/114</td>
</tr>
<tr>
<td>Signs suspicious of infection</td>
<td>42/228</td>
<td>3/107</td>
</tr>
<tr>
<td>Failure to perform satisfactorily</td>
<td>39/244</td>
<td>1/125</td>
</tr>
<tr>
<td>Off feed</td>
<td>29/228</td>
<td>3/106</td>
</tr>
<tr>
<td>Febrile periods</td>
<td>23/210</td>
<td>2/113</td>
</tr>
<tr>
<td>Weakness and incoordination</td>
<td>23/231</td>
<td>2/127</td>
</tr>
<tr>
<td>Edema, chest, body wall, sheath or udder</td>
<td>11/222</td>
<td>3/115</td>
</tr>
</tbody>
</table>

Table 10.

**BRED MARES**

<table>
<thead>
<tr>
<th>Maintain good condition during pregnancy?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>Did she deliver a live foal?</td>
<td>20</td>
<td>8</td>
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</tbody>
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**BIBLIOGRAPHY**

COMMITTEE OF INFECTIOUS DISEASES OF HORSES

Chairman: Dr. C. L. Campbell, Tallahassee, Fla.

Vice Chairman: Dr. W. O. Kester, Golden, Colorado


The Committee on Infectious Diseases of Horses received a progress report on equine infectious anemia from the Chief Staff Veterinarian for Equine Diseases of USDA, APHIS, Veterinary Services. This progress report indicated that the AAVLD has been requested to review and recommend appropriate amendments to the present criteria for approval of laboratories to perform the AGID test for equine infectious anemia. Currently 104 laboratories have been approved, 60 of which are state labs, three Army, and 41 private. Seven of the private laboratories are under supervision of a practitioner. The AAVLD has voiced objection to private practitioners having an interest in or supervision over such laboratories. It is anticipated that an early complete report from AAVLD will be forthcoming.

A report was received on the current status of controlled studies conducted at the University of Florida concerning the treatment of EIA reactors with thiouracil. The results of these studies indicate that thiouracil treatment of 16 animals was ineffective in eliminating infectious anemia virus from this group of inapparent carriers. Attempts are being made to substantiate or refute claims that six of approximately 70 horses treated with thiouracil under field conditions became AGID test negative. The identity, history, and current AGID status of these animals will be carefully examined. However, in consideration of the lack of efficacy of thiouracil under controlled conditions, the College of Veterinary Medicine, University of Florida does not at this time recommend the use of thiouracil in treatment of EIA.

It was reported that experimental vaccine trials at Texas A & M University failed to protect equidae against the virus of EIA.

Since April 11, 1973, 34 states have adopted requirements for negative EIA tests for entry into those states. Twenty-four specify
a six-month test, nine a 12-month test, and one requires a 90-day test. One additional state has adopted requirements for a 6-month negative test to become effective in January 1976. This compares with 17 states which had adopted interstate requirements at this time last year. Nine states of the 34 have additional regulations requiring negative tests for animals entering assembly points or for public or private sale.

In fiscal 1975, 9,614 EIA reactors were found in 613,396 equidae tested, an infection rate of 1.57%. Similarly, in fiscal 1974, 9,089 reactors were revealed from 354,412 animals tested, for an infection percentage of 2.56. The greatest incidence of infection is located in states bordering the Gulf of Mexico and the lower Mississippi River, which is consistent with the known insect vectors.

The figures submitted to the Committee indicated that proper disposition or accounting for known EIA reactors in some instances is inconclusive. More emphasis in such areas should be placed upon compliance with accepted identification procedures and disposal of infected animals.

In the 1974 report, the Committee provided an outline for a State Control Program for Equine Infectious Anemia which recommended that horses entering the state be AGID negative within six months prior to entry. This recommendation has been confirmed by a USDA biometrician following a study of EIA exposure probability and consideration of all factors which contribute to the chance of serious spread of disease.

Extensive discussion ensued surrounding the problem of falsification of EIA test records accompanying horses moved for various purposes. Several ideas were suggested, including providing singleton horse charts, but because of the diversity of problems inherent with this particular area of concern, it was deemed advisable to appoint a subcommittee to work out the details of deterring fraud in the identification procedures. The subcommittee will report to the parent body its recommendations at an early date.

The Committee received a report from University of Florida workers indicating that prior administration of tissue culture vaccines as a means of protecting horses from certain diseases might have a tendency to mask the results of EIA tests later made on these animals. In evaluating this work replicated by Veterinary Services Diagnostic Laboratory scientists, their conclusion is that this finding does not constitute a real problem but will be kept under surveillance. As antigen production becomes more sophisticated, the deletion of bovine serum in its production will eliminate this problem.

Recent findings by researchers at Cornell University have indicated in some EIA carrier animals that at times it is impossible to demonstrate febrile response with test ponies when whole blood is used. Their research reveals that both the removal of serum and re-
peated inoculation of large numbers of leukocytes appeared neces-
sary in assuring the detection of EIA virus in the blood of inapparent
carriers. With the foregoing in mind it is deemed necessary that
the protocol for conducting horse inoculation tests for equine infec-
tious anemia as presented in the 1971 Report needs to be revised,
and to this end a subcommittee was appointed to review the protocol
and report to the full committee at its next meeting.

It was reported that the film on Equine Infectious Anemia which
was prepared in 1968 is being updated by the United States Depart-
ment of Agriculture with the assistance of Pitman-Moore Biological
Company, and it is anticipated that the revision should be available
by early 1976.

It was brought to the Committee's attention that two cases of
refusal to identify as reactors infected horses in the states of Mary-
land and Virginia were successfully prosecuted in the courts by the
state officials seeking compliance with recommended EIA procedures.
It is felt by the Committee that these two instances attest the validity
of previous committee recommendations and should serve as prece-
dents in the event of future altercations wherein the owner of in-
fected animals would similarly refuse to comply with program
standards.

The 1974 report of this Committee urged the Secretary of Agri-
culture to immediately exercise interim authority to require that
all horses and other equidae offered for importation into the United
States be officially tested for equine infectious anemia and, if found
negative and otherwise eligible, be allowed to enter. The Committee
deplores the fact that this action has not been taken by the Secretary
in protecting our domiciled horse population and wishes to iterate its
posture in this matter of last year. A resolution has been presented
to the Committee on Nomination, Resolutions, and Internal Affairs
for appropriate action by this Association this week.

It was reported to the Committee that apparently there is a di-
versity of methods of identification of EIA reactors among the sev-
eral states, which is not in keeping with recommendations previously
made by this Committee in the interest of maintaining uniformity.
Since this condition does exist, however, the USDA has agreed to
compile the various methods now being employed by the several
states for identification of reactors, to be published and distributed
to all states.

In 1973 a subcommittee was appointed to help develop practical
means of individual horse identification. This subcommittee has, since
its inception, continued efforts toward improving such identification
procedures and reported to the parent body that, although slow, prog-
ress is being made in these endeavors.

The Committee was privileged to receive a detailed and compre-
hensive report of the extensive preventive and surveillance measures
being carried on in Mexico in connection with Venezuelan Equine Encephalomyelitis by Dr. Victor Schroeder, Chief of Equine Health, VEE National Campaign, Direccicon General de Sanidad Animal, of Mexico, D. F. It is gratifying to know through the extensive vaccination of horses which is taking place in Mexico that the chances of this disease again being introduced into the United States are greatly minimized. In view of the foregoing, upon evaluation by USDA, the existing regulations of those few states still requiring VEE vaccination prior to entry should be reviewed as to necessity for continuance.

Respectfully submitted,

C. L. Campbell, Chairman
PROBLEMS ASSOCIATED WITH IMPORTATION OF EXOTIC BIRDS

George P. Pierson*

Summary

The importation of exotic birds became a problem for the U. S. Department of Agriculture in 1970-71 when such importations were found to be associated with the introduction of velogenic viscerotropic Newcastle disease. This finding resulted in a total prohibition of bird importations in 1972. Provisions for resuming importations were adopted in late 1973. Problems with the exotic bird importations have been numerous. Some examples are: court action against the Department, diagnosis of chlamydiosis in USDA employees, limits on the availability of funds and manpower to operate the import program, and detection of the exotic Newcastle disease virus in a relatively high percentage of the lots of birds offered for entry. This is a report of some of these problems.

Recent History

In 1970 and 1971 when the U. S. Department of Agriculture (USDA) reported the isolation of velogenic viscerotropic Newcastle disease virus from psittacine and myna birds and asserted that such importations represented a potential threat to the United States poultry industry, many individuals expressed considerable skepticism concerning this threat. This skepticism was expressed by several individuals associated with the pet bird industry when they sought a court injunction to prevent the Department of Agriculture from regulating the importation of psittacine and myna birds. This injunction was initially sought in April 1972. After testimony by USDA experts, the presiding Federal judge ruled that an injunction would not be granted.

A proposal to regulate the importation of psittacine and myna birds was published in March 1972. The amendment called for a 45-day isolation period in the country of export in a facility approved by the Department. Standards for the facilities were published in the Federal Register in May 1972. After inspection of facilities in Europe and Southeast Asia, which were originally approved as treatment centers under the U. S. Public Health Service regulations pertaining to the importation of psittacine birds, the USDA was ready to approve two facilities—one each in India and Singapore. However, collection of samples from birds offered for entry at the Los Angeles International Airport, with subsequent virus isolations, indicated other avian species could be associated with the introduction

*Chief Staff Veterinarian, Import Birds and Poultry, Veterinary Services,APHIS, USDA
of exotic Newcastle disease. This finding, coupled with virus isolations from other imported birds, resulted in a total prohibition on commercial bird importations in August 1972. Concurrently with the prohibition, a provision allowing the importation of two birds per individual as personally owned pets was adopted.

The prohibition of commercial shipments of birds resulted in considerable pressure being exerted upon the Department to provide a means for such shipments to be resumed. The basic concepts and knowledge concerning the procedures to be followed to safely import animals were readily available to the Department because of the Department of Agriculture's involvement with regulating animal importations since the 1880s. Poultry import regulations were promulgated in 1950. Space for the purpose of quarantine at the USDA operated animal import facilities was limited. Funds to acquire land and/or building facilities to handle additional animal importations were also limited. Therefore, a decision was made to allow the construction of privately owned and operated facilities for the commercial importation of birds. An amendment providing for the importation of birds in privately operated facilities at or near designated ports of entry was adopted in October 1973.

General Information

The first facility was approved in December 1973. By July 1, 1974, ten additional facilities had been approved. Numerous requests for approval of additional facilities were pending. It was obvious that the Department's ability to provide minimum supervision of these facilities would be exhausted if approval of facilities continued. Therefore in August 1974, it was decided to hold in abeyance the approval of additional facilities with an understanding that those individuals to whom a verbal or written commitment had been made would receive consideration for the approval of a facility. Even with the new policy, eight more facilities were approved by January 1975. Since then, four facilities have been approved making a total of 23 approved facilities. Construction of one facility is being delayed because of local zoning problems. The 23 approved facilities are located at the following designated ports of entry: Chicago, Illinois (1); Detroit, Michigan (1); New York, New York (1); Miami, Florida (9); Brownsville, Texas (1); San Ysidro, California (1); Los Angeles, California (8); San Francisco, California (1).

Recently we were informed that legally it may be necessary to reconsider the Veterinary Services policy of not approving additional facilities. However, should it become necessary to approve additional facilities, availability of manpower to supervise said facilities will limit the number of facilities that can be operational at a given time.

Commodity Credit Corporation (CCC) monies are being utilized to fund the bird import program. During fiscal year 1975, $985,349...
was expended for this purpose. For fiscal year 1976, $1,417,000 has been budgeted for this program. Needless to point out, should additional facilities be approved without some mechanism being utilized to limit the number of man-years available for servicing these facilities, the expenditure of funds would increase proportionally.

**Program Information**

The first lot of commercial birds imported under the USDA regulations was released from quarantine in January 1974. During fiscal year 1974, 19 lots of birds were offered for entry, of which 13 lots (27,696 birds) were found eligible for entry, while 6 lots (31.6% of lots offered) were refused entry. In fiscal year 1975, 90 lots were offered for entry. Seventy-one lots (124,597 birds) were found eligible for entry, while 19 lots (21.1% of the lots offered) were refused entry. All of the lots refused entry occurred because a Newcastle disease virus was isolated. Most of the isolates were characterized as velogenic viscerotropic.

It is of interest that in approximately 40% of the lots refused entry, the classic clinical signs of Newcastle disease were not observed in the imported birds. The lack of clinical signs in one group of birds precipitated a court case being brought against the Department by one of the importers. This occurred in September 1974, in Miami, Florida. In January 1975, the presiding Federal judge, after hearing considerable evidence from witnesses for both parties, ruled that the Department should release the involved birds. In his findings the judge agreed with the importer's contention that the "two isolations of theVVND virus be regarded as spurious." The Department appealed this ruling to the U. S. Court of Appeals for Fifth Circuit, New Orleans, Louisiana. In June 1975, the appeals court ruled: (1) the U. S. Department of Agriculture acted within its authority, and (2); canaries (2); starlings (2); giant coots (1); myna (1). The period involved was July 1, 1973, through June 30, 1975, during which time 26 lots of infected birds were detected.

Psittacine species are most often involved when VVND virus isolations are made. They account for over 50% of the isolations. A summary of VVND virus isolations by species from commercial shipments of birds is as follows: Psittacine (17); finches (5); cranes (2); canaries (2); starlings (2); giant coots (1); myna (1). The period involved was July 1, 1973, through June 30, 1975, during which time 26 lots of infected birds were detected.

The number of countries which shipped infected lots of poultry or birds to the United States increased from 14 in fiscal year 1974 to 23 in fiscal year 1975. Commercial shipments of infected birds accounted for 7 of the countries in fiscal year 1974, while in fiscal year 1975, this increased to 15. On July 1, 1975, the Department adopted a policy whereby import permits for a given country will not
IMPORTATION OF EXOTIC BIRDS

be issued for a minimum 90-day period following the isolation of a VVND virus from birds from that country. Issuance of permits will be resumed, providing the national veterinary services of the involved country present an acceptable plan of action to prevent the shipment of VVND infected lots of birds to the United States.

Presently permits for commercial shipments of birds are not being issued for the following countries: Belgium, Honduras, Indonesia, Malaysia, Nigeria, Paraguay and India. Since this policy was adopted, 2 infected lots (11.1% of those offered for entry) of birds have been detected. The figure of 11.1% covers the first quarter of fiscal year 1976 and compares to 31.6% and 21.1% respectively for fiscal year 1974 and 1975. If the reduced number of infected lots continue then the new policy of not issuing permits will prove to be a wise decision.

One of the reasons for adopting regulations for importing birds was to reduce smuggling of birds, which had increased during the prohibition. Apprehension of individuals attempting to illegally enter commodities into the United States is the responsibility of the U. S. Customs Service. Contacts between USDA and U. S. Customs Service developed during the prohibition have continued. Anytime information becomes available to USDA personnel concerning smuggling it is furnished to the U. S. Customs Service. This cooperation has resulted in the apprehension of several individuals attempting to illegally enter birds into the United States. During fiscal year 1975, 106 investigations of suspected smuggling were made by Customs agents, resulting in 19 arrests and 13 seizures of birds.

Prior to 1967, the importation of psittacine birds into the United States was prohibited. In 1967 the U. S. Public Health Service adopted regulations which required that psittacine birds be treated with chlortetracycline for a minimum period of 45 days prior to their importation into the United States. When the USDA became involved with bird importations, the U. S. Public Health Service decided to allow the antibiotic treatment of such birds to be accomplished during the 30-day quarantine period here in the United States. Since the chlamydial organism may be shed for several days following the feeding of properly medicated feed, USDA field personnel working closely with such birds received instructions to wear gauze surgical face masks and to take other precautions as an aid to prevent them from becoming infected. Even with these precautions, several USDA employees have developed symptoms compatible with a diagnosis of psittacosis. Post infection serum titers in at least two employees were indicative of recent exposure to chlamydial organisms. The precautions to be followed to prevent infection have been re-emphasized to all personnel working with imported psittacine birds. In fact the entire problem of psittacosis in psittacine birds is under review. Because of the human health problem and the high number of VVND isolates associated with this species, it may be necessary to consider a special import requirement for this species.
**Conclusions**

The USDA avian import program has presented many problems. Some of the problems have been resolved completely, some only partially. Adequate solutions for some of the problems are still being sought. USDA personnel, especially field personnel, have made the program work successfully in spite of many handicaps and obstacles.

Various criteria could be used to evaluate the bird import program. If the criteria was the number of birds imported, then the program is only partially successful. This is especially true when a comparison of the number imported during the past fiscal year (about 125,000 birds) is made to the number imported prior to the USDA regulations between 2 to 3 million birds annually). If the criteria for evaluating the program is preventing the introduction of communicable diseases of poultry, especially exotic Newcastle disease, then the program is successful. To date, no outbreak of VVND has been associated with birds released from USDA approved quarantine facilities. The Department effort is directed to continue this success and to insure that VVND is not introduced into domestic avian population through importations.
VIRAL ISOLATIONS FROM EXOTIC BIRDS OFFERED FOR IMPORTATION INTO THE UNITED STATES

J. E. Pearson, DVM; D. A. Senne, BS;
E. A. Carbrey, VMD, MS; G. A. Erickson, DVM, MS
and L. D. Miller, DVM, PhD

Summary

Exotic birds are held in private quarantine stations for at least 30 days prior to importation into the United States. Specimens are collected for isolation of viruses that might be detrimental to domestic poultry, particularly fowl plague virus and velogenic viscerotropic Newcastle disease virus (VVNDV).

Viruses have been isolated from 65 of 127 lots of birds offered for importation into the United States. Newcastle disease virus was isolated from 32 lots: 28 of these isolates were VVNDV, 1 was a mesogenic strain and 3 were lentogenic strains. The other isolates were hemagglutinating viruses, such as Yucaipa and influenza.

The virus-isolation results are described in this paper.

Introduction

Velogenic viscerotropic Newcastle disease has been reported in parts of Asia, Africa and eastern Europe since 1926. In the mid 1960's, this highly virulent Newcastle disease virus was spreading to new areas. It caused high mortality in vaccinated and unvaccinated chickens. By 1970 the disease had been reported throughout most of the world. Epidemologically, the movement of pet birds, particularly psittacines, appeared to be responsible for this spread. Velogenic viscerotropic Newcastle disease virus (VVNDV) was isolated from quarantine stations in Holland, Germany and Republic of South Africa in 1970 and epornitics in England, Europe and South America were attributed to imported birds.

In 1971 an epornitic of velogenic viscerotropic Newcastle disease occurred in southern California. Contact of infected imported birds with domestic poultry was believed to have initiated the outbreak. Importation of exotic birds into the United States was prohibited in August, 1972. In October 1973 the importation of birds was resumed but only through private quarantine facilities approved by Veterinary Services, Animal and Plant Health Inspection Service, United States.

From Diagnostic Virology Laboratory, Veterinary Services Laboratories, APHIS, USDA, Ames, IA.

The authors wish to thank members of the Equine and Avian-Ovine Viruses Sections, Diagnostic Virology Laboratory, Veterinary Services Laboratories, Ames, IA for technical assistance.
Department of Agriculture (U.S.D.A). Avian species classified as poultry are imported through the USDA facilities.

The birds are held in quarantine for at least 30 days or until all laboratory tests are completed. Specimens are collected from the birds for the isolation of viruses detrimental to domestic poultry. Primary emphasis is placed on the isolation of fowl plague virus and VVNDV.

A brief review of the methods for isolation and identification of viruses, and virus-isolation results through September 1, 1975 are presented.

Materials and Methods

Sampling Technique — Tissues from all the birds that die, up to 150 per day, between the first and twenty-first day are collected and sent to the laboratory. Small pieces of brain, lung, trachea, spleen and terminal intestine are submitted in brain heart infusion broth. Between the seventh and fourteenth day of the quarantine, cloacal swabs are collected from 150 live birds. Swabs are pooled 5 per tube.

Virus Isolation — Virus isolation and identification technique has been described. Each of four susceptible 8 to 10-day-old embryonated chicken eggs is inoculated with 0.3 ml of the broth from the tissues submitted plus antibiotics. Embryos that are alive after five days are discarded, and the specimen is reported as negative. If the embryos die, the allantoic fluid is checked for virus.

If Newcastle disease virus (NDV) is identified, its pathogenicity for chickens and its mean death time in chicken embryos are determined as previously described.

Viral isolates other than NDV are inoculated into 4 chickens and 4 turkeys and, if an isolate is not pathogenic for chickens and/or turkeys, the quarantined birds may be released. These isolates are also screened by the hemagglutination-inhibition (HI) test against Yucaipa antiserum, 8 subtypes of avian influenza antiserum and 3 subtypes of fowl plague antiserum.

Confirmation of Results — The tissues submitted are stored at —20 C in the original tube until the case is reported. The supernate broth from the tissues is stored at —70 C for 30 days after the case is reported. If a pathogenic virus is isolated, reisolation is attempted from the supernate and the tissue. If the pathogenic virus cannot be reisolated, the submission is reported as negative.

Results

Through September 1, 1975, 127 lots of birds have been offered for importation into the United States. An average of 223 specimens have been submitted from each lot. Viruses were isolated from 65 lots of birds.
VIRAL ISOLATIONS FROM EXOTIC BIRDS

Newcastle disease virus was isolated from 32 lots. Twenty-eight of these isolates were VVNDV. A mesogenic NDV isolation was made from a canary in a lot of 1150 birds. One lentogenic isolate was made from each of two lots of finches. The third lentogenic isolate was from 21 birds in a lot of 632 of the Family Psittacidae. All the lentogenic strains were non-pathogenic for chickens and the chicken embryos inoculated had a mean death time of greater than 100 hours. Lentogenic NDV was isolated from an additional 3 lots but could not be confirmed by reisolation.

Sixty-four percent (18/28) of the VVNDV positive lots and 75% (301/453) of the VVNDV positive birds were the Family Psittacidae. Clinical VVNDV was observed only in the Psittacidae Family 54% (411/760) of the specimens submitted to the laboratory were positive. Birds of the Order Kakatoeinae (cockatoo) had a larger percent of positive specimens than other species—91% (126/138). Cockatoos also developed the most acute-severe clinical signs.

VVNDV was isolated from 5 lots consisting primarily of birds of the Family Fringillidae—4 lots of finches and 1 lot of canaries. Only one isolation was made from each of 2 lots of finches and one lot of canaries. The other 2 lots of finches had 3 and 6 isolations each. From the positive lots containing birds of the Fringillidae Family, 0.9% of the specimens were positive. The VVNDV isolations from the Family Fringillidae usually did not kill all eggs on initial isolation and were difficult to confirm by reisolation.

Single isolations were made from 2 of the 5 other VVNDV positive lots. Both lots contained a variety of different birds which had been obtained for zoological parks. These isolations were from a crane and a coot. The remainder of the birds were held for 6 months with cloacal swabs collected 3 times from all birds. All virus isolation attempts from other dead birds and swabs were negative and the birds were released to zoos approved by the USDA.

Another group of zoological birds was depopulated after VVNDV was isolated from the tissues of 3 birds—a velvet backed starling, a wattled starling and a crowned crane.

VVNDV was isolated from the tissues of 3 starlings in a lot of 98 starlings and 4531 finches. Virus was not isolated from the finches.

The remaining VVNDV positive lot consisted of 4392 birds of 18 different species. The first isolations were from tissues of turacos with subsequent isolations from parrots and parakeets.

All the velogenic isolates from quarantine stations were also viscerotropic. The range of the mean death times in embryonated chicken eggs was between 50 and 70 hours.

The first VVNDV isolation in all lots was made from tissues of dead birds collected within 14 days after the birds entered quarantine and about 60% of these isolates were made within 7 days after the birds entered the facility. Twelve isolations were made from swab
pools, all of which were collected from birds of the Family Psittacidae. In all cases, there had been previous isolations from tissues of dead birds.

Other hemagglutinating viruses were isolated from 45 lots of birds; however, NDV was also isolated from 8 of these same lots. A total of 2169 isolations were made with 76% (1652/2169) from finches and canaries (Table 3). Hemagglutinating isolations were made from 83% (33/40) of the finch lots and 59% (10/17) of canary lots, and 19% (14/75) of the lots made up of other species. Hemagglutinating-virus isolations have been made from 11% of the tissues from finches as compared to 1.5% of the tissues of other birds. The most isolations made was 473 from the tissues of 1455 finches which were in a lot of 14,668.

If death is excluded as a clinical sign, there was no correlation observed between clinical signs and the isolation of hemagglutinating viruses other than VVNDV. Hemagglutinating viruses were isolated from one group of clinically normal birds for 7 of the 12 months they were in quarantine.

Two hundred eighty of the hemagglutinating viruses have been completely serotyped (Table 3). An additional 86 have been typed as influenza but due to cross reactions, the specific type has not been determined. The hemagglutinating activity of the isolates has not been inhibited by the fowl plague antiserums. None of the isolates other than VVNDV have been pathogenic for chickens or turkeys. In several cases more than one type of hemagglutinating virus, has been isolated from the same lot of birds. A hemagglutinating virus that was negative on NDV HI test was isolated from 2 lots of birds; however, VVNDV was recovered from the chickens that died following inoculation.

Discussion

The movement of exotic birds appears to play an important role in the spread of VVNDV. Almost 25% of the lots offered for importation into this country were infected. The members of the Family Psittacidae, which includes cockatoos, parrots, parakeets, macaws, etc., appear to pose the greatest potential threat for the spread of VVNDV. Most isolations were made from these species and clinical signs were frequently observed. Clinical signs were also observed in laboratory studies. Other species from which VVNDV was isolated could also play a role in spread of the disease. Fewer isolations were made from the clinically normal birds, particularly the finches. However, the infected birds in these lots could be as important a threat to other birds as the clinically affected birds. The fact that they appeared normal would have allowed them to move freely and be put in with groups of NDV susceptible psittacines.

The virus isolation technique was developed to rapidly screen
large numbers of specimens for the presence of viruses that kill chicken embryos within 5 days. Less pathogenic viruses such as infectious laryngotracheitis, avian adenoviruses, many of the influenza strains and the lentogenic strains of NDV will not be detected. The technique does detect VVNDV.

It is of interest that all the velogenic strains of NDV isolated produced visceral lesions. Only one mesogenic strain and no velogenic neurotropic or pneumotropic strains were isolated. These strains have been assumed to be widespread; however, they were not prevalent in the exotic birds. The mean death time of chicken embryos inoculated with VVNDV averaged about 63 hours which is higher than indicated in the literature. However, most of these strains were as virulent for chickens as any strain that has been described.

The large percentage of isolations from tissues of dead birds indicates that many of the infected birds do die. It also indicates that this is the most effective way to screen for presence of the virus.

The large number of hemagglutinating viruses isolated is also of interest. It appears that the finch is a carrier of Yucaipa virus and several influenza virus strains. Viruses that have been isolated were not pathogenic for chickens or turkeys. In the one group of birds that was held for a year, hemagglutinating viruses were isolated from the tissues of the birds found dead for the first 7 months. This would indicate that if new birds are not introduced the infection will be self limiting. However, this may have occurred because the virus caused the deaths of infected birds.

Since the quarantine program was established, it has stopped the importation of a large number of VVNDV infected birds that could have resulted in the reintroduction of the disease into the United States.
Table 1. Velogenic Viscerotropic Newcastle Disease Viral Isolations from Import Quarantine Stations - Oct 1973 - Sept 1975

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<th>Family</th>
<th>No. of Lots</th>
<th>Positive lots</th>
<th>VVNDV with Clinical Positive for</th>
<th>VVNDV Reported</th>
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<td>16</td>
<td>18</td>
<td>16</td>
<td>54</td>
</tr>
<tr>
<td>Fringillidae</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>(Finches &amp; Canary)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sturnidae</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.0</td>
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<td>(Starlings)</td>
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<tr>
<td>(Crane)</td>
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<td>(Touraco)</td>
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Table 2. Velogenic Viscerotropic Newcastle Disease Virus Isolations from Import Quarantine Stations - Oct 1973 - Sept 1975

<table>
<thead>
<tr>
<th>Birds Examined (common name)</th>
<th>Number of Isolates</th>
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<tr>
<td>Parrot</td>
<td>180</td>
</tr>
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<td>Conure</td>
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</tr>
<tr>
<td>Cockatoo</td>
<td>126</td>
</tr>
<tr>
<td>Parakeet</td>
<td>43</td>
</tr>
<tr>
<td>Macaw</td>
<td>1</td>
</tr>
<tr>
<td>Toucan</td>
<td>6</td>
</tr>
<tr>
<td>Canary</td>
<td>1</td>
</tr>
<tr>
<td>Starling</td>
<td>5</td>
</tr>
<tr>
<td>Crane</td>
<td>2</td>
</tr>
<tr>
<td>Giant Coot</td>
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</tr>
<tr>
<td>Mynah</td>
<td>1</td>
</tr>
<tr>
<td>Sparrow</td>
<td>1</td>
</tr>
<tr>
<td>Honey Creeper</td>
<td>3</td>
</tr>
<tr>
<td>Finches</td>
<td>17</td>
</tr>
<tr>
<td>Touraco</td>
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</tr>
<tr>
<td>TOTAL</td>
<td>453</td>
</tr>
</tbody>
</table>
VIRAL ISOLATIONS FROM EXOTIC BIRDS

Table 3. Virus Isolations, Other Than Newcastle Disease Virus, From Import Quarantine Stations - Oct 1973 - Sept 1975

<table>
<thead>
<tr>
<th>Yucaipa</th>
<th>Avian Influenza</th>
<th>Avian Influenza (cross type)</th>
<th>Hemagglutinating virus</th>
<th>Total</th>
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<tr>
<td>Finch</td>
<td>124</td>
<td>127</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Canary</td>
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<td>1</td>
<td>80</td>
<td>83</td>
</tr>
<tr>
<td>Parrot</td>
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<td>21</td>
<td>258</td>
<td>261</td>
</tr>
<tr>
<td>Parakeet</td>
<td></td>
<td>24</td>
<td>89</td>
<td>113</td>
</tr>
<tr>
<td>Conure</td>
<td></td>
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<td>20</td>
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</tr>
<tr>
<td>Flamingo</td>
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<td></td>
<td>2</td>
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<tr>
<td>Lovebird</td>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mynah</td>
<td></td>
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<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Nightingale</td>
<td></td>
<td>11</td>
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<td>13</td>
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<tr>
<td>Macaw</td>
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<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Species not identified</td>
<td></td>
<td>37</td>
<td>43</td>
<td>80</td>
</tr>
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</table>

Total 129 148 3 86 1803 2169

REFERENCES


Newcastle Disease

Two outbreaks of Velogenic Viscerotropic Newcastle Disease (VVND) during the last year were reported by the APHIS poultry disease staff. The national surveillance program for exotic Newcastle disease including good lines of communication with poultry and bird industries and practicing veterinarians was responsible for the rapid detection of the outbreaks in Pharr, Texas, and Bay Shore, New York.

PHARR, TEXAS

On June 6, 1975, information was obtained from a veterinary practitioner that a small mixed poultry flock of 28 chickens and fighting chickens in Pharr, Hidalgo County, Texas, was sick and dying. An investigation of the birds on the premises revealed highly suspicious lesions of VVND. Specimens were collected and sent to Veterinary Services Laboratories, Ames, Iowa. On June 12, Newcastle disease was isolated and the 11 remaining chickens were depopulated and indemnity paid to the owner. Cleaning and disinfecting was done on June 13.

The Federal quarantine of the Pharr area was released July 24, at a total cost of $15,226.

LONG ISLAND, NEW YORK

On February 26, 1975, the Area Veterinarian in Charge in New York reported that there were sick birds in a pet shop in Bay Shore, Long Island, New York. Specimens were taken to the veterinary
diagnostic laboratory at Cornell University. On February 28 it was reported that embryos had died in 48 hours and were HA positive. Embryonic material was dispatched from Cornell to Veterinary Services Laboratories, Ames, Iowa, at once. On March 5 exotic Newcastle disease was confirmed by Veterinary Services Laboratories.

The pet shop received birds from nine sources. All sources were placed under surveillance and virus isolation attempts were from birds at the premises when deemed necessary. No disease was disclosed from these investigations. There were dozens of calls to the task force from people who had purchased birds from the pet shop. The calls were stimulated by the news media's reporting the outbreak. All purchases from the pet shop within the time frame were observed and many cloacal and tracheal swabs were taken. Newcastle disease was not found in birds other than those in the pet shop where the outbreak occurred: 415 birds were destroyed, and the owner was paid indemnity. The total cost of the outbreak was $166,661.

**COOTS**

Late in February 1975 a severe dieoff in American coots (*Fulica americana*) occurred in the Back Bay area of Virginia, south of Norfolk. The cause of the outbreak was determined to be fowl cholera (*Pasteurella multicauda*). In excess of 18,000 birds died from fowl cholera and an additional 7,000 were destroyed. The reason given by Fish and Wildlife officials for destroying the coots was to avoid infecting other free flying waterfowl that were in their migratory path moving north.

To establish the possibility of other pathogens in the coots, Fish and Wildlife personnel shipped 229 tissue specimens and 119 blood serum specimens for viral and bacterial examination that arrived at Veterinary Services Laboratories, Ames, Iowa, on February 27. The following day, there also arrived 101 broths containing tracheal swabs, spleen and brain tissues. By March 10 Veterinary Services Laboratories had identified nine isolates as Newcastle disease and, by March 15, inoculated chickens began to die with lesions typical of VVND.

Immediately, a survey was initiated by Veterinary Services and State regulatory veterinarians in North Carolina and Virginia but no evidence was disclosed that suggested disease had been introduced into poultry.

By the middle of April, a survey in coots for VVND along the Atlantic flyway, Texas and California had been completed. Thirty coots each were sampled off the coast of Florida, South Carolina, Georgia, Virginia, Maryland, and New York, as well as in Texas and California. Specimens collected were tracheal and cloacal swabs, trachea, spleen, lung, and brain tissues. Whenever possible blood
serum was collected for the HI test and Chalmydia testing. All specimens were negative.

A study was made at Veterinary Services Laboratories, Ames, Iowa, to determine the pathology, transmission, and duration of VVND infection in coots.

The Department of the Interior, Fish and Wildlife Service, furnished the coots for the project. One lot of coots was artificially infected with the “Back Bay” isolate and another lot with the “Fontana” isolate. The study has been completed but, generally, the coots were (1) equally susceptible to both isolates, (2) both isolates produced mortality in the coots, (3) contact normal coots and susceptible chickens became infected, (4) artificially infected and contact coots shed the virus for varying lengths of time. Complete results of this project will be published at a later date.

**PUERTO RICO**

Puerto Rico remains under Federal quarantine for VVND.

USDA inspected passenger handbaggage and luggage on all air flights from Puerto Rico and some of the flights from the Virgin Islands to the United States to remove live poultry and poultry products.

*Mycoplasma Report*

The USDA, APHIS, Veterinary Services filled all official requests for Mycoplasma gallisepticum and synoviae reference materials during the fiscal year 1975. There were 391 shipments made to 58 laboratories in 32 states. Veterinary Services is prepared to distribute these reference materials at approximately the same levels in 1976.

In response to numerous requests from the poultry industry, epidemiologic studies of testing problem flocks will be conducted in chicken and turkey breeder flocks. Techniques to be used in this study are those recommended by the Mycoplasmosis Committee of the American Association of Avian Pathologists.

Dr. Walter Martin has developed an HA antigen from “Klevin’s 508” isolate that will be used in serologic studies in problem flocks. APHIS does not plan to distribute this antigen at this time.

*Importation of Avian Species*

Dr. G. Pierson reported on avian importations into the U.S.

In the Proceedings of the last USAHA meeting this committee expressed concern that there is less than daily surveillance of bird quarantine facilities. At the present time the entrance to all bird quarantine stations are sealed 24 hours a day and no one is permitted to enter except when a Veterinary Services employee is present.

The committee also urged USDA officials to consider establishing
a Virus Characterization Center for Newcastle disease and other viruses. Veterinary Services recognizes the importance of such a facility and it has been discussed at various staff meetings. When the necessary biological security is considered along with other budget limitations, such a center does not appear to be imminent.

Since July 1974, eleven additional privately operated bird quarantine facilities were approved by the U. S. Department of Agriculture, bringing the total of approved facilities to 22.

Approved facilities are located at New York, New York; (1); Detroit, Michigan (1); Chicago, Illinois (1); Brownsville, Texas (1); San Francisco, California (1); San Ysidro, California (1); Los Angeles, California (8); Miami, Florida (8).

Ninety lots of commercial bird shipments were offered for entry at these facilities during Fiscal Year 1975. 71 lots (124,597 birds) were allowed entry. All of the lots (19 lots or 21.1%) refused entry were due to the isolation of velogenic viscerotropic Newcastle disease virus. This compares to 31.6% of lots being refused entry because of VVND in Fiscal Year 1974.

Psittacine species account for over 50% of the VVND isolations made. Virus isolations, other than NCD virus, have been made from commercial bird importations. Usually such isolates involve finch-type birds, and none of these isolates have been pathogenic for chickens or turkeys.

The number of countries involved with VVND infected groups of birds and poultry increased from 14 in Fiscal Year 1974 to 23 countries in Fiscal Year 1975. The number of isolations increased from 29 to 40. Effective July 1, 1975, a policy was adopted whereby import permits are not issued for a minimum period of 90 days following the finding of a VVND infected lot of birds. Issuance of permits are resumed only when the national veterinary services of the involved country informs the USDA of the action they will take to prevent the same from occurring again. Presently permits for commercial birds are not being issued for the following countries: Paraguay, Belgium, Indonesia, Malaysia, Nigeria, and Honduras. In addition, permits for any avian species from India are not being processed because of the inability of the Indian national veterinary services to issue the health certificate as required by the USDA regulations.

A more detailed report on viral isolations from exotic birds offered for importation into the U. S. may be found in a presentation by Dr. J. Pearson et. al. in this proceedings.

In view of shortage of funds available to Veterinary Services to provide adequate and needed diagnostic capabilities this committee supports APHIS Veterinary Services in the projected "user fee" system in helping to defray the cost of maintaining quarantine stations not only for pet birds and commercial poultry but for all species imported into the U. S.
**Ornithosis**

Veterinary Services, APHIS, proposed to assist in studies in an attempt to determine the pathogenesis and reservoirs for the Chlamydia agent in endemic areas where the agent appears to be more virulent, such as in Texas. It was also proposed that Veterinary Services would produce and distribute diagnostic reagents to interested turkey growing states to monitor for the presence and/or prevalence of ornithosis in turkey flocks.

Between May 6 and June 25, 1974, there were 102 confirmed and suspected human cases of sickness and one death from ornithosis in plant employees and poultry inspectors in three states attributed to turkey flocks originating in Texas.

Early in July 1974, a cooperative program was started in Texas to detect flocks infected with ornithosis before they were presented for slaughter. During an eight-week period, 7 flocks were classified as positive and were treated before going to slaughter. No additional human cases were reported after the program was initiated.

Alarmed by the appearance of 5 cases of ornithosis in turkeys and a Texas veterinary diagnostician becoming ill from acute ornithosis in early April 1975, a determination was made to reestablish a Texas turkey flock program similar to that of the summer of 1974 to avoid a repetition of the human involvement at turkey slaughter plants.

Beginning May 26, 1975, all Texas turkey flocks, before going to slaughter, are required to be certified that they have been inspected, blood tested, and are free of ornithosis. Approximately 10 to 14 days before a flock is scheduled for slaughter, it is inspected by a federally employed veterinarian and, at the same time, blood samples are collected for laboratory testing. If the flock is not showing illness and the blood samples are negative for ornithosis, a certificate is issued giving the flock a clean bill of health to accompany it to slaughter.

Dr. L. C. Grumbles of Texas A & M University presented a summary of a program for serologic testing of turkey serums and isolation of the chlamydial agent since the disease was confirmed in Texas turkeys in 1974.

Direct microscopic examination of tissues was done in an attempt to obtain a rapid, presumptive test in support of the clinical diagnoses made upon examination of turkeys at necropsy. The latter test was found to be quite useful because the test was able to predict the outcome of isolation attempts (except for one case which was positive in mice although no typical organisms were observed upon direct examination).

Although early in the outbreak some infected turkeys did go to slaughter and produce human infections, this was prevented following initiation of the testing prior to submission for slaughter. The program was completely successful in preventing human infections and adverse publicity for the industry.
Antigen production for serological testing was resumed in 1974 in order to keep up with the demand. The micro-direct complement fixation (DCF) test which was developed by Dr. Grimes in this laboratory was further tested and developed into a rapid, easily performed procedure. Results on serums by the micro-DCF were compared with results obtained by the agar gel precipitin (AGP) test. On a flock basis the two tests were in agreement. However, on an individual bird basis the micro-DCF test was found to be more sensitive than the AGP test and is an ideal procedure for testing large numbers of samples. The major problem has been in standardizing and producing adequate supplies of the special antigen required for the test.

Both serologic testing and agent isolation attempts have continued in 1975 in cooperation with APHIS, USDA in an attempt to detect turkey flocks with orinthosis. In 1975 all flocks that were suspicious by serology were tested by mouse inoculation; and, if negative after ten days, were released for Slaughter. This procedure has apparently been satisfactory.

One isolation was made, however, from tissues of birds that were negative by serology. A few other birds in the flock had low titers and had been considered suspicious. The reason for this finding is not understood although it is presumed that the flock had been only recently infected or the incidence of infection in the flock may have been very low.

During the past fifteen months of study it has been apparent that the indiscriminate use of low levels of chlortetracycline clouded the picture as far as clinical disease was concerned and also made it necessary to resort to performing serological tests and isolation attempts before certifying a flock for slaughter. The antibiotic, as used, apparently may not prevent infections from occurring and may allow an infection to become chronic which continually stimulates antibody formation at a low level. Turkeys which become chronically infected may undoubtedly serve as sources of infection for other turkeys which is believed to have occurred in at least one instance.

Personnel of USDA, APHIS report that as of October 10, 1975, a total of 418 flocks containing 4.7 million birds have been certified eligible for slaughter. Of these, 15 flocks were found serologically positive and the ornithosis agent was recovered from four. No human ornithosis has been reported in turkey slaughter plant employees since the program began on May 26, 1975, to date. This program is scheduled to be terminated on or about December 31, 1975.

_Crates as Dissemimators of Disease_

Dr. Everett Bryant expressed the concern of the New England Poultry Industry as to the dangers of dissemination of avian diseases within states and interstate with uncleaned crates and equipment as
the vehicle of transmission. The New England Poultry Industry goes on record in support of crate cleaning and disinfection.

This committee recommends to the USDA Veterinary Services and other regulatory services that appropriate action be taken to prevent the dissemination of diseases from mechanical carriers such as crates, trucks, vehicles, etc. The committee further recommends that all dressing plants under USDA supervision institute cleaning and disinfecting procedures for equipment before leaving the plant.

_Salmonellosis in Turkeys_

A report of a five year epizootiological study of Salmonella and Arizona hensawii infection in a commercial primary breeding turkey flock was presented by Dr. B. C. Zecha. The manuscript will be published in its entirety.

_National Poultry Health Programs_

A progress report was made by the subcommittee on current national poultry health programs and relationships with APHIS. The subcommittee submitted the following statements:

1. Effective and representative state poultry industry involvement in decision making processes affecting APHIS poultry health programs, presently lacking, is necessary for the success and industry support of present and future APHIS programs. Industry desires such a voice. We recommend that a program be developed to provide for this needed industry-APHIS relationship on a state and national level.

2. We note and support the recent decision of the General Conference Committee (NPIP) to study the potential of more formalized relationships between NPIP and APHIS poultry health programs. We urge that this study determine the feasibility of NPIP serving as an industry organization advising USDA (ARS-APHIS) on endemic poultry disease control programs.

3. We support the formation of the U. S. Poultry Health Advisory Committee to APHIS as a step towards achieving the needed industry-APHIS relationships in the area of poultry disease control.

This committee commends the members of this subcommittee for their efforts and excellent progress towards a solution of this important matter. The committee further requested that the subcommittee continue their efforts to devise a program to provide for a much needed industry-APHIS relationship on a state and national level to be reported at our next meeting.

_Report of the Subcommittee on Avian Influenza_

Dr. Walter Butterfield presented the report on avian influenza, stressing problems encountered with an influenza-Mycoplasma syn-
drome in Minnesota turkey flocks, and that isolates of influenza virus from turkeys had the same hemagglutin markers of isolates recovered from guinea hens, white Embden geese and wild waterfowl in Minnesota. Influenza viruses have been isolated from wild waterfowl on the three major flyways of the U. S.

Recent findings question the use of the agar gel precipitin test as the sole diagnostic tool. Hemagglutin-inhibition tests should be applied in parallel with agar gel tests.

Reports of new recombinant influenza viruses produced in the laboratory and experienced in the field emphasized the importance of this group of agents and their relationship between human, mammalian and avian species.

The importation of exotic strains of influenza viruses and other agents or materials that are infectious to animals, birds, and humans into any laboratory in the U. S. without prior permit and adequate laboratory security threatens the livestock industry. This committee has submitted a resolution for consideration requesting that the National Institute of Health and the USDA establish joint procedures and protocols prior to issuing permits for the importation of such viruses and materials and establish a laboratory security program adequate to protect the nation's livestock industry from these agents.

Subcommittee on National Poultry Disease Control Programs

REPORT OF SUBCOMMITTEE MEETING, NOVEMBER 3, 1975

Chairman: R. A. Bankowski
Co-Chairman: W. Butterfield


A meeting of the subcommittee was held at the Hilton Hotel, Portland, Oregon, November 3, 1975, with members E. Bryant, R. L. Hogue, R. McCapes (Chairman), I. Peterson, and T. Ryan present. A letter from B. Pomeroy, unable to be at the meeting, is enclosed.

The items on the October 21 announced agenda (enclosed) were discussed. The subcommittee submits the following statements as its report:

1. Effective and representative state poultry industry involvement in decision making processes affecting APHIS poultry health programs, presently lacking, is necessary for the success and industry support of present and future APHIS programs. Industry de-
sires such a voice. We recommend that a program be developed to provide for this needed industry-APHIS relationship on a state and national level.

2. We note and support the recent decision of the General Conference Committee (NPIP) to study the potential of more formalized relationships between NPIP and APHIS poultry health programs. We urge that this study determine the feasibility of NPIP serving as a broad-based industry organization advising USDA (ARS-APHIS) on endemic poultry disease control programs.

3. We support the formation of the U.S. Poultry Health Advisory Committee to APHIS as a step towards achieving the needed industry-APHIS relationships in the area of poultry disease control.

Committee members: E. Bryant, H. Goldstein, R. Hogue, I. Peterson, B. Pomeroy, T. Ryan, R. McCapes
No reasonable person can doubt the complicated issues involved in controlling salmonellosis. Salmonella is widely distributed in the environment and can reach man from numerous sources, including pets, drugs, and contaminated water and foods that have been improperly handled or processed. Because of the high frequency of salmonella in animals, it is obvious that meat and poultry and other animal origin product can be hazardous to public health.

During the 1960's, public health officials were alarmed at the reported increase of human salmonellosis in the country and focused more Federal attention on the problem of salmonella in raw meat and poultry.

In June, 1967, at the request of FDA and USDA the National Academy of Science undertook an indepth study of the Salmonella problem. The study, completed in 1969, concluded that although it is unreasonable to expect salmonellosis to be eradicated in the foreseeable future, a great deal could be done to reduce salmonella in our food supply and thereby minimize the likelihood of infection.

As many of you know, the Federal Meat and Poultry Inspection Program (MPI), which is part of USDA's Animal and Plant Health Inspection Service (APHIS), is charged by the Secretary with responsibilities to prevent the sale and distribution of adulterated or misbranded meat and poultry products. The issues raised in the Academy's report were particularly timely concerns for MPI. For some time, the meat and poultry processing industry had been following suite with the rest of the food industry by producing more "table ready" cooked convenience foods. Obviously, many of the concerns for cross contamination possibilities of raw and cooked meat and poultry had moved from the family kitchen to the processing plant, giving a new dimension of public health responsibilities to regulatory officials.

The Department went forward in 1970 with new policies aimed primarily at maintaining the wholesomeness of the cooked, ready-to-eat meat and poultry products. Obviously, many of these items would be consumed with little or no additional heating. For example, cooked products are to be cooled through the incubation zone of 120°-40° F, in two hours. Cooked and raw meat and poultry products are to be stored in separate areas of the processor's cooler. Food contact equipment is to be washed and sanitized at midshift intervals or a microbiological monitoring program may be used to verify microbial build-up on such equipment does not occur.

Precautions are to be taken to prevent employees from handling raw and then cooked products. Equipment cannot be used for cooked items without proper cleaning and sanitizing. We also help and en-
courage processors to implement a training program for employees so they can better understand the importance of their actions in handling food.

In many cases, meat and poultry processors were already ahead of the regulations in prescribing actions in their plants to reduce possibilities of microbial contamination. In house laboratories capable of monitoring sanitation programs and shelf life studies became common tools used by meat and poultry processors. These efforts all have been working. To my knowledge, in the 3 years of microbial surveillance conducted thus far on cooked frozen ready to eat products not one salmonella positive sample has been encountered.

But more needs to be done, if we are serious in a commitment to reduce the microbial load, including salmonella, of all raw poultry and meats.

In July of 1974, the General Accounting office's report to Congress intitled, "Salmonella in Raw Meat and Poultry; an Assessment of the Problem," was published. This renewed again the Department's efforts to revitalize plans to reduce salmonella contamination in all meat and poultry products. We included in our responses back to GAO an outline of additional actions the Department intended to pursue. Rather than focusing on specific meat and poultry products, we believe there is a general need for all elements of the food production, processing, and marketing chain to move towards eliminating all possible sources of bacterial contamination, including but not limited to salmonella.

Let me go over a few of the recommendations that have been made to us as regulatory officials recognizing the further evaluations of efficacy and priority are needed.

Salmonella contamination and/or infection of the Nation's meat and poultry products begins with breeding flocks and herds, then continues in rearing areas where endemic infections spread from one animal to another. Contaminated feeds also contribute to the cycle. During feedlot operations and during transport from the farm to the processor; crowding, poor sanitation, and physical stress increase the likelihood of infection among uninfected animals so that the incidence of infected animals at slaughter is much higher than it is on the farms. Whereas, inspectors sort out obviously sick animals at ante-mortem inspection, the typical adult carrier has no symptoms of illness. Thus, inspectors are powerless to prevent the entry of carrier animals into the slaughter plant. Investigators have described the varying incidence of asymptomatic carriers at slaughter to be from 1 to 28 percent, depending on the animal species and the method of analysis.

Since 1971, the Department has encouraged red meat slaughters to utilize carcass chlorination procedures. One such process utilizes an intermittent spray of a mild chlorine solution. We presently have
50 plants either on board with an approved system or in the test stage. We are pleased with the enthusiasm the chlorination programs have received and believed it is an important part of an action plan for microbiological controls.

We believe there is also an opportunity for cooperative programs both Federal and State to include specific measures for the prevention of salmonellosis in flocks and herds. Possible areas for such cooperative programs could include maintenance of breeding flocks and herds negative to the common serotypes considered to cause a carrier state in each species; monitoring livestock and poultry for salmonellosis prior to slaughter; and correction of environmental conditions that contribute to salmonella transmission in livestock and poultry. I am aware of the sophisticated records poultry growers and processors have. Computer use in common place and in many cases lays out flock histories in infinite detail. It seems such records could be better utilized in our disease surveillance and control programs.

Other quarters advocate all water used on the poultry lines from the exit of the scald tank to the chill tank exit should contain at least 20 ppm residual chlorine as measured at point of use before it contacts the birds.

In addition to the above, the following specific steps have been proposed in the red meat area: It has been suggested that swine de-hairing machines be flushed and sanitized at midshift. (This is, of course, in addition to present requirements for daily cleanup.) The machines should be constantly flushed during use, with water containing at least 100 ppm chlorine. Calf carcasses could be hot skinned as other bovine are processed. Historically, calves have been slaughtered and shipped hide on. How serious it this practice when considering a total salmonella control program?

To reduce the possibility that dust or aerosols from hides or feathers will contaminate finished product, it has been proposed all new construction should be designed to prevent air movement from the killing operation toward the finished product.

The surface of red meat carcasses support high bacterial loads because of contamination and long storage. Contamination could be reduced by wrapping in appropriate materials for shipment. We should determine whether this is feasible and what kinds of materials would be suitable. I am sure the environmentalists would be interested in this one. Preliminary data has already determined that this approach shows promise. Traditionally, red meat items have been shipped naked, into warehouses, out of warehouses, to another processor, back on a truck, etc., and eventually on to the retailer.

MPI should possibly require the chlorination of all water supplies used in meat and poultry plants. The amount added to the water should be adjusted so that at least one part per million residual oc-
curs at each water tap within the plant. During cleanup, the chlorine residual should be raised to at least 100 parts per million.

Chlorine toxicity allegations are still unresolved; however, we are routinely asking GRAS list approval from all participating plants.

We are cooperating with FDA in an effort to resolve the acceptability of chlorine. Toxicity and efficacy studies are being conducted by ARS. FDA has indicated that chlorine use may be approved for all existing uses in meat and poultry plants. Any new uses for chlorine will require the submission of a food additive proposal. With respect to chlorine levels, FDA toxicologists have not yet made a final decision, but discussions to date indicate they would not be restrictive and deny or ban existing systems that are now in effect in the meat and poultry industries.

Chlorination, however, cannot do the entire job since salmonellae may find their way into hair follicles, feather follicles, and fatty layers. Chlorine cannot penetrate under the surface of organic foods but it may reduce cross-contamination in washing and chilling.

Thorough cooking, the best way to destroy salmonella, is foolproof provided, workers or homemakers do not recontaminate the cooked food from the raw product.

The Department already has an extensive ongoing, consumer educational program directed primarily at the housewife and food service employees. I am sure many of you have seen the T.V. "spots"—many directed to salmonella specifically. Since most of the food poisoning outbreaks occur because of mishandling at this level, education efforts should be expanded.

These are just some of the areas to consider when discussing the salmonella problem. I am sure if we as a regulating agency went forward with all these recommendations, we would be accused of "over regulating" and rightly so. So how do we proceed? First, it must be stated that, to demand changes from the very efficient methods and pieces of equipment used in today's slaughtering and processing operations, the requested changes must be viable alternatives. Changes which will result in skyrocketing costs and production slowdown will gain little support from industry or consumers. For example, to make changes in processing that would prevent all cross-contamination between carcasses would introduce costs that would be excessive. We should consider a cost-benefit relationship unless we are clearly talking about a procedure affecting public health. I do not believe we have any one procedure that qualifies. One approach that has been suggested over the years is to compare standard and experimental equipment and procedures under commercial conditions for their effect on salmonella levels by operating parallel slaughter and/or processing lines.

So the "shopping list" of potential answers to salmonella problems gets longer and longer. These are just a few of the issues that
have been raised. As we pondered such actions, it became obvious some central leading force was necessary to direct and elevate the proposed actions. Numerous salmonella work groups had progressed to some stage of development since the National Academy of Science report in 1969 and the GAO report of 1974.

In January 1975, a decision was made in favor of a task force approach for studying ways to lower the incidence of salmonella, during the hatching, feeding, growing, processing, transportation and distribution of poultry and poultry products. The membership was balanced with technical regulatory and industry participants. The response and interest was overwhelming. A similar task force was to be organized for the red meat problem following this general format for organization.

Meanwhile, the Department had been discussing the prospect of creation of an Advisory Committee on Salmonella for both meat and poultry. The reason for taking this step is very clear. The Federal Advisory Committee Act requires the deliberations of an advisory committee whenever a group is convened to advise the Secretary.

A notice appeared in the Federal Register on October 23 announcing the Secretary's intentions and soliciting comments until November 7. A press release will be distributed in Washington soon.

I would like to take this opportunity to solicit the council and support of USAHA in this advisory committee venture. Certainly your Salmonella Committee will be particularly interested and kept advised of its activities. And if the Commitment of the Advisory Committee is to look at the total salmonella issue, I am sure other USAHA committees may also be involved.
INDUSTRY CONCERNS
Harold J. Buyens

My discussion today has been titled "Advisory Committee on Salmonella: Industry Concerns".

The subject of salmonella and its control or eradication has been the subject of discussion for many years in the raw agricultural product industry.

The meat and poultry industries have extensively considered the problems relative to salmonella because the meat and poultry industries have been extensively involved in the eradication or control of all disease organisms for many years. Far reaching changes have been made in all phases of the meat and poultry industry over the past 20 years in attempting to reach the goal that all animals will be healthy animals.

The work of researchers, veterinarian, industries, government agencies, is continuing to stop animal diseases. U.S. Animal Health Association has addressed itself ambitiously to the disease control problem for many years. The U.S. Animal Health Association's disease control programs are increasing in intensity in the plans for the future. There are also programs by the American Association of Avian Pathologists. There is a National Poultry Improvement Plan. Other associations and organizations have plans in effect that are all aimed at animal disease control.

One of the diseases involved is the pullorum organism. This organism can cause high baby chick mortality. A national program was launched years ago to provide pullorum-free breeding stock to the industries. Procedures were established to maintain the pullorum-free status through the meat animal processing operation. The national program was successful and is continuing in effect in order to maintain pullorum-free animals.

There are now 13 states designated as "U.S. pullorum-typhoid clean state" under the National Poultry Improvement Plan (NPIP). This classification means that the state has had no outbreak of pullorum or fowl typhoid diseases in turkey breeding or commercial chicken flocks in the last 12 months and that regulations are in effect to ensure the detection and elimination of any future outbreaks.

As is true with many of the diseases associated with animals the industry has found it necessary because of potential economic losses to maintain an effective eradication/control program.

A similar organism is well known to people involved in the production of raw agricultural products. This organism is ubiquitous. It comes in almost 2,000 varieties. Its activities are a matter of record in the the annual reports of public health officials and the Communicable Disease Center. The 2,000 varieties are all classed under the name "salmonella".
Scientists have debated for years both the similarities and the differences between “pullorum” and “salmonella”. Physiological classifications, growth characteristics and other criteria are all used in the debate over whether pullorum is a salmonella, other whether it is not a salmonella. One of the most important distinctions between pullorum and salmonella lies not in a scientific criteria but in the fact that in the raw agricultural production system pullorum can be a more serious economic loss factor causing death losses while salmonella will only cause decreases in feed conversion, result in lower weight animals, and increase contamination losses at the processing plant.

These basic facts have given impetus to the control of pullorum. There have been good steps taken to control salmonella but there still remains a great deal of opportunity to institute stricter measures to reduce the numbers salmonella on raw agricultural products.

The difficulty over the years in implementing the desired control of this organism has been due to the fact that the problem is so vast and the organisms are so universally distributed. The sources of salmonella are so many that there is an actual tendency for every segment of the meat and poultry industry to point their fingers at others in the industry as a main or key source of salmonella on the raw meat and poultry products. The breeders and the hatchery people point their finger at the feed people. The feed people point their finger at the renderers. The renderers point their finger at the processing plant. The processing plants point their finger at the animal producers and growers. The animal producers and growers point their finger at the feed people and at the breeders. The finger pointing goes on and on. It is an endless ring.

Overall the problem of the presence of salmonella continues not only because of the foregoing described ring of possible sources of salmonella but also because the entire ring of transmission of the organism is being constantly and heavily reseeded by nature herself. Predatory animals, birds, insects, dust, ranges, etc. are all fountains of salmonella that feed the organism into the entire raw meat production system.

The efforts that the various disciplines of the raw agricultural production industry have made so far have basically been individual efforts by groups that represent specific segments of the entire industry. They have pointed fingers but each segment has made improvements. However, individual improvements made seem insignificant in the face of the re-seeding that occurs throughout the industry. The improvements made were accomplished in spite of the fact that the reduction of numbers of salmonella organisms could not be expected to produce an economic improvement.

In searching far away to solve the salmonella problem the Secretary of Agriculture has established an "Advisory Committee on Sal-
monella.” The advisory committee will consist of representatives of regulatory agencies (both federal and state), industry, research and educational facilities. The advisory committee will consist of a dozen or so people. Representation in that committee will cover the breeding, the hatching, the meat production, the processing plant, the rendering, the feed and feed ingredients and the research and marketing sections of the meat and poultry industry. You might very well ask how can this committee of a dozen or so people hope to control the problem that hundreds of highly qualified individuals have not yet been able to control in the past.

The answer is really quite simple. A dozen or so people will not be able to control it by themselves. The dozen or so people will only be able to coordinate the efforts of all the several hundreds of qualified individuals in all phases of the meat production business over the next coming years.

The advisory committee will constantly have before it the duties that have been assigned to it by the Secretary of Agriculture. These duties are:

1. Studying measures to reduce the incidence of salmonella organisms in the live poultry and animals and to limit the spread of salmonella during slaughtering, eviscerating and further processing operations.
2. Recommending and soliciting the cooperation of effected industries in implementing measures which are developed.
3. Recommending regulatory requirements needed to apply critical control procedures.
4. Considering means of disseminating information on preventative practices to all segments of industry and to consumers.

It becomes apparent that the advisory committee will be able to implement its recommendations (A) voluntarily or (B) by regulation. There is adequate regulatory representation on the advisory committee so that in the event that voluntary participation is not completely effective the committee can make recommendations for regulations.

There is adequate representation on the committee by industry, research and educational people that there will be careful consideration given to each recommendation, voluntary or regulatory, from an economic impact standpoint and from a public health standpoint.

This does establish the tool, the means, the system that can coordinate the individual efforts of people and groups and achieve control over this vast and widespread problem.

It is anticipated that the salmonella committee will be organized into 6 coordinated divisions. Those 6 divisions will be:

1. Breeders and Hatchers
2. Feeds and Feed Ingredients
3. Production Growers
4. Processing
5. Research
6. Education

The people involved on the advisory committee will possibly be the representatives for their entire section of the industry. The recommendations, suggestions, ideas, principals that they expound will be developed through careful coordination of the knowledge, opinions, desirable effort that they will develop from many varied sources within their respective division of the industry. Research, industry practices, economics, etc. will be considered in bringing forth recommendations. These recommendations will come from many and long discussions between the advisory committee coordinators and representatives—their section of the industry division and related interests.

There are areas of practical knowledge already developed that will be used to develop the recommendations of the advisory committee.

For example, the hatchery division may consider information such as printed in leaflet 2629 of the University of California in August 1975, some of these sanitary practices are as follows:

1. Collects eggs frequently (at least 4 times a day).
2. Keep nests filled with clean nesting material.
3. To keep floor eggs to a minimum provide one nest for every 4 hens. Be sure nests are in place before egg production starts.
4. Exclude hens from nests at night.
5. Maintain dry litter under hen's feet at all times.
6. Separate cracked, stained and heavy dirty eggs as you collect them, and don't set them.

These are sanitary practices that might be utilized. These are sanitary practices that are not universally practiced at the present time. This type of information will be typical of that considered for recommendation to the advisory committee meetings by the division coordinators.

There are many examples that can be cited as possible practices that might be utilized by each division. The processing division will probably be carefully considering in-plant chlorination of the surfaces of meat & poultry products to reduce the numbers of microorganisms. The feed division will probably be considering some means of truck and car sanitation and protection. The education division might be giving consideration to obtaining commitments from cook book publishers, food editors, recipe authors etc. to include a simple cleaning/sanitizing statement in an appropriate place in each recipe that involves handling of raw agricultural products in the home or institutional kitchen.

Everyone is looking forward to the initial advisory committee meeting which will be announced in the Federal Register. We sin-
cercely hope that everyone will be willing to cooperate and help co-
ordinate the entire effort so that the incidence of salmonella on our
raw agricultural products can be drastically reduced.

Thank you for your attention.
REPORT OF THE COMMITTEE ON SALMONELLOSIS

Chairman: H. G. Geyer, Washington, D. C.
Co-Chairman: John W. Walker, Hyattsville, Maryland


Seven members of the Committee on Salmonellosis and fifteen guests met on November 5, 1975 to address the salmonella problem.

Your committee has repeatedly emphasized the need to address salmonellosis as a total environmental problem in lieu of merely attacking only segments of the problem. Here reference is made to the program instituted by the Food and Drug Administration (FDA) aimed at the control of salmonella in animal and marine products intended for use in animal feeds. This is not meant to minimize this aspect of salmonella control, but rather to emphasize that it represents only one area of concern. Your committee has been advised by a memorandum from FDA that another task force has formed within the agency to prepare a position paper for salmonella operations. This task force is charged to conduct a study and respond to two specific questions:

1. “What is the contribution of salmonella contaminated rendered animal and marine by-products to animal and human salmonellosis?” and,

2. “Does the contribution of Salmonella in animal feed ingredients to animal and human salmonellosis warrant the resources required to eliminate such contamination?”

Until the report of this special task force is made public, no conclusions or recommendations are available for consideration. A copy of the FDA memorandum is attached to the report (attachment no. 1).

Your committee notes with pleasure the announced intent of the Secretary of Agriculture to establish an Advisory Committee on Salmonella. Notice of this intent was published in the October 23 Federal Register. Interested persons will have until November 7 to submit comments to the Administrator, Animal and Plant Health Inspection Service, U. S. Department of Agriculture, Room 316-E, Administration Building, Washington, D. C. 20250.

During the committee’s deliberations, information was provided to substantiate the need to address salmonellosis as a total environmental problem. In addition to livestock and poultry concerns, other sources of environmental contamination by salmonella such as that
of land and water stemming from urban-derived wastes, warrants equal consideration and assessment.

If the problems stemming from salmonella are to be controlled or diminished, your committee recommends that efforts be made to give appropriate emphasis to the roles of the following agencies: The USDA Animal and Plant Health Inspection Service (APHIS), the Agricultural Research Service (ARS), the Meat and Poultry Inspection Programs (MPIP), the Cooperative State Research Service (CSRS), and the Cooperative Extension Service (CES); the Food and Drug Administration (FDA), the U. S. Public Health Service (USPHS), the Department of Commerce (DOC), the Department of Interior (DOI), and the Environmental Protection Agency (EPA). Concomitant consideration should be also given to relevant agencies at the state level.

In addition, an objective assessment should be made of the following to ascertain the role of each as contributors to the problem: cattle, swine, sheep, horses, poultry, and aquatic animal producers; feed manufacturers and processors; meat, milk, and egg processors; the transportation industry; retailers, public food services; companion animals; and the consuming public.

In light of the complexity and gravity of the salmonella problem, your committee recommends that the State-Federal Relations Committee take such actions as may be necessary to encourage a close working relationship between the Committee on Salmonellosis of this association and the Secretary's Advisory Committee on Salmonella, and further, that the following resolutions be favorably considered by the Executive Committee and adopted for transmission to the Secretary of Agriculture: (Resolutions sent to Resolutions Chairman.)
INTERNATIONAL MOVEMENT OF SHEEP AND GOATS —
LEGAL AND POLITICAL IMPLICATIONS*

Warren C. Foote, Ph.D. and Jay W. Call, D.V.M.

Requirements for control of animal diseases are well enough understood that effective precautions can be maintained in most instances to prevent the spread of old diseases and detect the occurrence of new ones. Historically, recognition that movement and mixing of animals increased the incidence and spread of disease preceded man's knowledge of the principles of pathogenesis.

The more and more stringent health requirements that have evolved which influence animal movement have not changed the desire of animal scientists to acquire new and better genetic material from different parts of the world for improvement of animal production. It is essential that selection and development of livestock be continued if production is to be improved. One objective of the International Sheep and Goat Institute is to accumulate, evaluate and select sheep and goats with improved adaptive and production capability. Programs should be developed to better evaluate animal performance without endangering the health of any population.

Each country and many local units of government within them have developed their own individual health requirements for importation or movement of new animals. The health requirements for importation of sheep and goats are extremely variable and range from no restrictions, to requirements so stringent that entry is impossible. In general, the main cause for the restrictions are diseases such as Blue Tongue, Foot and Mouth, Rinderpest and Scrapie. These types of diseases are difficult to diagnose and are possibly enzootic in many regions of the world and yet they remain to a great extent unknown or undiagnosed.

From our experience, when shipping animals to other countries the veterinarian is well advised to become familiar with their import requirements. This information can be acquired from the Veterinary Service, Animal and Plant Health Inspection Service, United States Department of Agriculture. The Federal Veterinarian in charge of the State of Origin, also, will often have foreign health requirements. It is very important that contact be made with the foreign Veterinary Service or Quarantine Officer to determine special procedures or requirements that must be completed to facilitate import. Adequate time should be allowed to conduct all necessary laboratory and clinical tests.

It is necessary to use an official federal health certificate and include all necessary information and test results. An accredited

*Published as Utah Agricultural Experiment Station Paper No.
**International Sheep and Goat Institute, Utah State University, Logan
Veterinarian must complete and sign the health papers. These papers must then be authorized by a federal veterinarian. Each animal should carry identification and be listed on the health papers.

Many countries have developed health regulations for importation of animals that cannot be met and thus have precluded import of any new animals. In some instances the regulations have been circumvented by illegal entry. In other cases, politics have been used to gain entry. The other extremes exist where there are no import regulations and movement of animals appears to be based on economic considerations rather than health requirements.

Many of the health and political regulations which govern international movement of animals are inconsistent and appear to a great extent to fail in the prevention of the spread of disease or otherwise serve the livestock industry.

It would seem that these purposes can be achieved by the establishment of international regulations which each country could recognize and modify if required in terms of their national regulations for the mutual benefit of their own and other countries involved. Obviously, great political and technological problems as well as lack of knowledge of pathogenesis of many diseases limit such a program.

A limiting factor in effective disease control is failure to recognize diseases and develop programs for their control. As an example, in the United States anaplasmosis has spread to many areas of the country, yet some individuals do not appear to want to recognize its presence. An essential prerequisite for effective disease control is the use of a full diagnostic and reporting system. Recognition of the presence of a disease must precede its control.

It is apparent to people working with international movement of animals that a need exists for a more efficient and effective system that will allow movement of selected animals. A great deal of effort is being made to develop new procedures and upgrade others in order to import new genetic material into the United States without endangering the present livestock populations. In a world where animals can be moved from country to country within hours, a better monitoring system is needed. This would include wild as well as domestic animals.

In The International Sheep and Goat Institute program our major goals can be fully reached only as we are able to exchange animals among countries. These goals include (a) identification, characterization, and perpetuation of genotypes of wild and domestic sheep and goats, (b) increasing production of animal protein (meat and milk) and (c) fiber (wool, mohair, cashmere), (d) developing sports hunting and game ranching potential and (e) fostering training programs and exchange of information including information on disease.

Wild sheep and goats and their close relatives represent the greatest and most diverse gene pool among animals for production of
food and fiber. This provides for selection of animals with high production potential that are adopted to practically every environment where man lives. Crossing and selection programs involving selected genotypes together with appropriate management programs will result in increased production of food and fiber for each environment. We estimate that there are nearly 1200 genotypes of these animals distributed throughout the world. One of our greatest challenges is to take advantage of this great potential for adaptation and production, which requires international exchange, within the restrictions imposed on animal movement.

The United States should be one of the countries measuring the value of genotypes of sheep and goats selected from throughout the world and to profit from introduction of animals with greater production potential. Yet, there are very few countries from which domestic sheep and goats can be imported. Also, it is impossible at present to import semen or fertilized ova. Wild genotypes can be imported into zoological parks where they must remain permanently in a post entry quarantine. Their offsprings, however, are not restricted to permanent quarantine.

Recently three countries have declared genotypes of sheep, which occur almost exclusively within their boundaries, as essential resources and deny export. This was apparently done for political and economic reasons. In one case the reason given for such action was to allow for adequate measurement of production and adaptation under domestic conditions before releasing them for export. In other cases the restrictions were apparently imposed to increase numbers for domestic use and to increase the value of animals for export purposes. While such action is clearly within their sovereign rights, the result is that these genotypes are not now available even in small numbers to other parts of the world for use in increasing production of food and fiber. In other countries ministries of agriculture or comparable organizations are very willing and seem almost eager to facilitate export when the use of the animals is known to be of a more altruistic nature.

Politically oriented relations among agencies or organizations within a country which has responsibility for or which can exert an influence on animal movement have acted to make impossible or impractical import or export even though the regulation does not appear to intend such.

Our experience over the last few years with international movement of sheep and goats has demonstrated the lack of disease control in many areas of the world and the debilitating affect that it has on production. We have also become more keenly aware of the relative freedom from disease that we enjoy in this country.

We support the USDA in its efforts to prevent introduction of disease. The USDA is aware of the need to import domestic sheep
and goats and is seeking ways of making this possible, at least on a limited basis. We believe that the necessary information is mostly available and hope that required programs and facilities will soon be provided.

In the few cases in which we have introduced new genotypes of animals through inter-country transfer, increased production from crossbreeding or from better adapted straightbreds have been demonstrated. In one program it is estimated that adoption of our crossbreeding and related management programs, including animal health, would increase meat production by as much as 50%.

We have already selected several breeds of sheep for use in the United States for purposes of increasing productivity and are waiting now for development of procedure for their import. The introduction of some of these breeds, however, must also wait upon changes in political-economic policy which deny export in countries where they are now being produced.

Livestock are natural, renewable resources common to and prized by every country in the world. They are major basic sources for production of food and fiber for man and as such have unique importance as import-export commodities. We believe this is particularly true of sheep and goats because of their great genetic diversity and their ability to self-harvest energy from many areas of the earth otherwise largely wasted as food. Optimistically, these pressures coupled with increasing capability in control of animal disease will help overcome politically oriented restraints to international animal movement.
REPORT OF THE COMMITTEE OF SHEEP AND GOATS

Chairman: F. James Schoenfeld, Salt Lake City, Utah
Co-Chairman: C. C. Beck, Manchester, Michigan

Dr. Howard Whitford, Mr. Ward Van Horn, Dr. Thomas B. Snodgrass, Dr. A. L. Klingsporn, Dr. H. E. Metcalf, Dr. C. E. Terrill, Dr. M. E. Macheak, Dr. W. A. Hickman, Mr. John Neimi, Dr. H. A. Hancock, Dr. William W. Hawkins, Jr., Mr. O. H. Timm, Dr. J. E. Pearson, Dr. Richard F. Hall, Dr. D. W. Baker, Dr. R. E. Simmons; and guests totaling 34.

Met as requested by the president to consider the business of the committee and submit the following report:

Dr. R. F. Hall of Moscow, Idaho, announced that there would be a symposium on neonatal calf and lamb mortality at the University of Idaho on November 13-14, 1975.

An item of interest is the fact that viral agent associated with diarrhea in lambs has been isolated and will be put back into lambs this coming spring as part of a continuing research effort. This discussion was lead by Dr. R. E. Simmons.

The committee passed a resolution commending the State of Idaho for their ovine research effort and encouraged the Land Grant Colleges to continue research effort on diseases of economic significance of the sheep industry.

Discussion of Foot-Rot and related research ensued. A resolution commending Oregon State University for their Foot-Rot research efforts and encouraged to continue their efforts. Dr. Goode commented that efforts are underway by USDA toward funding on Foot-Rot research within the current budgeting limitations.

The National Wool Growers, National Lamb Producers, American Association of Sheep and Goat Practitioners support Resolution No. 4 of the Intermountain Veterinary Medical Association 1975 which encouraged USDA to consider assigning a high priority and adequate funding to Foot-Rot research efforts.

Dr. Barber, USDA—Denver Bluetongue Laboratory, spoke on Bluetongue: Of 16 known serotypes of Bluetongue virus, four have been typed in the United States utilizing the plague neutralization test. Vaccination with a vaccine containing one serotype does not result in any protective immunity to other serotypes.

Epizootic Hemorrhagic Disease (EHD) of deer is caused by a virus that appears to be serologically related to Bluetongue Virus but is a distinctly different virus which infects deer and cattle but not sheep.

Dr. Hugh Metcalf, Denver, commented that the Culicoides vector is ubiquitous in the United States. Research would indicate that vaccines should not contain more than four serotypes. Annual vaccination with a Modified Live Virus (MLV) is recommended. The
presently available vaccine has distinct problems due to serotype inadequacies and lack of cross immunity.

Dr. Hall commented on Dr. Bushell's (California) report on Bluetongue in California lambs causing a significant Laminitis problem.

The committee recommends that the Committee on Biologics be supported as regard to their resolution relative to Bluetongue Virus Vaccine.

The problems related to approval of biologics for minor species and minor indication of other species was discussed.

Dr. Goode commented on the possibility of certain designated federal laboratories doing some of the efficacy testing toward the goal of availability of specific needed products in the best interest of the industry.

Discussion relative to Enzootic Abortion of Ewes (EAE), Ram Epididymitis (REO), and related vaccines ensued. Dr. Hall indicated that in Idaho, Montana, Utah, and Oregon EAE is endemic in nearly 100 percent of the flocks resulting in 2-5 percent abortion which does not respond to antibiotic therapy as does Vibrio. The Epididymitis and EAE vaccine is effective and commercial availability would be advantageous.

Dr. Reynolds, supported by Dr. Drake, indicated the need for an approved effective fluke preparation. Clostridial infections due to Cl. novyi and hemolyticum are of significance.

Mr. Laird Noh, President of National Lamb Feeders, spoke on the needs of the industry and the desire of their organization to cooperate toward the solution of ovine health problems.

Dr. Reynolds pointed out the need to carry the message of this committee and USAHA to the sheep industry.

Mr. John Morrison, of the American Sheep Producers Council, indicated the desire and willingness to cooperate with the committee toward executing their goals and desires.

Chairman Schoenfeld commented on the Virginia Dairy Goat Breeders Association and their survey relative to Caseous Lymphadenitis. A discussion ensued.

Dr. M. E. Macheak gave information pertaining to studies on predators and endangered species.

Dr. J. L. Hourrigan reported on the 1975 activities and results of Mission Texas—Scrapie Research Project. There has been two outbreaks of Scrapie in 1975.

Dr. A. L. Klingsporn submitted written reports on Scrapie and Bluetongue to be included in the committee report.

A brief discussion ensued on infertility, and Polyarthritis of sheep, and EHD in New Jersey deer.

The committee members adjourned at 4:30 p.m. This report is submitted for the Executive Committee consideration.
Bluetongue

During fiscal year 1975, bluetongue was confirmed by virus isolation in 6 cattle herds, 3 in Colorado, 1 in Idaho, 1 in Texas, and 1 in Washington; and in 23 sheep flocks, 3 in California, 2 in Idaho, 1 in Oregon, 10 in Texas, 1 in Utah, and 6 in Washington.

In addition 83,446 modified complement-fixation (MCF) tests were run at Veterinary Services Laboratories, Ames, Iowa, and at 13 approved laboratories with the following results:

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Tested</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>74,099</td>
<td>70,216</td>
<td>3,329</td>
<td>554</td>
</tr>
<tr>
<td>Sheep</td>
<td>733</td>
<td>670</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Goats</td>
<td>475</td>
<td>458</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Wildlife</td>
<td>200</td>
<td>185</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Zoo Animals</td>
<td>311</td>
<td>289</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Equine</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unreported</td>
<td>7,613</td>
<td>7,482</td>
<td>120</td>
<td>11</td>
</tr>
<tr>
<td>Totals</td>
<td>83,446</td>
<td>79,315</td>
<td>3,518</td>
<td>613</td>
</tr>
</tbody>
</table>

Positive MCF samples were collected from the States of Arizona, Arkansas, California, Colorado, Florida, Idaho, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Montana, Nebraska, Nevada, New Hampshire, New Mexico, North Carolina, North Dakota, Oklahoma, Oregon, South Carolina, South Dakota, Tennessee, Texas, Utah, Washington, Wisconsin, and Wyoming.
Scrapie Field Trial, Mission, Texas

The scrapie field trial has been underway since November 1964 (129 months). During this period scrapie has been confirmed by histopathological study or by mouse inoculation in 362 animals. The 362 cases have occurred on infected premises No. 3 in the following category of animals either taken to Mission or born on the premises:

a. Scrapie bloodline exposed sheep .......................... 266
b. Field suspects held for observation ....................... 12
c. Nonbloodline exposed sheep ................................ 42
d. Nonbloodline exposed goats ................................ 42

The findings at the scrapie field trial study have demonstrated that bloodline animals exposed on infected and source-flock premises will continue to develop scrapie and provide foci of infection for continuing spread of the disease unless slaughtered. The losses in bloodline animals continue to occur in succeeding generations of such animals reared on the infected premises.

The work at Mission has also confirmed that scrapie can be transmitted to previously unexposed nonbloodline sheep and goats and their succeeding generations when such animals are placed on infected premises in contact with scrapie-affected sheep and goats. Without exception, scrapie has been transmitted naturally to every breed of previously nonexposed non-bloodline sheep and goats used in the field trial study and some of their crosses. These breeds include Hampshire, Rambouillet, Suffolk, and Targhee sheep and Angora, Nubian, Angora X Nubian X Toggenburg, Nubian X Toggenburg, Nubian X mixed dairy, and Nubian X Toggenburg X mixed dairy goats.

From a program and disease eradication standpoint, it is important to determine at what ages animals born and reared on infected premises can safely be removed from exposure without later developing scrapie. To determine the answer to these questions, animals at Mission born on infected premises were removed from exposure at birth (with and without having suckled their mothers), at 4 months of age, 6 months of age, 9 months of age, and 20 months of age. To date 17 animals have been confirmed scrapie which were removed from exposure at the following ages: Removed from exposure at 20 months, 11 animals were confirmed scrapie at 28, 34, 35, 36, 37, 39, 40, 45, 46, 51, and 51 months of age; removed at 9 months, 1 animal at 38 months of age; removed at 6 months, 3 animals at 39, 40 and 41 months of age; removed at 4 months, 2 animals at 39 and 40 months of age; and removed at birth, no animals. This study clearly demonstrates that animals sold from infected flocks at 4, 6, 9, and 20 months harbor the scrapie virus and progress to clinical scrapie;
therefore, they must be destroyed if we are to eradicate this disease. If such animals are allowed to live, they will continue to be a source of new foci of infection and spread of the disease. These studies are continuing.

Scrapie

Scrapie was reported in two flocks in Illinois and West Virginia during fiscal year 1975. The Illinois outbreak occurred in a Suffolk flock in De Kalb County and the West Virginia outbreak in a Suffolk flock in Pendleton County. There were three outbreaks each reported in fiscal years 1971 and 1972, two in 1973, and one in 1974. This compares with an average of 11 outbreaks per year during the 10-year period of 1961 through 1970. The number of flocks under surveillance has decreased from 148 in 1974 to 116 in 1975.

The West Virginia outbreak was reported to a regulatory official by the owner. The infected flock has been slaughtered, and regulatory officials have endeavored to locate and slaughter all bloodline sales from the flock and locate all exposed sales from the flock and place them under surveillance in some States and slaughter them in others. The source of this outbreak has not been determined; however, the flock has been under surveillance on different occasions over the past 12 years due to the purchase of bloodline and exposed sheep which have died or been slaughtered in the flock.
The Iowa Legislature passed a law effective June 15, 1974, requiring the practicing veterinarians of the State to report all cases of known or suspected poisoning of domestic livestock. The law specifically states that the reports shall be made to the Veterinary Diagnostic Laboratory, Iowa State University, and the Iowa Department of Environmental Quality. Pursuant to this authority the chemical technology commission of the Iowa Department of Environmental Quality has adopted a rule requiring that any person practicing veterinary medicine in the State of Iowa, encountering a case of poisoning, or suspected poisoning, of domestic animals through injury from contact with, exposure to, or ingestion of any biological or chemical agent or compound shall immediately report by telephone or telegraph such poisoning to the Veterinary Diagnostic Laboratory, Iowa State University. Iowa is apparently the first state to undertake such activities.

The toxicology section of the Diagnostic Laboratory felt that this initiated not only a challenge, but also an opportunity to gain valuable information through the investigation of field problems of agricultural chemical accidents as they affect animal and human health. We have attempted to investigate all poisoning reports to determine the circumstances associated with the problem, the number and type of animals involved, specific information relating to the drug or chemical, the type of reaction manifested by the affected animals, and results of any therapeutic measures.

The Iowa Veterinary Diagnostic Laboratory receives from 12,000 to 14,000 cases annually and in about 20% of the submissions during this study period, the referring veterinarian suspected a toxicosis. A few more than 1 out of 10 suspected cases are confirmed as toxicoses. Approximately 20% of the confirmed cases are from out of state; however, the data that is presented here is derived entirely from Iowa case studies. Also, two human cases involving mycotoxins were omitted from these tabulations.
ANIMAL TOXICOSES

Table 1:

Iowa Veterinary Diagnostic Laboratory

Classification of Animal Toxicoses Investigated in the State of Iowa (June 1974 thru June 1975)

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. Cases</th>
<th>No. Confirmed</th>
<th>% of Total Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organophosphorus-Carbamate Compds</td>
<td>49</td>
<td>27</td>
<td>22%</td>
</tr>
<tr>
<td>Chlorinated Hydrocarbon Compds</td>
<td>17</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td>31</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Rodenticides</td>
<td>32</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Feed Related</td>
<td>35</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Plant Related</td>
<td>14</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>17</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Herbicides</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Other Agri. Related Compds</td>
<td>11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Adverse Drug Rx</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>224</strong></td>
<td><strong>157</strong></td>
<td><strong>101%</strong></td>
</tr>
</tbody>
</table>

In these times of greater public awareness of liability, chemical toxicity, and environmental pollution, we in the occupation of animal disease diagnosis often receive more opinion than specimens from those victimized in the suspected poisoning incident. Therefore, we must stress the importance of involvement of all various fields of diagnostic veterinary medicine. Expert evaluation in the disciplines is the only way we can have confidence in these results. Seventy percent of the cases during this period received presumptive or conclusive diagnoses. The remainder were reported by veterinary practitioners. The clinical signs and circumstances surrounding these cases were such that we felt confident in including them in these data.

In Table 1 of the cases recorded from June, 1974 through June, 1975 have been classified by compound type. Most of the organophosphate and carbamate insecticides were field use products, while most of the chlorinated hydrocarbon insecticide cases resulted from animal use materials. (Insecticide residue cases involving meat or milk are not included in these figures.) Those recorded under heavy metals primarily involved lead in cattle. Three incidents involved inorganic arsenic poisoning in cattle and another involved thallium poisoning in a dog. Strychnine and sodium fluoroacetate poisoning in dogs made up the majority of the cases under rodenticides. The feed related category includes the accidents involving feed additives such as urea, copper, and the organic arsenicals in cattle, sheep, and swine, respectively. For lack of a better category cases of sodium ion toxicoses—or water deprivation—were also listed here. Because much of its land is under cultivation, Iowa has relatively few plant related prob-
lems. Most of these cases involved acorns (Quercus sp.) and pigweed (Amaranthus retroflexus).

Interest in the chemical identification and elucidation of field problems associated with mycotoxins has increased in the Iowa Veterinary Diagnostic Laboratory over the last several years. We are presently screening routinely for four or five of the mycotoxins and have the capability to analyze for approximately 34 of the compounds. Most of the identifications made in association with field problems involved the Fusarium sp. mycotoxins primarily zearalenone and T-2 toxin.

Problems caused by materials such as ammonium nitrate fertilizer, anhydrous ammonia, and creosote; and dermatitis associated with petroleum distillates are listed under agriculture related compounds. The miscellaneous category includes two cases of ethylene glycol and one case involving a broken pipeline when eleven cows died from drinking gasoline.

The adverse drug reactions were unrelated incidents and involved a variety of compounds. Two were anthelmintics, one a suspected anaphylaxis after bacterin administration, and the other two were associated with chemotherapeutic agents.

Table 2

| Animal Toximes Investigations in the State of Iowa (June 1974 thru June 1975) |
|-------------------------------|-----------------|-----------------|
| Percentage of Cases by Species (224 Total Cases) |                          |
| Bovine                         | 47%             | Canine          | 16%             |
| Porcine                        | 23              | Feline          | 4               |
| Ovine                          | 6               | Poultry         | 1               |
| Equine                         | 1               | Falco Sp.       | 1               |
| Caprine                        | 1               | All*            | 1               |

*all animals on farm exposed via water

Table 2 was included to demonstrate how the toxicology cases during this period were distributed by species. For the laboratory as a whole there were a few more swine and equine cases at the expense of bovine. “All” includes two incidents when herbicide spray mixture was accidently siphoned into the farm well. No ill effects were noticed in the animals or farm families.

The cases are under statistical study from several aspects. However, one parameter which might be of particular interest here, is source.

In Table 3 of the first two major categories, involuntary and animal access, describe the case distribution in terms of option on the part of the animals exposed. When exposure is involuntary, the animal had no option since there was contamination of feed or water...
sources, or sprays were improperly used. To the contrary, with animal access, the animal was exposed by another means and had the option to consume the material or leave it alone. The subclassifications indicate how these exposures occurred by describing the involvement of the manager. In the remaining 21% of the cases investigated during this period the source was found to be intentional, unknown, or involved an adverse drug reaction.

Table 3: Iowa Veterinary Diagnostic Laboratory
Animal Toxicoses Investigated in the State of Iowa
(June 1974 thru June 1975)

Source Classification:

<table>
<thead>
<tr>
<th>Source Classification</th>
<th>Insecticides</th>
<th>Heavy Metals</th>
<th>Feed Add.</th>
<th>Bio. toxin</th>
<th>Rodenticide</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involuntary Water</td>
<td>(16)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Feed Contamination</td>
<td>(12)</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Faulty formulation</td>
<td>(13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mineral imbalance</td>
<td>(9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>(17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Misuse</td>
<td>(15)</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>82(37%)</td>
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</table>

Animal Access

<table>
<thead>
<tr>
<th>Animal Access</th>
<th>Insecticides</th>
<th>Heavy Metals</th>
<th>Feed Add.</th>
<th>Bio. toxin</th>
<th>Rodenticide</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accidental access</td>
<td>(46)</td>
<td>14</td>
<td>10</td>
<td>1</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Discarded material</td>
<td>(36)</td>
<td>13</td>
<td>4</td>
<td>16</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Storage</td>
<td>(9)</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Misuse</td>
<td>(3)</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94(42%)</td>
</tr>
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</table>

Intentional

<table>
<thead>
<tr>
<th>Intentional</th>
<th>Insecticides</th>
<th>Heavy Metals</th>
<th>Feed Add.</th>
<th>Bio. toxin</th>
<th>Rodenticide</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Adverse drug reaction

<table>
<thead>
<tr>
<th>Adverse drug reaction</th>
<th>Insecticides</th>
<th>Heavy Metals</th>
<th>Feed Add.</th>
<th>Bio. toxin</th>
<th>Rodenticide</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(4)</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Unknown

<table>
<thead>
<tr>
<th>Unknown</th>
<th>Insecticides</th>
<th>Heavy Metals</th>
<th>Feed Add.</th>
<th>Bio. toxin</th>
<th>Rodenticide</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(40)</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

Water was a minor source of poisoning and animal loss, especially, when we consider that half of these listed cases involved no water at all, namely, sodium ion toxicosis (water deprivation). The others in this group primarily involved herbicides and the few deaths reported were not confirmed.

Feed was contaminated by various and sundry means. Insecticide was accidently incorporated into feed sources after being used to kill insects and rats around the corn crib, and bags stored with feed were broken. The one case involving heavy metals was a storage battery that went through a feed grinder. The faculty formulation category
involved feed additives that were part of the formula but in the wrong concentration. These were brought about by feed mill errors, metric conversion errors, the use of equivalent amounts of anhydrous arsanilic acid in lieu of the 20% concentrated feed additive, an attempt to accurately weigh one pound on a bathroom scale, and not realizing that a new batch of pellets was not soybean oil mean, but urea. Although problems with trace elements other than copper were encountered by this laboratory from other states, the Iowa problems listed under mineral imbalance all involved copper poisoning in sheep. Both field and storage related mycotoxin problems were encountered during the study period. Misuse is defined here, as those cases which occurred because of and during the faulty use of a material directly on an animal. Most of these involved spraying errors.

In the first two subclassifications of "Animal Access", the poison was found in the confines of the animal, ie., within the pasture, dry lot, etc. When "Storage" was the source, the animals had to break from their confines and into the area of storage for exposure. Most of the accidental access insecticide cases involved spillage or planters stored in the pasture with leaking insecticide boxes. Several occurred after cattle or swine were put on the stalk field after corn harvest. A predominant occurrence involving insecticides under discarded material involved empty bags which were piled in the field for future disposal or allowed to blow across the planted field and into a pasture. Again, misuse included instances where materials were used contrary to labelled recommendations and animals gained access to excessive amounts of the toxicant.

We recorded the rodenticide cases in the intentional category only when conclusive evidence of food contamination was found, such as when meat products spiked with strychnine granules were found in the lawn. If the whole truth were known, perhaps more of these cases from unknown would fall into the intentional category.

Attack rate (number affected) and case fatality rate (deaths per number affected) statistics have been recorded on these cases along with the compounds involved. The cases in this period were distributed in the above manner in order to ascertain the role that the animal might have in determining his own fate. From studies such as this, one may estimate the affect of certain additives to the agricultural chemicals which would render them unpalatable. There is a significant difference in the attack rates between these two general groups but this is probably due to the larger potential for actual exposure during feed contamination than the usual point source involved with "Animal Access" cases. There was no significant difference between the case fatality rates for these two general groups. In other words, the animal was as apt to die when the option was his as when the toxicant was administered to him involuntarily. We are continuing to accumulate case data and develop methods to more closely define circumstances.
These cases record 1,104 dead animals and another 5,000 that were affected but survived.

As the results from our first year's efforts are analyzed, we would have to question whether the proposed pesticide law controlling the applicator use of these agriculture chemicals will have any significant affect on poisoning occurrences and animal death loss in the State of Iowa. We are hoping for more support which will allow the continued evaluation and scrutiny of the livestock field poisoning situation.

We are indebted to Dr. Vaughn Seaton, Director of the Iowa Veterinary Diagnostic Laboratory, and Dr. Fred Wertman, Executive Secretary of the Iowa Veterinary Medical Association, for their part in helping this legislative act be more beneficial to the profession of veterinary medicine in the State of Iowa.
DEVELOPING DATA TO SATISFY DRUG AND PESTICIDE APPROVAL REQUIREMENTS OF FDA, EPA, AND USDA; ARE CATTLE, SHEEP, AND POULTRY MINOR SPECIES?

Roland A. Gessert, D.V.M.

Some 15 years ago during one of my frequent business trips to FDA in Washington, I expressed my concern over the high cost of government as reflected in the high income tax I was paying. To my complaints, a good friend in the agency jokingly replied, "Just be thankful you're not getting as much government as you're paying for". During the ensuing years, our contributions to the government coffers have steadily increased, while the amount of "government" and regulation we've been getting has increased at even a greater rate.

What are some of the benefits we who are interested in animal health and agriculture have witnessed during the past 1½ decades?

1. We have witnessed nearly total elimination of hog cholera from the United States.
2. We now have compulsory meat and poultry inspection.
3. We have experienced continued enforcement of the Delaney amendment, which forbids the addition of carcinogens to food.
4. We have enjoyed a review of all so-called "new animal drugs" by the National Academy of Science, National Research Council, to determine whether they are effective for their intended claims. And animal drugs now are approved for efficacy as well as safety.

In the case of drugs for food animals, safety must be established for food derived from treated animals. Generally speaking, no drug residues are permitted in food, and much expense goes to developing tissue residue assay methods and residue data.

In some instances, residues may persist for extended periods. Then, safe levels must be determined for long term consumption of these drug substances, and tolerances established for residue levels in the tissues, generally between 1/1000 to 1/3000 of the level demonstrated to be safe.

The cost of developing data to support establishment of a tissue residue tolerance is between one quarter million and one half million dollars. So the total cost of getting a drug for food animals approved by FDA will cost from a bare minimum of $50,000 up to the millions of dollars.

Faced with such costs, we can hardly expect pharmaceutical manufacturers to develop new and needed pharmaceuticals for use in minor species of livestock, such as ducks, geese, bison, pheasants, goats, fish, rabbits, even sheep, or creatures such as honey bees.

Because of the high cost of developing pesticides for plants, Congress made provision for the USDA to assemble data to support ap-
proval of pesticides for minor uses. This is designated the “IR-4 Program”. A similar program has been suggested for developing pharmaceuticals for use in minor species of livestock. But what about our so-called major species of livestock, such as cattle, swine, chickens, and turkeys?

I recall my early days as a veterinarian, when I was in practice—before the days of concern with tissue residues. I was called to a dairy farm where I was confronted with two veal calves severely ill, and, I thought, dying, of the calf scour-pneumonia complex. I provided my usual best treatment, which experience previously had demonstrated to be inadequate in such severe cases. And then I administered and dispensed a new investigational antibiotic I had been given—chloramphenicol. Then leaving the farmer with a poor prognosis, I left the farm.

A few months later the farmer caught me in town and yelled, “Hey, Doc! Remember those sick calves you treated last Spring?” I expected him to chide me for being an inefficient quack. “Well, they recovered in fine shape, finished real well, and I just got top dollar for them on the market last week.” In the ensuing years, many veterinarians have found chloramphenicol to be a very effective antibiotic for food producing animals as well as for dogs and cats. But now regulatory people in FDA and USDA are faced with an enforcement problem in that chloramphenicol is not approved for use in food animals, and it has been hypothesized that antibiotic residues due to chloramphenicol might be found in meat.

The pharmaceutical manufacturer holding the patent on chloramphenicol attempted to develop a tissue assay method, but were unable at that time to develop a method of adequate sensitivity to satisfy the desires of FDA. Or rather, they developed methods, conducted tissue residue studies, and then FDA demanded methods of ever increasing sensitivity. So after spending significant funds, the manufacturer abandoned this superior antibiotic for food animal use. Their patent now has expired, and several manufacturers now market the antibiotic—all for non-food producing animals. With current chemical technology, an adequately sensitive chloramphenicol residue method probably could be developed, and I understand such a method is in the final developmental stages by a governmental agency. I have no doubt that toxicological studies would demonstrate that residues, if any, would be safe, so that a residue tolerance could be established. But who would pay for all this research? Not the firm whose patent has expired, and not those who currently market the antibiotic for dog and cat use. Who, then, are the losers in the case of chloramphenicol? The losers are agriculture and the veterinary profession.

Here’s another example. A human pharmaceutical company markets a cholinesterase reactivator useful in treatment of organophosphate poisonings. The amount which they could expect to sell to the
veterinary profession is miniscule. In order that a poison antidote might be available to veterinarians, FDA requested the firm to submit an NADA, which they did, submitting data which they had on hand and which they extracted from the literature. The NADA was approved for use in dogs, cats, and non-food horses, but not for cattle or other food-producing animals. The antidote is very safe, huge oral doses being used for man. A couple years ago the firm got a phone call from a veterinarian in the West asking if the antidote could be used to treat some 300 head of cattle poisoned with an organophosphate. The veterinarian was told that the antidote was not approved for use in cattle. I understand the veterinarian rounded up stocks of the antidote and treated the cattle anyway and saved their lives. He then consulted with an FDA official who recommended the cattle be held for a prolonged period before marketing. While we occasionally may criticize some of our governmental agencies, we also must recognize that they are manned by good men, capable men, who are practical and sensible, as well as conscientious. So that in recommending observance of the long withdrawal period of the organophosphate, this FDA person knew very well any possible residues of the safe antidote would be dissipated by the time the cattle were sent to slaughter.

The basic ideas behind these laws are good. Of course, our food should be pure. I don’t want a slug of mercury compound in every fish I eat. And I’m also for God, for America, and for motherhood. But I think we have observed a bit of stage play on the part of the lawmakers. The laws, when passed, usually aren’t exactly as would seem best if we had written them. Then, the government lawyers take a new law and write the regulations, there frequently is a great distortion of the original intent of the law.

I recall attending a banquet honoring the retirement of a former General Counsel of a government agency. Next to me sat one of the finest of gentlemen, a former top agricultural and animal health administrator, a top-notch leader in veterinary medicine. As the speakers—also lawyers—droned out plaudits about the retiring General Counsel, this fine veterinary leader leaned to me and whispered, “Why do you suppose they’re so honoring him?” Surprised, I mumbled something like, “I guess it’s probably because he’s had such a great influence on the food and drug industries.” To which my esteemed friend replied, “He’s been nothing but a damned nuisance to me.”

Reflecting on this comment, it does frequently seem that medical, scientific, and social progress take place in spite of, if not because of, laws and their interpretation and administration by the legal profession.

So, admitting that many of the laws governing pharmaceuticals for food-producing animals are desirable, let us consider how this
nation might have pure and safe food and at the same time have economical food and healthy animals, cared for by veterinarians armed with the most up-to-date supply of pharmaceuticals and biologicals, and owned by livestockmen who can freely and legally use production tools, chemical or otherwise, to obtain the greatest possible production efficiency.

These laws that have greatly increased our taxes have also increased our workloads, and have greatly increased the cost of developing new pharmaceutical and production products for livestock. As a result, many products we might have had will not be developed. To counteract the effect of past laws which have stifled food-animal research at an ever-increasing rate, possibly we need yet another law to provide some government support to food-animal drug and production research. Let the government pay for research for animal health products and chemical production tools that would have been paid by private industry if the government, through its laws and regulations, hadn’t made the research and development costs so unbearably high. The research could include both efficacy and safety studies; residue assay development; tissue residue studies, and long-term safety studies on which residue tolerances might be based. Thus, controlled breeding compounds abandoned by pharmaceutical companies because of research costs, but desired by beef and swine producer associations and by the USDA-ARS (Beltsville), could be developed for extensive use. A true anti-stress agent useful in shipping livestock and also useful as a growth promoter could be developed for animal agriculture. The company owning this agent will not spend $50,000 to $250,000 to develop a drug for a veterinary market of less than a million dollars when the same product in the human market will bring annual sales of $10,000,000 to $50,000,000 or more.

Maybe a government sponsored program could at last make chloramphenicol and other superior antibiotics available and legal for food producing animals.

Having little experience in holding my hand out to the government, I’m not certain how the project might be funded. My first thought might be a grand explosion of the IR-4 Program. Perhaps a great expansion of the USDA-ARS might be a logical site — or maybe a new department in Meat and Poultry Inspection. Certainly Congress would need to provide for the program and its funding. But, as the Congressional investigating committees turn the screws tighter and tighter on FDA and USDA, and as EPA gets ever friskier and more suppressive, in its new day, I feel it’s the duty of this same Congress to assure this nation, and the world who depend on us, of a continuing and increasing supply of pure, safe, wholesome, economical food.
METHYLENE BLUE VS. NITRITE

G. E. Burrows and J. L. Way

The investigation was supported from the National Institute of Health, General Research Support Grant FR 5465 administered through the College of Veterinary Medicine, Washington State University.

Nitrite toxicity in domestic farm animals occurs primarily secondary to nitrate ingestion. Nitrate exposure occurs following inadvertent availability of farm fertilizers or nitrate accumulating toxic plants. The rumen fermentation microbes convert the nitrate to nitrite, with nitrite ion acting as the toxicant. The primary mechanism of toxicity associated with nitrite intoxication is considered to be the formation of excessive methemoglobin (MHb) as a result of the oxidation of ferrous hemoglobin (Hb) to ferric Hb. The oxidized ferric MHb is incapable of acting as a reversible oxygen carrier, hence the animal essentially suffocates. Methylene Blue (MeBl) has long been recognized as an effective antidote for methemoglobinemia both in man and domestic animals. Traditionally, however, the dose recommended is quite low, i.e., 4 mg/kg, since MeBl is considered to be a reducing agent at low doses and a direct oxidizing agent at higher doses. The reductive activity is associated with a naturally occurring NADPH dependent enzyme system in the body which results in the conversion of the MeBl to the reduced leuco form of MeBl. This reduced dye then becomes reoxidized back to MeBl while in the process reducing the MHb back to Hb. In higher doses the MeBl is thought to oxidize the Hb directly to form MHb. More recently several investigators have been unable to demonstrate any direct oxidation of Hb associated with high doses of MeBl in vivo. The present investigation was carried out to determine the optimal dose of MeBl as an antidote for nitrite toxicity based on reduction of toxic levels of MHb and protection against lethality.

Materials and Methods

Mature ewes of mixed breeding (both white and black faced) and good health were utilized. These ewes were maintained in dry lots on a mixed alfalfa-grass hay diet. Sodium nitrite (NaNO2) prepared as a 3% or 5% solution in physiologic saline was administered intravenously. MeBl chloride (NF) was administered I.V. as a 1% or 3% solution (Solubility = 3.55% in water) in 14% sorbitol. Nitrite tox-
city was produced by administration of either 50 or 100 mg/kg NaN0₂, with MeBl therapy beginning 30 minutes and 10 minutes later, respectively. Either of these doses of sodium nitrite resulted in the death of ewes if no antidotes were available. Four ewes were utilized for each dose of nitrite and MeBl. The MHB analysis was carried out utilizing the method of Leahy and Smith, with a Bausch and Lomb spectronic 20. MHB values are reported as percentage of total circulating Hb.

Results

The effects of varying amounts of MeBl in reducing a lethal level of MHB are shown (Fig. 1). The administration of 50 mg/kg NaN0₂ intravenously resulted in the formation of 70-80% MHB in approximately 30 minutes and was lethal in four out of four ewes, 45-50 minutes following administration. The lethality associated with this dose of NaN0₂ was successfully antagonized by any of the three doses of MeBl used in this experiment, 2.2, 4.4 or 22 mg/kg. However, the rate of reduction of MHB to Hb increased as the dose of MeBl was increased. The time required to reduce the lethal level of 70-80% MHB down to 20% MHB was significantly shortened by increasing the dose of MeBl from 2.2 or 4.4 mg/kg up to 22 mg/kg.

The effects of MeBl in counteracting a large excess of NaN0₂ are shown (Fig. 2). The dose of 100 mg/kg NaN0₂ provides considerable excess nitrite ion over and above that required to oxidize Hb to a lethal level of 70-80% MHB. Doses of MeBl of up to 8.8 mg/kg were not effective in antagonizing the lethality of this amount of NaN0₂ in any of four ewes. A dose of 22 mg/kg MeBl was effective in antagonizing the lethality associated with 100 mg/kg NaN0₂ in all of four ewes. However, 85 minutes were required for the reduction of the lethal 70-80% MHB levels down to 20% MHB level as compared to the 15 minutes required when only 50 mg/kg NaN0₂ was administered.

The LD₅₀ of NaN0₂ I.V. was in the range of 40-45 mg/kg since 35 mg/kg was not lethal for any of four ewes while 50 mg/kg was lethal in four out of four ewes. The LD₅₀ of MeBl was in the range of 40-43 mg/kg and no lethality was noted at 35 mg/kg. The direct oxidation of Hb to MHB by the MeBl itself did not appear to be an important factor as the maximum MHB produced by 22 mg/kg MeBl was only 6%.

Discussion

The lethal level of MHB in sheep appears to be approximately 75% which is similar to that reported by other investigators. In sheep the dose of NaN0₂ required to produce the lethal MHB level is approximately 40-50 mg/kg. This is equivalent to 30-37 mg/kg for nitrite ion or 11-14 mg/kg for Nitrite-N. The lethal level of 75% MHB is read-
ily reduced to non-lethal levels by doses of MeBl conventionally used in the treatment of nitrite toxicity in ruminants, 4 mg/kg.\textsuperscript{3,6} The rate of reduction of the MHb can be markedly increased by increasing the dose of MeBl. A dose of 50 mg/kg NaN\textsubscript{2} while provoking the formation of lethal levels of MHb probably does not result in appreciable amounts of excess nitrite ion over and above that required to produce the lethal MHb effects. This is shown by the slope of the curve of MHb reduction (Fig. 1). The nitrite apparently continued to oxidize the Hb for about 45 minutes while the MeBl is reducing the MHb. During this period of time, at low doses of MeBl, the MeBl reduction of MHb and nitrite oxidation of Hb are in approximate equilibrium. Increasing the dose of MeBl seems to effectively shift the equilibrium to favor MHb reduction. At 100 mg/kg NaN\textsubscript{2}, sufficient nitrite ion is available to effectively antagonize the reductive activity of MeBl on the oxidized Hb molecule. A competitive situation seems to exist with the nitrite ion oxidizing the Hb at least as fast or faster than the MeBl can reduce the MHb back to Hb. Increasing the dose of MeBl up to 22 mg/kg apparently shifts the equilibrium in favor of MHb reduction back to Hb to such a degree that the MHb levels are reduced rapidly enough down to nontoxic levels to antagonize the lethality. Direct oxidation of Hb to MHb by the MeBl at these levels (22 mg/kg) while possibly occurring in very small amounts (6\% or less) is apparently inconsequential. This is in agreement with recent investigations which have been unable to confirm the earlier reports of methemoglobinemia following the use of high levels of MeBl in vivo.\textsuperscript{11,14,15} Methemoglobinemia as produced by MeBl is primarily an \textit{in vitro} phenomenon.

From this data it appears that increasing the dose of MeBl in cases of nitrite toxicity is very likely to produce positive results. The lack of formation of MHb with these high doses of MeBl would seem to alleviate the apprehension of some individuals concerning the use of the high doses of MeBl. Therefore, the recommended dose of MeBl should be increased to about 20 mg/kg in sheep in confirmed cases of nitrite toxicity. This represents about 100 ml of the commercially available 1\% solution of MeBl in an adult sheep. It should be pointed out, however, that this recommendation applies only to ruminants since other species may differ considerably as regards susceptibility to MeBl toxicity.
Fig. 1

Methemoglobin levels following 50 mg/kg sodium nitrite 0, and 2.2 •, 4.4 □, or 22 △, mg/kg methylene blue. Expressed as a percentage of the total circulating hemoglobin.

Fig. 2

Methemoglobin levels following 100 mg/kg sodium nitrate 0, and 8.8 •, or 22 △, mg/kg methylene blue. Expressed as a percentage of the total circulating hemoglobin.
REFERENCES


INFLUENCE OF AMBIENT TEMPERATURE, HUMIDITY, VENTILATION, AND OTHER PESTICIDES ON NEURAL AND DERMAL TOXICITY OF DDVP

R. Keith Farrell,* Thomas G. Bell,** George A. Padgett**

A report to the Committee on Pharmaceuticals of the U.S.A.H.A. on observations of ataxia, depression and death in laboratory cats apparently associated with dichlorvos (DDVP) flea collars was presented at the annual meeting in 1973. This report will clarify some of the observations reported at that time and confirmed later by our observation on additional animals.

Of 50 laboratory cats wearing commercial flea collars impregnated with 2,2-dichlorovinyl dimethyl phosphate (dichlorvos or DDVP) in a warm, dry environment, 42% exhibited an ataxia-depression syndrome. Of the affected cats, 8% died. The signs observed resembled those described in field cases of suspected DDVP flea collar toxicity. Anorexia, gastrointestinal hypermotility, salivation, diarrhea, and dyspnea were common early signs. Muscle twitching and incoordination were readily apparent when the cats previously trained were induced to negotiate a suspended 2 x 4 board. The more severely affected animals showed progressive weakness, flaccidity, and partial paralysis. Central nervous system symptoms of apprehension and atypical anger with a tendency to bite the handler were observed in previously placid cats. The more severely affected cats progressed to a somnolent state. Many of the severely affected cats were observed to have pale mucous membranes and were found to be anemic. The anemia appears in the more chronic cases and has not been described in the literature for other organic phosphates. It appears in the collared cats only at high dosage, and not in the litter mate controls.

Cervical contact dermatitis occurred in 74% of 50 dichlorvos collared cats and appeared on both cats that showed ataxia-depression and those that did not.

The muscarinic, CNS, and nicotinic symptoms occurred in cats only in a warm, dry environment and not in cats deliberately exposed to two and three collars in high humidity.

Multiple collar exposure on single animals showed a marked variation in individual susceptibility between cats.

The high occurrence of systemic and cutaneous abnormalities in these experiments, when compared with superficially similar trials conducted by others, must be explained. The major difference to be considered is the warm, dry environment.

*Pioneering Research Laboratory, Western Region, Agricultural Research Service, US Department of Agriculture, **and the Department of Veterinary Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA 99163.

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The acute dermal LD_{50} of dichlorvos in a xylene carrier for the female rat has been determined as 75 \, \mu g/kg body weight; death is reported in rats exposed to 31 to 118 \, \mu g of dichlorvos/L of air over exposure periods of 4.8 to 17.5 hours. Humans have withstood 6 to 9 \, \mu g of dichlorvos/L of air for 30 to 60 minutes without change in plasma or erythrocyte cholinesterase (ChE) activity or other clinical effect, whereas 0.15 \, \mu g of dichlorvos/L of air kills 99\% of houseflies during a 30-minute exposure period. A cat collar contains roughly 500 ± 40 mg dichlorvos (4.56\%). This dose is delivered over a period of 2 to 3 months, but it is not clear what part might be delivered in the first 5 to 15 days after collar application.

In any solid formulation of dichlorvos, factors that may influence the rate of release are (1) environmental temperature, (2) concentration of dichlorvos in the formulation, and (3) humidity. Dichlorvos has a high vapor pressure compared with that of other organic phosphates. The vapor pressure is 0.032 mm of Hg at 32 C and 0.296 mm of Hg at 60 C. It is not surprising, therefore, that the rate of vaporization from PVC resin in strips approximately doubles for every 11.1 C increase in room temperature. More specifically, all other factors being constant, with relative humidity less than 20%, a change of temperature from 25 C (77 F) to 38 C (100 F) increases the output of dichlorvos 2.5 times.

As the dichlorvos collar ages during wear, the concentration of dichlorvos decreases within the resin. The initial rate of vaporization is high at any temperature. Eventually, an equilibrium is established, and then molecules of dichlorvos must diffuse to the surface before vaporization.

After dichlorvos is vaporized, hydrolysis and consequent detoxification occur most rapidly in the presence of moisture. For every 30\% rise in relative humidity, the dichlorvos released from a given application will be reduced by about 50\%.

In this study, cats in warm, high humidity evidenced no abnormalities, whereas cats in warm, dry environments evidenced both dermatitis and systemic effects.

The neurologic manifestation, not previously reported in dichlorvos toxicosis in any species to our knowledge, is the syndrome known as the dying-back process, or delayed neurotoxic effect, and has been reported as a result of exposure to a number of organic phosphate compounds, such is di-isopropyl phosphorofluoridate, N,N'-di-isopropylphosphorodiamidic fluoride, and tri-orthocresyl phosphate. The production of neurotoxic lesions is enhanced when survival has been maintained by antidotes that suppress cholinergic crisis. The clinical syndrome in cats varies from unsteadiness of hindlimbs and clumsy gait to marked hindlimb weakness and depression, indistinguishable from signs manifested by cats in this study. Urinary and fecal incontinence are also noticed.
The essential difference between the neurotoxic properties and the cholinergic properties of organic phosphates is neither chemically nor biologically well defined. Although dichlorvos will not cause delayed neurotoxic effects in a single oral or injected dose, it has not been extensively tested at doses approaching the LD₅₀ of the slow-release formulations (trials at least 90 days in duration) for neurotoxicity in susceptible species (cats, birds, and primates). Such trials are necessary, inasmuch as organic phosphate compounds may cause delayed neurotoxic effects only if doses above the LD₅₀ are given at the same time as treatments for the cholinergic effects. Inasmuch as acute cholinergic effects limit the dose that can be given, it is difficult to state categorically that any organic phosphate is not neurotoxic.

Our studies of more chronic nature have shown muscle degeneration in cats typical of that described for neural involvement.

However, our study only superficially exposes the nature of systemic abnormalities. From the data presented, we can conclude that ambient temperature and relative humidity may have profound influence, particularly when cats are confined, as in this study, under laboratory conditions. The danger of a build-up of vapor from multiple collars was evident from cholinesterase drop in caged contact controls. Cats in catteries, veterinary hospitals and some homes share the danger of more than one flea collar in an enclosure and frequently, a more powerful (20% DDVP) gas generator in the form of an anti pest strip.

One of the safety claims for DDVP resin strips in relatively confined spaces, that the user virtually cannot alter rate of release, must be put aside. A task force report of the Environmental Health Science center, Oregon State University at Corvallis, Oregon (Gillett, et al.: “Evaluation of human health hazards on use of dichlorvos (DDVP), especially in resin strips.” Residue Review, 44 (1972):115-159), describes the release of DDVP from resin strips and warns that “the combined conditions of high temperature, low humidity and restricted ventilation may create an environment in which DDVP air concentrations could exceed established safety standards”.

We feel that additive dosage problems are real under both laboratory and field conditions and the problem is likely to increase with increased use of systemic anthelmintics, anti pest strips, spray insecticides, back rubbers, roach killers, and other products, not at present on the market, but contemplated.

We are skeptical about the use of marketing surveys indicating a high degree of acceptance as a criteria of an absence of adverse reaction. Subjective interpretations are no substitute for laboratory experimentation.

We feel veterinarians observing ataxia and depression should immediately remove the flea collar and consider flea collar toxicity as
an entity to be excluded before making a diagnosis.

We respectfully request the Committee on Pharmaceuticals and Toxicology to encourage change in testing of DDVP related products to include observations specifically on neurological parameters under severe environmental conditions known to affect release or activity of the product and encourage additional research with more objective methods such as electromyography to assess neurological activity.

REFERENCES

REPORT OF THE COMMITTEE ON PHARMACEUTICALS AND TOXICOLOGY

Chairman: Dr. Roland A. Gessert, Vineland, N. J.

Co-Chairman: Dr. Fred J. Kingma, Fairfax, Va.

Dr. Dan J. Anderson, Ft. Worth, Tex.; Dr. William B. Buck, Ames, Iowa; Dr. E. E. Denlinger, Ronks, Pa.; Dr. R. Keith Farrell, Pullman, Wash.; Dr. James Fox, Ashland, Ohio; Dr. Hardin E. Gouge, St. Joseph, Mo.; Dr. William Knapp, Rockville, Md.; Dr. G. A. Mitchell, St. Louis, Mo.; Dr. William A. Rader, Washington, DC.; Dr. Sam F. Scheidy, Bryn Mawr, Pa.; Dr. Clifford C. Beck, Manchester, Mich.; Dr. W. C. Burnett, Kansas City, Mo.; Dr. George T. Edds, Gainesville, Fla.; Dr. James Hanson, St. Paul, Minn.; Dr. G. D. Lindsey, Carmel, Ind.; Dr. Fred Oehme, Manhattan, Kans.; Dr. Jane Robens, Rockville, Md.; Dr. Norman Tufts, Boston, Mass.; Dr. Clyde Wilson, Cedar Rapids, Iowa

Others Present:

Dr. Max L. Crandall, San Juan, Puerto Rico; Dr. James W. Glosser, Helena, Mont.; Dr. Richard F. Hall, Caldwell, Idaho; Dr. Carl Norden, Lincoln, Neb.; Dr. F. Edward Sterner, Denver, Colo.; Dr. R. F. Weidner, Chicago, Ill.; Dr. C. D. VanHouweling, Rockville, Md.; Dr. F. D. Wertman, Des Moines, Iowa; Dr. Gavin L. Meerdink, Ames, Iowa

The committee has been expanded to include toxicology in addition to pharmaceuticals, and several prominent veterinary toxicologists were added to the committee membership.

In the past two reports of this committee, the concept of Peer Group Review to settle differences of scientific evaluation as outlined in briefs prepared by a special task force of the Animal Health Institute, was endorsed. (See Page 231, Proceedings, 77th Annual Meeting of the USAHA) The Director, Bureau of Veterinary Medicine, FDA, states that there is no provision in the FD & C Act for a Peer Review Group as suggested by AHI, but states that a three man Board of Inquiry might be considered in lieu of the much more expensive and unwieldy official hearings provided in the regulations.

A paper on the use of methylene blue in treatment of nitrite poisoning was presented by George E. Burrows, DVM, PhD, University of Idaho. His study indicates that the recommended dose of methylene blue should be increased to about 20 mg/kg in sheep in confirmed cases of nitrite toxicity. Further, this recommendation applies only to ruminants, since other species may differ considerably as regards susceptibility to methylene blue toxicity.

The committee continues to recognize that many drugs are not made available for use in food producing animals because the limited
use the drugs would receive does not justify the cost of the research necessary to support FDA approval. This is particularly true of research to determine tissue residue levels, residue tolerances, and drug withdrawal times.

In order to make such drugs available to the livestock industry (and particularly for the minor species), the committee urges Congress to provide for the development of these data by the USDA, FDA, or other governmental agency.

The Chairman and Co-Chairman of the Committee on Biologics expressed interest in obtaining similar data to support registration of biological products for minor species or minor uses. A program similar to the IR-4 Program (Interregional Project 4) for registration of pesticides for minor uses seems worthy of consideration. (The IR-4 Project was activated to compile required information through the State and Federal Agricultural Experiment Stations and other sources to obtain pesticide tolerances and registered labels.)

The FDA position that all animal drugs are regarded as new animal drugs under the FD & C Act was criticized by the committee. This position is defended by FDA chiefly on the basis of concern with tissue residues in food producing animals.

Dr. Gavin Meerdink, Iowa State University, briefly described Iowa's law which requires reporting cases of toxicity due to agricultural chemicals and veterinary drugs. Iowa's law may be used as a model for other states seeking information and methods of control of such adverse reactions. A detailed report of Iowa's program is presented elsewhere in this volume.

Dr. R. Keith Farrell, U.S. Pioneering Research Laboratory, Pullman, Washington, reported on his continuing studies on toxicology of dichlorvas (DDVP) in cats. As a result of his studies, he suggests that testing of DDVP related products include observations specifically on neurological parameters under severe environmental conditions known to affect release or activity of the product and encourages additional research with more objective methods such as electromyography to assess neurological activity.

The following new animal drug entities were approved by the Food and Drug Administration during the past year:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenperone HCL</td>
<td>An injectable tranquilizer</td>
</tr>
<tr>
<td>Megestrol Acetate</td>
<td>An oral progestogen for postponement of estrus and alleviation of false pregnancy in dogs.</td>
</tr>
<tr>
<td>Bambermycin</td>
<td>An antibiotic to increase growth and feed utilization in swine</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>An antibiotic to increase growth and control dysentery in swine.</td>
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</tbody>
</table>
Ampicillin  First food animal approval of this antibiotic: to treat bacterial pneumonia, colibacillosis, and salmonellosis in swine.

The following are the first products for intramammary infusion approved under the revised stringent guidelines for establishment of safety and efficacy:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novobiocin and Procaine</td>
<td>Treating mastitis in lactating cows</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Mastitis treatment</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td></td>
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</table>

Withdrawal

Vincophos  Anthelmintic for dogs; NADA withdrawn by Shell Chemical Co. because of a high incidence of adverse reactions.

The committee recommends dissemination of this report, after acceptance by the Executive Committee, to interested officials in governmental agencies and to appropriate leaders in Congress.
CONVERTING GARBAGE (FOOD WASTE) INTO A SAFE ANIMAL FEED INGREDIENT (BIOMEAL™)

Dr. Thomas W. Powell*

In our society today we are faced with the disposal of waste materials in volumes never before encountered. It is estimated that over 800 million tons of food waste are being destroyed in the United States each year. Probably 3/4 of this amount can be collected and processed.

My colleague, Dr. Charles N. Dobbins, Jr. and I have been involved for about five years in the development of a process that converts food waste into a safe, valuable feed ingredient. I am happy to say that we can now collect, process and utilize our product Bio-meal.

Disease eradication programs, increased emphasis on ecology, the cost to cities for solid waste disposal and the world food situation have all influenced the development of the new feed ingredient, Bio-meal.

This project has been supported by the University of Georgia, USDA, Department of the Army, our Georgia Department of Agriculture and others. Since we are in the Cooperative Extension Service, it has truly been a cooperative effort to say the least.

The Hog Cholera Committee of USAHA has made significant contributions to the successful eradication of Hog Cholera. They should be commended and recognized for this effort. The problems associated with garbage feeding have been noted in recommendations from this committee. The utilization of food waste for the production of animals, maintaining safety factors, is another step we should take and recommend as advisors to the animal health community.

By providing an alternate method of feeding garbage or food waste we solve many problems. The least of these may be disease control, but will certainly aid and enhance the control of hog cholera, trichinosis, salmonellosis and others.

Cities are faced with ever-increasing costs of solid waste removal. By removing the food waste portion from the total solid waste to be collected, cities can reduce the frequency of pickup by at least one-half. This can mean tremendous savings to the cities involved.

It has been demonstrated that food waste does have nutritional value. It is merely a resource out of place. Traditional garbage feeding is not compatible with hog cholera control. Changing food waste in character and providing a safe, nutritious feed ingredient does meet the criteria for its utilization.

*Extension Veterinarian, University of Georgia


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Biomeal, derived entirely from food waste, is not a complete feed ration. It is not a supplement or feed additive. It is a feed ingredient the same as corn, soybeans, oats, milo, etc.

The analysis of Biomeal is very uniform although there may be variations from individual plants due to the input of different raw materials. Military food waste yields a very high analysis. A protein level of 18-20 percent, 10-12 percent fat with a fiber content of $1\frac{1}{2}$-2 percent is our experience at Ft. Benning, Georgia.

Biomeal is produced utilizing various pieces of equipment to remove fat, moisture and grinders to size the material. The final product after heat treatment contains 10 percent moisture and is in meal form. The identity of the raw material is lost. It is no longer garbage but processed food waste. The yield is between 32-35% of the initial input of food waste after dehydration. All known pathogens are killed by this treatment.

Our controlled feeding trials have been with poultry. A recent trial using Biomeal, as 20% of the ration produced birds at $1/4\$\text{/lb.}$ less than the base1 ration.

The collection of the raw material by utilizing vacuum and containers that can be kept clean along with the process that produces a safe, feed ingredient makes for a successful program. While large volumes of material are necessary to produce Biomeal, the economics of production indicate that a ton of Biomeal can be produced for about $\$75-\$85/ton. Additional free fats and oils removed from the material provide an additional source of income from the process. The nutritional value of Biomeal will make its sale price/ton between the price of corn and soybean meal.

"Time and Tide Waits for No Man," is very true, and a "giant step for mankind" is an appropriate quote. Let us all concern ourselves with problems of disease, our environment and a feeling of responsibility toward efficient production of animals. We feel that the production of Biomeal will contribute to these goals.
Fiscal year 1975 was an excellent year for the State-Federal Hog Cholera Eradication Program. Hog cholera was not diagnosed during the entire fiscal year.

Plans were being made to officially declare the United States free of hog cholera when the virus was detected in a small livestock market located in Hereford, Texas, on July 4, 1975.

During April 1975, Great Britain removed a 20-year ban and began to again import pork products from the United States, since hog cholera had not occurred in this country for over one year. This importation abruptly ended with the reappearance of the virus in Texas.

On July 1, 1975, all 50 States and Puerto Rico were in hog cholera "free" status. The year had begun in July 1974, with 50 States in free status and Puerto Rico in Phase III; having had hog cholera occur in May 1974, Puerto Rico lost its free status because of spread of the disease. Puerto Rico progressed through Phase IV status and then back into the "free" category on November 16, 1974.

During July 1975, Texas lost its free status because of spread of the disease. The State re-entered Phase IV this October and would have been eligible to regain "free" status during January, 1976.

However, an amendment to Title 9, CFR, Part 76, entitled "Hog Cholera and Other Communicable Diseases of Swine" which deletes references to Eradication (Phase IV) and "free" States, is pending signature and is expected to become effective sometime this month. This terminology is being eliminated upon the recommendation of the USAHA and the Secretary of Agriculture's Advisory Committee on Hog Cholera Eradication. The original intent that the States meet certain step-like goals (phases) while proceeding toward eradication of the disease, has been fulfilled.

Hog cholera was not detected in the United States during FY 1975. Prior to the hog cholera outbreak in Texas in July of this year, the last previous cases were in Puerto Rico (May 1974), Mississippi (February 1974), and Indiana (June 1973). This is a total of three isolated outbreaks during a period of 28 months extending from July
1973 through October 1975. This is in marked contrast to just five years ago (FY 1970) when 1,231 cases were confirmed.

The longest periods of freedom from the disease were 225 days between the Indiana and Mississippi cases, and 426 days between the cases in Puerto Rico (May 1974) and Texas (July 1975). Hog cholera was not detected in the continental United States for a 512-day period (February 1974 through July 1975).

Along with the decrease in incidence of hog cholera has come a steady decline in reporting and investigation of sick swine. The 821 investigations conducted during FY 1975 represent a twofold reduction from FY 1974 and a fivefold decrease from FY 1973. (Investigations were conducted at a rate of 349, 126, and 68 per month during fiscal years 1973, 1974, and 1975, respectively.)

Epidemiology

Had it not been for the hog cholera outbreak in Texas on July 4, 1975, the United States would have become eligible to be declared officially "hog cholera free" today, November 4, 1975.

The southern Regional Emergency Animal Disease Eradication Organization (READEO) was quickly activated to stamp out the disease in Texas. The epidemiology involved was quite extensive. The herd which was initially found infected had recently received swine from 198 herds which were sampled in an attempt to locate the source of infection. All were negative except one which was depopulated on July 21, 1975. It was concluded that this herd was the most probable source of infection based on herd history, serology, histopathology, and postmortem lesions.

Twenty-two herds (23 owners) in Texas and six in Oklahoma were depopulated because of exposure to the first diagnosed case. In addition to this, slaughter animals moved from the herd through three stockyards into eleven slaughter plants in five States. Known distribution of the result pork products was to 27 States, Puerto Rico, England, and Tokoyo, Japan. An alert was immediately sent to all States to intensify garbage inspections to assure proper heat treatment of garbage being fed to swine.

In addition to the extensive tracing, 243 investigations were conducted where diagnostic work was performed, and over 18,000 swine were inspected on 194 premises in Texas and Oklahoma.

The source of the outbreak is unknown.

Surveillance

The previously mentioned decrease in reporting of sick swine has made it necessary to place greater emphasis on other surveillance methods.

One of the most important is the routine screening of swine
tissues for hog cholera by the fluorescent antibody (FA) test (figure 3). Forty laboratories in 31 States are approved to conduct this test. Veterinary Services Laboratories, Ames, Iowa, serves as the reference laboratory and also screens tissues submitted from States which do not have hog cholera FA capability. Four of the last five hog cholera outbreaks were initially detected through laboratory screening (they were not reported as being suspicious of hog cholera).

Surveillance statistics for FY 1975 (figure 4) indicate more than 95 million swine were either inspected or screened by laboratory methods during the year. Since this is almost double our swine population, it is obvious that many were checked at least twice.

Over 77 million head received both antemortem and postmortem inspection at slaughter. Ten million were inspected in markets and 8 million on-the-farm. The on-the-farm category includes swine inspected prior to and following movement, during garbage feeding inspection, or while State or Federal veterinarians are on a farm for another purpose.

Tissues or sera from approximately 50,000 head were screened for hog cholera virus or antibodies in diagnostic laboratories during FY 1975.

Outlook

Those who have been associated with the hog cholera eradication program cannot consider it without feeling a sense of pride and accomplishment. We remember what the disease can do in a herd of hogs and how it can spread from farm to farm.

We remember the obstacles in getting the program started, and the detours along the way. We also remember the long hours and the hard work.

We remember the difficult years like FY 1970 when hog cholera occurred in 37 States and we experienced 195 positive cases in North Carolina, 175 in Texas, 157 in Missouri, and 130 in Tennessee. We even remember at times calling Washington to see if USDA had sufficient funds to help depopulate infected herds.

We remember hearing "it can't be done", and, in retrospect, marvel that it was ever started.

We remember many things about the program, but what we must remember is that we are not finished. We still have a job to do. We must continue to be vigilant and to do the things which have brought us this near accomplishment. We still need to remind folks to "suspect hog cholera first".
Laboratories Approved for Conducting FA Testing for Hog Cholera

* Veterinary Services Laboratory, Ames, Iowa
40 Laboratories in 31 States

HOG CHOLERA SURVEILLANCE
FY 1975

<table>
<thead>
<tr>
<th></th>
<th>HERDS</th>
<th>SWINE</th>
</tr>
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<tr>
<td>ON-THE-FARM INSPECTION</td>
<td>129,755</td>
<td>8,344,082</td>
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<tr>
<td>MARKET INSPECTION</td>
<td>521,957</td>
<td>10,328,259</td>
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<tr>
<td>SLAUGHTER INSPECTION</td>
<td>—</td>
<td>77,163,400</td>
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<tr>
<td>LABORATORY SCREENING:</td>
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<tr>
<td>Reactive Antibody (FA) Tests</td>
<td>5,265</td>
<td>15,282</td>
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<tr>
<td>Serum Neutralization (SN) Tests</td>
<td>11,461</td>
<td>35,742</td>
</tr>
<tr>
<td>TOTAL</td>
<td>668,438</td>
<td>95,886,765</td>
</tr>
</tbody>
</table>

U S DEPARTMENT OF AGRICULTURE  VETERINARY SERVICES  ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONWIDE ERADICATION OF HOG CHOLERA COMMITTEE

Chairman: D. L. Smith, Rushville, Ind.

Co-Chairman: J. B. Taylor, Montgomery, Ala.


The committee again emphasized the outstanding contribution of individuals and members of the swine and allied industries and organizations, and state and federal regulatory agencies to the hog cholera eradication program.

In consideration of the long time periods between the past few hog cholera cases, the committee recommends that the nation be declared officially hog cholera free one year after depopulation of the last positive case, followed by a three year surveillance period. The committee recommends that this organization, the state, the industry, and USDA cooperate in formulating plans to properly recognize this achievement.

The latest hog cholera outbreak in Texas emphasizes the need for increased surveillance along the US-Mexico border. The committee reminds the Association that this problem is a national responsibility, and not a problem to be faced solely by the states along the border.

It was noted that steps are being taken to eliminate references to the various phases of the hog cholera eradication program in Title 9 CFR, Part 76 in line with previous recommendations of this committee to consider states as either infected or free.

It was also reported that a proposed rulemaking has been published in the Federal Register concerning identification of sows and boars. The committee had recommended the identification of all swine, but recognizes the importance of the above proposed rulemaking.

The committee recommended that proper market standards be maintained as previously recommended.

The committee again recognized the importance of surveillance and recommends continued emphasis on the following:

1. The reporting and investigation of sick swine.

2. Screening of all swine tissue submitted to diagnostic laboratories for hog cholera by the fluorescent antibody (FA) technique.
The committee recognized that four of the last five hog cholera outbreaks were detected through this means.

The committee recommended that the use of positive control tissues be continued in approved laboratories, and noted that laboratory security should be conducted annually. The committee will also re-examine this position annually.

3. Adequate enforcement of state laws and regulations pertaining to garbage (food waste) feeding.

The committee commends the efforts taken to publicize the dangers of introduction and spread of foreign animal diseases by foreign visitors or other persons leaving the country and returning, and recommends continued activity of this nature.

The committee again recommends that the inspection of garbage (food waste) premises be continued after the hog cholera eradication program ends, in order to prevent re-infection with hog cholera or other foreign animal disease; or until such time that the practice has been converted to an approved garbage dehydration process. The committee finds the latter method more desirable and encourages the states to work toward that objective.

It was noted that during the most recent hog cholera outbreak that there was a delay in getting the task force in full operation. The committee wishes to call this to the attention of the president so that he can refer this to the proper USAHA committee.
The identification and discovery of hitherto unknown herpes-viruses in vertebrate animal life are being made with sufficient frequency that uninformed persons may be deceived into the view that herpesviruses are actually rather new. It also seems to surprise many that within the herpesviruses group there are serologically and chemically identifiable viruses that affect not only man, primates, and other mammals, but also lower vertebrates such as fish, amphibians, and birds. This rather sizeable group of viruses are bound together on the basis of common or similar chemical and physical properties, and to a lesser extent on biological properties. Perhaps it has been a consequence of the broad spectrum of life that herpesviruses may infect that much attention has been given to them individually and as a group. Many molecular biologists around the world have built their careers on studies of the physical, chemical, and biological characteristics of a variety of the herpesviruses. And they continue to do so. Much has also been accomplished in the study of the immunological reactions of animal life to these viruses. Vaccines have been developed to cope with some infections of birds and other economically important animals with varying measures of success. The measure of success is related in some important respects to the commercial or economic objectives involved. Most cattle, swine, and poultry are marketed early in their potential life span. Consequently, the effective period required for immunity is relatively short. In contrast, live vaccines against herpesvirus infections of humans find few supporters because of the possibilities of establishing latent infections over the long span of life. The most unhappy consequence of herpes latency in humans has been in type 2 *Herpesvirus hominis* infections of females; as it has been frequently associated with carcinoma of the cervix. Latent infections of herpesviruses in animals are important principally because they result in the spread of disease and maintenance of the virus in the environment.

A prototype herpesvirus in the virus of pseudorabies or Aujeszky's disease. Much of the study alluded to has been accomplished using pseudorabies virus (PrV) as the model and much is known about it. One unusual feature of PrV is the rather broad spectrum of life that it infects naturally and under experimental conditions as opposed to the narrow host range of other animal herpesviruses. Natural infections have been reported in swine, goats, cattle, sheep, dogs, raccoons, deer, cats, mink, foxes, badgers, hares, rats, and mice.
This fact is of concern when methods for control of the disease are being considered. Infections were confirmed by fluorescent antibody tests in six Indiana raccoons in the first four months of 1975. It is especially interesting to note that the disease was confirmed in a raccoon from each of two farms on which no evidence of the infection in swine was found. In each case the raccoon was fatally infected. There are probably yet unrecognized natural hosts for the disease. Vaccinations of swine with a live-virus preparation could result in latent infection of free living wild life and, at worst, become a means of establishing or expanding reservoirs of the virus and therefore, sources of virus for infection of swine or other susceptible species. This point of view has support from basic research information on PrV. The virus has been a model for the study of the events of replication of herpesviruses. The components of PrV are not made in equal quantities and all reactions in assembly do not go forward to completion of the virion (the complete infectious viral unit), for this and other reasons. Subvirion components are restricted to the nucleus; these are such units as empty capsids, capsids, nucleocapsids, and cores. Only complete virions are present in the cytoplasm of cells and only complete virions are released from cells under optimum circumstances. One conclusion derived from such studies is that infectivity is related to envelope proteins and another is that non-infective sub-assembly units are retained in the nucleus for an unknown length of time. This latter feature seems to be part of the establishment of latent viral infections in swine with PrV. Latent infections may be initiated by natural infection as well as by inoculation with a live-vaccine. In the field under natural conditions as well as in the laboratory it has been found that immune animals may be reinfected and intermittently shed the virus for variable periods, of at least 11 months. This suggests that PrV is present in endemic herds despite the presence of virus neutralizing antibodies in the serum. And further, once a herd is infected it will remain a potential source of virus for susceptible animals.

Pseudorabies infections have been reported in all of Europe, in the Middle East, Asia, South America, and Central America. While there are unconfirmed reports of the disease from North Africa no reports have been found of its occurrence in Australia or Japan. Essentially, the disease is present world wide.

The history of PrV infections in the United States was reviewed by Hanson in 1954. The earliest description of a syndrome in cattle which is suggestive of PrV infection was made in 1813 in Ohio. It is interesting that early reports are almost exclusively concerned with the disease in cattle. Between then and the 1930’s a relationship between the presence of swine and the development of the disease in cattle was recognized. The disease was mild in swine and most often went unrecognized. In the period from the 1930’s to 1962 the disease
in swine became somewhat more serious. Infections in baby pigs sometimes caused severe illness and death. So unusual were these outbreaks in the 1940's and 1950's that published case reports of such occurrences usually followed. The next change in virulence of PrV populations occurred sometime in the early 1960's. We published a record of our first observations, in late 1962, of PrV infections in swine which causes the death of fetuses, baby pigs, shoats, and mature sows. Since that time similar observations have been made in nearly every area where swine are raised in the United States. As veterinarians in the field became familiar with the syndrome, referrals to diagnostic laboratories became less frequent. Nevertheless, the increase in numbers of outbreaks has steadily climbed and their severity has been a cause for much concern. At the Symposium on Pseudorabies held at Peoria, Illinois, May 27, 1975, it was reported that the incidence of PrV had apparently increased in six midwestern states. Swine production in these states constitutes slightly more than 60% of the nation's total. For example, sixty-eight pseudorabies infections were confirmed in Indiana in calendar 1974, including one feline and five canines. Forty-three new infected premises were identified in the first nine months of 1975. In another survey for antibodies against PrV in swine sera of packing plant origin from Ohio, Indiana, and Illinois 77 PrV positive sera were found in 3002 samples. The severity has reached severe proportions in some instances. A producer in Illinois lost approximately 7000 swine in a single episode of the disease which lasted about seven weeks except for breeding problems among recovered sows. Breeding problems are manifested by difficulty in causing pregnancy in sows that recover from the infection. The problems occur among sows that were pregnant at the time of infection and subsequently aborted embryos or fetuses; expelled fetuses at term with some of them being macerated, expelled them all beyond the expected farrowing date in macerated states, or farrowed normal pigs. Another producer in the same area lost 25 to 30 percent of the farm's annual production. And an Iowa practicing veterinarian recorded experience with 19 cases of pseudorabies over a 15 month period. Losses included swine, cattle, dogs, and cats.

Gross pathological changes in infected swine have been observed which had not been previously reported in the United States. In 1968 severe conjunctivitis was frequent. More recently in 1974 and 1975 necrotic tonsillitis and small foci of necrosis in liver, spleen, and lungs of suckling pigs have been reported. Similar observations were reported in Europe in 1961. In some cases skin lesions resembling Herpesvirus hominis lesions have been found on the snouts of young pigs infected with PrV. In perspective, considering the presence of the disease in the United States for some 160 years, it would seem certain that this disease has suddenly become much more frequent, more virulent, and a menace to more forms of animal life. Human in-
Infections have not yet been detected. There have not been great losses among flocks of sheep in the absence of swine as has occurred in Eastern Europe where vaccination with live-vaccines is practiced. In the United States, those who have lost large numbers of swine or whose herd shows evidence of smoldering involvement are much concerned about the means of coping with a problem. One common reaction is to seek a vaccine. A second thought is to seek something that will lessen the infection that exists and to prevent further losses in those not yet affected. A third reaction is to suffer the losses and hope that it will not occur again. There are methods that can be employed to minimize the chances of infection and to reduce losses once an infection has become recognized.

A. **MINIMIZING CHANCES OF INFECTION**

Contrary to the statement of some, PrV can be brought to susceptible pigs by passive vectors and also on fomites through carelessness. Contrary to popular belief the virus will remain viable for significant periods when protected by protein such as found in skim milk or serum. The lower the temperature, the longer the survival of the virus. The management of a production unit is important in maintaining freedom from PrV infection. The following basic procedures are aimed at reducing the possibility of exposure to the virus.

1. Isolate additions to the herd until a virus neutralization test can be conducted on serum samples from 10% of the new acquisitions. If this is not feasible, keep the animals in isolation for at least 10 days. If none become ill then it will probably be safe to release the isolation.

2. Reduce the accessibility of the herd to visitations by feral cats, dogs, and raccoons.

3. Stop human and vehicular traffic between infected and non-infected premises.

4. Reduce the amount of equipment used to service more than one group of animals on the farm.

B. **IMMUNIZING AGENTS**

There are no vaccines licensed for use in the United States at this time. None are known to be in any stage of research or development. Market studies have been and are being made by commercial organizations to determine whether it is feasible to invest in the research and development necessary to obtain a license to market a vaccine. Currently, it appears that a profitable market for a vaccine does not exist nor does one loom on the horizon of such moment as to permit the commitment of corporate resources, especially in today's national economic climate. If a program were initiated and was successful the time lag to market availability would be a minimum of three
years. Similarly if a vaccine were to be imported it would not be immediately available at the market place. If such an importation were successfully pursued the time course for safety testing, research and development, licensure, production development, and unavoidable delays of one sort or another might not improve the time lag to market from conception in any material way.

Inactivated vaccines that have been developed in Europe thus far have not been successful in the environment in which they have been employed. The ultimate conclusion has been that inactivated vaccines that have been produced are too costly in terms of losses among swine exposed to the virus; that they are ineffective in the face of challenge, albeit innocuous.

Attenuated live vaccines have been produced and are in extensive use in Europe. Successful use of the best known vaccine is characterized by the induction of virus neutralizing antibodies in the serum of vaccinated swine, the transfer of antibodies to piglets from their vaccinated dams, and the improvement of the epizootiological situation in the general area of use. However, vaccination must continue in areas where it is used because the risk faced by non-vaccinates is high. Antibodies rise slowly in vaccinates and two inoculations is common practice. Vaccinates subsequently exposed to virulent virus maintain the virus for as long as three weeks in the tonsils. Under field conditions swine may harbor recoverable virus for at least 11 months. Thus, vaccinated animals can be reinfected with virulent virus, shed it intermittently for long periods even though they have circulating virus neutralizing antibodies. Colostral antibodies are not uniformly capable of protecting newborn pigs. Newborn pigs are maximally susceptible to PrV as opposed to mature swine, in keeping with the fact that, with age, swine become increasingly resistant to the virus. Therefore, attenuated vaccines are more dangerous for baby pigs. In endemic areas, or where vaccines have been used for cause, the risk becomes higher. Indeed, the use of live attenuated vaccines seems to ensure the maintenance of the virus in the environment. This is emphasized by losses in the presence of vaccination and the necessity for vaccination to prevent even greater losses where such is the practice in Europe.

C. PASSIVE IMMUNIZATION

The prevailing experience in the United States with pseudorabies infections in swine is sporadic, focal geographically, and gradually becoming obscure demographically. As veterinarians become confident of their diagnostic skill in PrV infections, they are less inclined to bother the demographic establishment. Consequently those in diagnostic services are becoming less sure of the accuracy of their figures on the number of cases in their area. They are sure that there is more than they have on record. Nevertheless, the numbers that are
involved at present has not caused a general demand for immunization but a strong desire for means to stop the spread of infection once it has been recognized or seems imminent because of a nearby infected group of swine.

In the short run the use of PrV antiserum is of value. Antisera produced in swine has been found to be of greater value than that produced in another species such as the horse. The half-life of heterologous antiserum is significantly less than homologous serum. We have found that antiserum is useful prophylactically but not therapeutically, as have many others. The use of antiserum is not likely to prevent infection but it reduces the severity of the disease and the affected but treated swine are likely to recover so they can be marketed. Passive immunization with antiserum is best utilized in baby pigs since the losses among shoats are not likely to be high because of the age-resistance factor.

D. CHEMOTHERAPY

The use of antiviral chemicals to reduce losses due to PrV has a potential value similar to the use of antiserum. Nucleoside analogs appear to hold the greatest promise for efficacy in ameliorating PrV infections in baby pigs. There is significant activity in research on the value of such chemicals in herpesvirus infections. Initial clinical trials in swine suggest that while these drugs probably will not prevent infection they may reduce the severity of infection by such measure as to avoid death and permit the pig to grow, as well as most, to market weight. At best the availability of such antiviral chemicals appear to be at least four years away.

Conclusions

Pseudorabies infections in the United States are increasing in numbers, severity, and the spectrum of animal life affected. Losses have reached threshold values in terms of swine producer concern for the future in many areas in the midwest. There are only two avenues for dealing with the disease at the present. (1) Good management and disease prevention practices are effective means to reduce losses and avoid involvement. (2) Antiserum is effective in ameliorating the effects of PrV infection especially in newborn baby pigs. No other means of dealing with the disease is currently available. Some prospects for the future may lie in an effective eradication program, antiviral chemicals, and/or safe and effective immunizing agents.
PSEUDORABIES IN PERSPECTIVE

BIBLIOGRAPHY

MYCOBACTERIUM AVIUM INFECTIONS IN GEORGIA SWINE

John R. Cole, Jr., PhD.; Charles O. Thoen, DVM, PhD.;
Frank E. Mitchell, DVM, MS

In Georgia during 1974, the number of abattoir condemnations of swine due to tuberculosis increased greatly over previous years. Because of industry concern about the economic loss, an investigation was made to document the etiology of the lesions. The findings presented here are the results of laboratory examinations on 78 lymph nodes with tuberculous lesions. These specimens were collected by meat inspectors from swine in Georgia from March 1974 through April 1975.

Materials and Methods

Specimens. Seventy-eight lymph nodes, considered tuberculous by inspectors, were collected from abattoirs located in 12 south Georgia counties. These specimens were transported in ice to the Veterinary Diagnostic and Investigational Laboratory (VDIL), Tifton, Georgia for pathologic examinations. A portion of each lymph node was placed in a saturated sodium borate solution at the VDIL and forwarded to the Veterinary Services Laboratories (VSL), Ames, Iowa, for mycobacteriologic culture.

Pathologic examinations. Direct impressions of the lesions were stained by the Kinyoun or Auramine O staining techniques, and examined for acid-fast bacteria. Lesions were also fixed in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin for histopathologic examination.

Mycobacteriologic examination. Tissue specimens were rinsed in a dilute solution of sodium hypochlorite as soon as possible after receipt at the VSL. All fat was removed from tissues using separate sterile scissors and forceps for each specimen. The tissues were submerged in fresh sodium hypochlorite solution and allowed to stand undisturbed overnight at 4°C. The tissue specimens were then transferred to a blender jar. Fifty ml of nutrient broth containing 0.4% phenol red indicator was added, and the tissue was blended for 2 minutes. Four tubes of culture media (1 tube each of Lowenstein-Jensen, Stonebrinks, Herrolds with malachite green, and Middlebrooks 7H10) were inoculated with untreated tissue suspension using cotton-tipped applicators. Five ml of tissue suspension was transferred to a 20 x 125 mm tube containing 5 ml of 0.5N NaOH. After shaking thoroughly

From the Veterinary Diagnostic and Investigational Laboratory, College of Veterinary Medicine, University of Georgia, Tifton, Georgia, 31794 (Cole and Mitchell); and Veterinary Services Laboratories, APHIS, USDA, Ames, Iowa 50010 (Thoen).
by hand, the mixture was allowed to stand 10 minutes at room temperature. The mixture was neutralized with 6 N HCl and allowed to stand for 5 minutes. It was centrifuged at 1500 RCF for 30 minutes, and the supernatant fluid was discharged. Four tubes of culture media were inoculated with the treated sediment using cotton-tipped applicators. The inoculated slants were incubated at 37°C and observed for growth at weekly intervals for 8 weeks.

Smears of colonies were stained with carbol fuchsin. Acid-fast isolates were characterized by colonial, morphologic, and biochemical tests. Mycobacterium avium isolates were identified by seroagglutination tests.

Results

Acid-fast bacteria were observed in 65 of 78 (83%) lymph nodes. Eleven tissues negative microscopically for acid-fast bacteria yielded mycobacterial isolates.

Mycobacteria were isolated from 69 of 78 (88%) lymph nodes (Table 1). Of these 69 isolates, 36 were either serotypes 1 or 2, and 9 isolates were serotype 10. Serotypes 4 and 8 were each isolated 7 times, and serotypes 11 and 12 were each isolated once. Double types, isolates sharing antigens with two or more serotypes, were isolated 6 times. Nonchromogenic slowly growing mycobacteria (Mycobacterium spp.) were isolated twice.

Mycobacterial infection was confirmed in 76 of 78 (97%) cases by either acid-fast staining or mycobacteriologic culture. The 2 (3%) cases in which mycobacteria were not detected, however, did show typical microscopic lesions.

Discussion

In a recent South Dakota investigation, mycobacteria were cultured from 97% of the tissues diagnosed by gross examination as swine tuberculosis. It is interesting that, in our survey, mycobacteria were also observed in, or cultured from, 97% of the lymph nodes collected routinely from swine with tuberculous lesions. Furthermore, the mycobacteria-negative cases showed microscopic lesions compatible with tuberculosis. These independent investigations validate gross examination as a means of detecting mycobacterial disease in swine.

The predominant serotypes isolated in Georgia swine, M. avium 1 and 2, are the same serotypes commonly associated with tuberculosis in chickens. These serotypes are also the ones most commonly isolated from swine in the United States. There is, however, no apparent relationship between tuberculosis in swine and poultry, since there have been no reported cases of tuberculosis in chickens in Georgia since 1959. The finding of serotypes 4, 8 and 10 in 23 of 78 (29%) of the samples coincides with a survey which indicated that these serotypes are important in the South Atlantic region.
11 and 12 have been isolated from man and swine.\textsuperscript{5,6} Epidemiologic studies should be made to elucidate the source(s) and mode(s) of transmission of these organisms.

Summary

Lymph nodes from 78 swine which originated in 12 south Georgia abattoirs were examined microscopically and by culture for mycobacteria. Acid-fast bacteria were observed in 65 of 78 (83\%) lymph nodes. Mycobacteria were isolated from 69 of 78 (88\%) lymph nodes. Mycobacterial infection was confirmed in 76 of 78 (97\%) tissues by acid-fast staining or culture. The predominant isolates (52\%) were \textit{M. avium} serotypes 1 and 2.

Table 1—Results of Mycobacteriologic Examinations Performed on 78 Swine Lymph Nodes with Tuberculous Lesions

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Mycobacterium avium} Serotypes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>1 &amp; 4</td>
<td>1</td>
</tr>
<tr>
<td>2 &amp; 4</td>
<td>1</td>
</tr>
<tr>
<td>3 &amp; 9</td>
<td>4</td>
</tr>
<tr>
<td>\textit{Mycobacterium} spp.</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
</tr>
</tbody>
</table>
REFERENCES

REPORT OF THE TRANSMISSIBLE DISEASE OF
SWINE COMMITTEE

Chairman: E. A. Butler
Co-Chairman: T. F. Zweigart


The Transmissible Disease of Swine Committee met Wednesday, November 5, 1975. Fourteen members and 36 guests were present.

Introductory remarks were given concerning the need for greater coordination and communication of information obtained by researchers and the need for comprehensive disease reporting systems.

A very interesting report was given by Dr. George Saunders on work being carried out at Los Alamos on diagnosis of disease with enzyme labeled antibody technique. This procedure promises to make the diagnosis of various diseases relatively simple and inexpensive.

Dr. E. A. Butler summarized the Iowa swine tuberculosis indemnity program. Dr. M. W. Vorhies discussed the swine TB symposium recently held at Madison, Wisconsin. There was a consensus of opinion at this meeting to consider swine TB as a Mycobacteriosis rather than tuberculosis. Since there is no evidence of transmission of this disease to man by consumption of pork, the committee recommends that consideration be given to relieve the swine industry of the economic burden caused by the meat inspection regulation requiring carcasses with lesions of this disease to be passed for cooking. This procedure caused an unwarranted loss of animal protein to the consumer.

Dr. Howard Hill gave a report on pseudorabies incidence in Iowa and other states. A preliminary report on a survey conducted in Iowa corresponding with work done in other midwestern states indicates that incidence of the disease is still quite low. Control of the disease using quarantines is difficult because of the presence of inapparent carriers which may shed the virus following stress. Investigations indicate that wildlife, especially raccoon, may serve as a reservoir.

Dr. Robert Glock gave a report on progress in swine dysentery control. The findings of investigators in several states and Europe were detailed and discussed. Several therapeutc agents which show promise in controlling the disease have not been given clearance by FDA for use in swine.

The National Feeder Pig Dealers Association's Swine Dysentery Committee submitted a communication to the committee expressing great concern with the growing incidence of the disease. Included
for consideration of the committee were recommendations for con- 
control of the disease. It was the decision of the committee to send a 
copy of their report to each State Veterinarian for consideration, 
asking them to implement control programs that would be feasible 
in their state.

Dr. Dan Farrington gave a report concerning infectious atrophic 
rhinitis in swine. His report called attention to the very high inci-
dences of the disease in swine. The work by Dr. Switzer and Dr. 
Farrington indicated that Bordatella bronchiseptica is the primary 
cause, while other organisms may contribute to the severity of the 
disease. He reported that experimental bacterins have shown promis-
ing results. USDA has given permission to conduct field testing of 
this product, which is in progress.

Dr. E. O. Haeltermon presented the report of the Subcommittee 
on TGE as follows:

Report of the Subcommittee on Transmissible Gastroenteritis (TGE)

TGE virus continues to be of major importance to the swine in-
dustry in the midwest, and outbreaks appear to be increasing in 
frequency and numbers of pigs involved with expansion of the in-
dustry in the South.

A paper given at this meeting and preliminary reports given at 
the annual meeting of North Central Regional Research Committee 
on enteric diseases of young swine describe an association of reovirus-
like virus with a disease resembling TGE in swine. In general, these 
reports describe a diarrheal disease that spreads rapidly between pigs 
from near weaning age to those 3 days old, often starting in the 
older pigs. With one exception, sows were not affected. Morbidity is 
said to be very high, but death losses, in contrast to TGE, were very 
low in uncomplicated cases. The reovirus-like agent has been demon-
strated by electron microscopic examination of stools and intestines 
of infected pigs. The same or similar virus has been isolated in cell 
cultures from pigs in which the major problem appeared to be edema 
disease, salmonellosis, colibacillosis or pneumonia as well as the 
diarrheal disease described above. Reovirus-like agents have also 
been associated with enteric disease in man, monkeys, foals, calves, 
and lambs. The committee recommends that this virus and the 
disease(s) it causes be further characterized.

The committee also recommends further research on TGE, par-
ticularly in the areas of epidemiology and immunology. The main 
problem in development of a highly efficient immunizing procedure 
appears to be stimulation of immunoglobulin A (IgA) in the milk 
of sows without resorting to planned infection with virulent virus.

With respect to epidemiology, a number of problems exist. Among 
them are definition of the role of swine and animals other than 
swine in the maintenance of the virus during interepidemic periods.
It has been reported that after several passages, TGE virus became virulent for puppies, producing a severe diarrheal disease. This suggests canidae could be more important epidemiologically than has been supposed. Stockmen continue to indict starlings as spreaders of TGE. The role of swine that have recovered from TGE should be explored further. One recent report indicates that swine may carry TGE virus in the lung for over 100 days. In other work, sentinel pigs placed in contact with infected pigs more than 2 weeks after infection did not become infected. It is possible that TGE virus carriers could be converted to active shedders under stress or subsequent infection with other agents.

A major factor in the development of control programs would be the natural prevalence of TGE. The committee recommends that efforts be made by the use of serological surveys (which may be more feasible since the development of the indirect fluorescent antibody test) using epidemiologically sound sampling methods to determine the extent of TGE infection in the swine population.

Subcommittee Members:

Chairman: E. O. Haelterman
E. H. Bohl, J. Black, G. Lambert, M. Ristic, N. R. Underdahl
EPIDEMIOLOGY OF THE BOVINE TUBERCULOSIS OUTBREAK IN GEORGIA

Daryl C. Johnson, DVM; Arnos N. Rogers, DVM; MPVM; James F. Andrews, DVM; James A. Downard, DVM; Charles O. Thoen, DVM, Ph.D.

Extensive tuberculous like lesions were found on routine slaughter in a beef animal by a Veterinary Medical Officer on August 29, 1973. The animal was condemned and the lesions sent to Veterinary Services Laboratory, Ames, Iowa. *M. bovis* was isolated from the granulomatous lesions. Traceback was initiated and the herd of origin (herd 1, Table 1) was tuberculin tested. All animals were negative by the caudal fold method. The herd was retested later by the 0.2 ml cervical method which revealed 4 reactors. No gross lesions were found during necropsy of these cattle. The condemned cow was purchased by herd 1 through a purebred beef cattle sale from herd 2 (Table 1).

A tuberculin test of herd 2 revealed 160 reactors of 350 cattle tested. Lesions typical of tuberculosis were found in 9 animals on slaughter inspection. This herd was depopulated as being grossly infected with *M. bovis*.

Herd 2 was a purebred beef herd from which 430 breeding cattle had been sold to 157 buyers mostly in Georgia from January 1969 to May 1974. Numerous animals had been purchased from several purebred herds scattered across the United States and introduced into this herd during the same time period.

Tracing and testing of exposed cattle was done by State and Federal personnel. The testing protocol required the locating quarantining and cervical tuberculin testing of all animals originating from *M. bovis* infected herds. If these animals were found negative to the cervical test, the remainder of the herd would be tuberculin tested by the caudal fold method. If the purchased animals were found infected with *M. bovis* the remainder of the herd would be cervical tested and held under quarantine. Animals in the primary infected herd 2 found to have grossly visible lesions and to have been introduced into the herd prior to October 1972, were traced to herds or origin. Some of these animals had up to six transfers of ownership. This required the testing of all existing herds of which such animals had...
been a member. Sales into and purchases from other States were re-
ported to those States for follow-up testing. Compliance with this
protocol required the testing of 23,000 cattle in 350 herds in Georgia.
A total of 551 reactors were revealed, of which 100 animals were
found to have grossly visible lesions on slaughter inspection.

The earliest date of sale from the primary infected herd of cattle
found to have *M. bovis* infection was in April 1971. Two cows were
sold into a north Georgia herd and resold into herd 11 (Table 1)
March 1973. One of these cows died 4 months later with a history
of debilitation and pneumonia. No postmortem mwas performed. The
other cow was a reactor with extensive granulomatous lesions on post-
mortem. The remaining 27 animals in herd 11 have remained negative
to the tuberculin test. This finding provided evidence that infection
did exist in herd 2 as of April 1971.

Two animals were sold from the primary infected herd into pure-
bred herd 4 in March 1972, and were found to be *M. bovis* infected
in August 1974. Complete herd testing of 264 animals in herd 4
(Table 1) revealed 79 reactors of which 19 contained gross lesions.
Epidemiology determined that 421 cattle were sold from this herd
to 167 buyers between January 1970 and August 1974. Tracing and
testing of sales from herd 4 located *M. bovis* infected herds 6 and 10.

Emphasis was placed on tracing purchases and sales of herd 2
and herd 4. Both were purebred herds selling primarily foundation
stock to other herds. Epidemiologic evidence indicated that herd 2
was the primary infected herd source for the Georgia *M. bovis* epi-
demic. Animals sold from this herd were traced to 8 other herds
where *M. bovis* infection was found (Table 1). It was not possible to
determine the source of infection into the primary infected herd.
Several purchased animals had died on the farm. One of these might
have been the source of infection. Many source herds were tested
negative to tuberculin tests while several other herds had been dis-
persed and no longer existed to test.

Many of the sales from herds 2 and 4 were young animals sold
to stockyards and feed lots where their identity was lost. Of the 815
cattle sold from these 2 herds, only 6 were adult animals sold to
slaughter in a manner that would allow a lesioned animal to be traced
to the herd or origin. Purebred beef cows are usually not culled as
long as they produce a calf. Therefore, many of them died on the
premises.

Five herds with evidence of *M. bovis* spread within the herd
were completely depopulated. Because of the restrictive regulations
for disposition of tuberculous cattle, it was difficult to find slaughter
establishments that would accept the reactors. This caused consider-
able delay in disposing of some of the reactors. Many of them had to
be enthanized, posted, and buried on the farm.
Discussion

Herds 2 and 4 were tuberculosis accredited, meaning all animals over 24 months of age were tuberculin tested annually by an accredited veterinarian. Three of the 30 *M. bovis* infected herds discovered during fiscal year 1974 were accredited. Apathy on the part of some veterinarians and the livestock industry has become a stumbling block in the bovine tuberculosis eradication effort.

The importance of constant vigilance by veterinarians doing routine inspections in slaughtering establishments cannot be overemphasized. The alertness of one of these veterinarians in Georgia started the chain of events that uncovered the epidemic described herein. Animal identification and record keeping are essential for successful tracing of animals to herds of origin. One can only speculate as to how wide-spread this epidemic might have been had another 2 or 3 years passed before being detected.

Table 1. Results of Tuberculin Testing and Postmortem Examinations of Cattle from *M. bovis* Infected Herds in Georgia.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Animals in herd</th>
<th>Reactors to tuberculin test</th>
<th>Animals with Gross Lesions</th>
<th>Source of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105</td>
<td>4</td>
<td>1 (+)</td>
<td>Herd 2</td>
</tr>
<tr>
<td>2(d) (p)</td>
<td>350</td>
<td>160</td>
<td>69</td>
<td>Unknown</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>5</td>
<td>1</td>
<td>Herd 2</td>
</tr>
<tr>
<td>4(d)</td>
<td>264</td>
<td>79</td>
<td>19</td>
<td>Herd 2</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
<td>11</td>
<td>1</td>
<td>Herd 2</td>
</tr>
<tr>
<td>6(d)</td>
<td>173</td>
<td>144</td>
<td>2</td>
<td>Herd 4</td>
</tr>
<tr>
<td>7(d)</td>
<td>252</td>
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<td>3</td>
<td>Herd 2</td>
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<td>9</td>
<td>86</td>
<td>1</td>
<td>1</td>
<td>Herd 2</td>
</tr>
<tr>
<td>10(d)</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>Herd 4</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>1</td>
<td>1</td>
<td>Herd 2</td>
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(d) — Herd depopulated as grossly infected with *M. bovis*.
(+ ) — Nonreactor animal with grossly visible lesions at slaughter and traced to the herd of origin.
(p) — Primary infected herd.
REFERENCES


EPIDEMIOLOGICAL REPORT OF A TUBERCULOSIS OUTBREAK IN ILLINOIS

Paul L. Spencer, D.V.M.*

One of the charts Dr. Bennett will show in the next presentation gives the States of Georgia and Illinois the joint credit for leadership in tuberculosis infected herds for fiscal year 1975, with 10 each, so it would appear appropriate that Dr. Johnson and I share this time period.

I will briefly describe, with the assistance of 2 overhead transparencies, the Illinois outbreak involving 8 of our 10 herds. To the best of our knowledge, the Svoboda outbreak did not involve the other 2 herds although continuing epidemiological investigations may eventually lead to a connection with one of them.

William Svoboda is an elderly man of foreign heritage residing in Madison County, Illinois, which is located just across the Mississippi River from St. Louis. In June, 1963, a dairy herd which he operated was tested for milk marketing purposes with the results of 42 negative and 7 suspects. A retest was conducted in January, 1964, with results of 31 negative, 2 suspects and 1 reactor. The reactor was slaughtered locally, was NGL, and as far as we can determine no tissues were submitted. A retest was conducted in March, 1964, 31 head, all negative. Under the protocol existing at that time, the herd was released from quarantine.

Mr. Svoboda made a transition from a dairy to a beef operation in 1963 through 1965, through purchase of a purebred bull and some purebred heifers, but still maintained a few of the dairy cows although he no longer milked them. No further contact was made with him until a fat heifer he sent for slaughter at a local locker for home consumption in 1974 revealed lesions indicative of tuberculosis. Tissues were collected, the diagnosis was confirmed at V.S.L., and the following investigation resulted.

(Refer to Transparencies for Balance of Presentation.)

Special credits for outstanding epidemiology on this outbreak must be given to Dr. Harold McCoy, APHIS, State Tuberculosis Epidemiologist; Dr. Charles Hertich, Bureau of Animal Health District Veterinarian, and Dr. Carl Rogers, Madison County Veterinarian, along with many other State and Federal veterinarians and livestock inspectors who worked many hours on tracing and testing in connection with this outbreak.

WILLIAM SVOBODA TUBERCULOSIS INFECTED HERD DIAGRAM

HENRY BULLOCK TUBERCULOSIS INFECTED HERD DIAGRAM
REPORT OF THE SUBCOMMITTEE TO STUDY THE UTILIZATION OF PARAMEDICAL PERSONNEL IN TUBERCULIN TESTING

At the 1973 United States Animal Health Association meeting, the Chairman of the Tuberculosis and Johne's Disease Committee appointed a subcommittee to make contacts, explore complications, and investigate the potential for the use of government-employed paramedical personnel for the tuberculin testing of livestock.

The subcommittee members were Dr. P. L. Smith, Chief, Bureau of Animal Health, California Department of Food and Agriculture (Chairman), Dr. C. E. Boyd, Director, State-Federal Livestock Disease Eradication Program, South Carolina, and Dr. R. J. Stadler, State Veterinarian, Connecticut Department of Agriculture.

Several states with large, concentrated dairy populations consider that routine "down-the-road" surveillance tuberculin testing is still necessary in achieving the goal of eradication of tuberculosis. Because of budgetary restrictions and the inability to hire veterinarians, other methods of continuing surveillance tuberculin testing must be explored. One possibility is to utilize paramedical personnel under a veterinarian's supervision to conduct surveillance testing. The veterinarian would be utilized to conduct diagnostic tuberculin tests.

Paramedical personnel would be used as follows:

1. Paramedical personnel would be highly trained veterinary technicians and full-time state or Federal employees. Many states are moving in the direction of implementing legislation to define animal health technicians and how they may be utilized.

2. Paramedical personnel would be under the supervision of a full-time state or Federally employed veterinarian.

3. Paramedical personnel would conduct surveillance tests only. This would be routine testing for reaccreditation purposes. They would only record the size of the response to the test when found and would not classify as positive, suspect, or negative. The test findings would be reported to the supervising veterinarian.

4. Paramedical personnel would not do diagnostic tuberculin testing, which is described below.

Veterinarians would be used as follows:

1. Supervise paramedical personnel and evaluate surveillance test results.

2. Conduct all diagnostic tests. The following are considered diagnostic tests:
   a. Classification of surveillance test responses as a reactor or suspect.
   b. Determination of which herds containing animals with responses to the tuberculin test need further evaluation.
c. Retest of suspects found on surveillance tests.
d. All comparative and other supplemental tuberculin testing.
e. Tests of infected herds.
f. Tests of exposed herds.
g. Tests of ANH 6-35 herds.
h. Tests for health certificate requirements (intrastate, interstate, and international movement).

During 1974, the subcommittee made a variety of contacts concerning the utilization of paramedical personnel. These contacts include state veterinarians, deans of veterinary medical schools, presidents of state veterinary associations, boards of veterinary medical examiners, extension service veterinarians, et al.

The subcommittee made a report of their findings to the Tuberculosis and Johne's Disease Committee at the 1974 annual conference. The subcommittee was instructed to continue its investigation by submitting its report to all appropriate state and Federal animal health officials for comments. It was anticipated that the full committee would act on the subcommittee's recommendation at the 1975 meeting.

During 1975, all state animal health officials and Veterinary Services, APHIS, were contacted regarding their opinions concerning the subcommittee's report. All but one state responded to the inquiry.

FINDINGS

The subcommittee recognized that the proposal would not be acceptable for implementation in all states for many understandable reasons, particularly in states where practice acts would prohibit the concept and in states where tuberculin testing is contracted out to the private practitioners. It was further recognized that surveillance tuberculin testing may no longer be required in states free of the disease. The decision to utilize surveillance testing procedures rests with the responsible state officials. However, the proposal would have its most beneficial application in states which consider routine area testing a necessary tool for achieving eradication. It would probably be most acceptable in states that are using full-time veterinarians for surveillance testing.

Veterinary Services, APHIS, endorsed the concept of the use of paramedical personnel for routine tuberculin surveillance under the restrictions outlined in the committee report. The opinions of the 50 state animal health officials can be summarized as follows: 35 approve; 11 opposed; 3 neutral, 1 no reply.

The subcommittee found that several states have veterinary practice acts which would prohibit the use of paramedical personnel for testing in their states. However, a majority of officials were sympathetic to problems encountered in states with large, concentrated, dairy populations where routine area testing is still considered neces-
It is the opinion of the subcommittee that, if states utilize paramedical personnel in accordance with the restrictions outlined above, testing for reaccreditation purposes would be accepted by other states.

The subcommittee further recognized a concern of the approving animal health officials in the assurance of control of uniformity, quality, and confidence in surveillance tuberculin testing. This assurance must be maintained through state-federal cooperative agreements with each state contemplating the use of paramedical personnel. Also, uniformity of training paramedical personnel must be achieved. It would seem appropriate that Veterinary Services, in cooperation with state officials, would play a vital role in securing uniformity in training.

**RECOMMENDATION**

The subcommittee recommends to the USAHA Committee on Tuberculosis and Johne's Disease that the Uniform Methods and Rules — Tuberculosis Eradication be amended to recognize approved full-time government-employed paramedical personnel for surveillance tuberculin testing.

In the event that the Uniform Methods and Rules are amended, the subcommittee recommends a study be made to formulate uniform training procedures and guidelines for state-federal cooperative agreements.
THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

Ralph W. Bennett, D.V.M.*

A full year has elapsed since I last stood before you and gave you a status report on the Cooperative Bovine Tuberculosis Eradication Program. In that report an attempt was made to point out some of the roadblocks in the program and some of the factors which have slowed the pace of eradication to a point where we are bordering upon, if not actually in, a bovine tuberculosis control program.

Where were we in the program on June 30, 1975, compared to June 30, 1974? What progress was made during that 12 month period toward removing some of the roadblocks and solving some of the problems in the program which are retarding and hamstringing our efforts to eradicate the disease.

Here is the box score!

For the third consecutive year it must be reported that there is no change in the status of the individual States (Figure 1). Five States and the Virgin Islands retained their accredited-free status with 45 States and Puerto Rico remaining in the modified-accredited category. Two States, Mississippi and Arkansas, which had not discovered any *M. bovis* infection in the past 5 years, fell victim to the slaughter surveillance program this year when one *M. bovis* herd in each State was found through traceback of a regular kill animal with lesions of tuberculosis. On the plus side, one State, South Dakota, was added to the list of States, now numbering 19, which have not found *M. bovis* in the past 5 years. In addition, the five accredited-free States Maine, Connecticut, New Hampshire, Rhode Island, and New Mexico have not reported *M. bovis* in the last 12 years or longer.

Forty-seven tuberculous herds were found in the United States in fiscal year 1975 (Figure 2). Forty-two of these herds were located in States east of the Mississippi River with Georgia and Illinois accounting for 20 of these herds. This statement is not intended to downgrade either of these States but rather to praise them for their program. It was only through thorough and complete epidemiology, dogged persistence, and the intestinal fortitude to classify tuberculin reactors that this was accomplished. Had not this dedicated effort been put forth, 20 additional *M. bovis* infected herds would remain in the country to serve as a source of infection to other herds. Of the 47 tuberculous herds found in this fiscal year, 10 were herds that

*Chief Staff Veterinarian, Tuberculosis Eradication, Cattle Diseases, Veterinary Services, Animal and Plant Health Inspection Services, United States Department of Agriculture. Presented at the Seventy-Ninth Annual Meeting, United States Animal Health Association, Portland, Oregon, November 7, 1975.
were known to have been infected with *M. bovis* in previous years. Thirty-seven of the herds were newly discovered this year.

It is revealing to analyze the methods by which these 37 herds were located (Figure 3). Even though we place great reliance on the traceback of tuberculous regular kill slaughter animals to their herds of origin as our primary means of locating tuberculosis affected herds, only 14 herds were located in this manner. Twenty affected herds were located by tracing exposed animals sold from known tuberculous herds. This is the method whereby the greatest preponderance of the affected Georgia and Illinois herds were found. It is imperative, therefore, that when a tuberculous herd is found by any method, we must trace all sales from this herd and tuberculin test these animals or depopulate them as exposed.

One affected herd was found through tracing of animals purchased, one through sale testing, and one through area testing.

It is also interesting to note that of the 47 affected herds this fiscal year, 35 or 74 percent were detected by epidemiological means (Figure 4). Only 12 herds were found by all other tuberculin testing such as area, sale and show, accredited herd, export testing, etc. One herd was discovered to be either reinfected or to have been infected at the time it was released from quarantine.

A very definite swing in the type of cattle found to be tuberculous occurred this year with beef types predominating (Figure 5). Dairy types have been considered over the years to be more at risk of tuberculosis primarily because of the direct contact afforded by milking parlors and loafing barns. The upswing of beef types this year is due, in a large measure, to the outbreaks of tuberculosis in Georgia and Illinois which were almost exclusively in beef cattle. Of the 47 tuberculous herds this year, 35 were of the beef type and 12 were of the dairy type.

Bovine tuberculosis was found in 16 States and Puerto Rico this year with Georgia and Illinois accounting for 20 of the 47 herds. Figure 6 shows the numbers of tuberculosis affected herds by State and the number depopulated in each State. Eight States depopulated every known infected herd in the State with Illinois leading the way by depopulating all 10 of the known affected herds in that State.

This year 29 of the 47 known tuberculosis affected herds were depopulated (Figure 7) in direct contrast with last years figure of 10 depopulations out of 30 affected herds. Percentage-wise 62 percent of the affected herds were depopulated this year against 30 percent depopulations last year. The fiscal year 1975 figure of 62 percent is the highest since the policy of depopulation was adopted. As has been said many times in the past, we must reach the 95 percent depopulation level to efficiently eradicate bovine tuberculosis. This year is encouraging, but we must continue to improve if we are to eradicate the disease.
Figure 8 depicts the ratio between depopulation of dairy herds and that of beef herds. Of the 12 tuberculous dairy herds this year, 7 or 58 percent were depopulated. Of the 35 beef herds found to be affected with bovine tuberculosis, 22 or 63 percent were depopulated. There does not appear to be any significant difference, therefore, in the difficulty of depopulating a dairy herd and a beef herd as had been formerly thought.

Perhaps the most significant fact in Figure 9 is not the degree of success we have in locating tuberculous herds of origin, but the alarming lack of individual animal identification indicated by these figures. Last fiscal year 98 of the 195 tuberculous regular kill slaughter cattle were identified by some means. This fiscal year only 59 of 195 tuberculous regular kill animals were identified. Since our degree of success in tracing lesioned animals to their herds of origin is largely dependent upon the amount and accuracy of the identification supplied, this figure needs to be improved drastically in the coming year.

There is a direct correlation between slaughter class and individual animal identification. Feeders, in general, tend to have a great deal less individual identification than adult cattle. Figure 10 shows that successful tracing of feeder cattle was accomplished only 18 percent of the time whereas adult cattle were successfully traced 59 percent of the time. The key is identification.

Of the 1,682 traceback cases closed in fiscal year 1975, only 195 were laboratory confirmed as tuberculosis (Figure 11). The majority of the balance of 1,487 cases determined not to be tuberculosis were administratively closed without a field investigation.

There is a constant effort to increase the numbers of submissions of thoracic granulomas. There were 1,436 granuloma submissions in fiscal year 1975 compared to a total of 1,101 in fiscal year 1974 (Figure 12). This is encouraging, but we must strive to have every thoracic granuloma submitted. Figure 12 shows that 32 establishments, each slaughtering over 100,000 cattle annually, submitted no thoracic granulomas this year, 41 establishments in this class submitted from 1-5 granulomas, and 21 establishments submitted from 7-98 granulomas.

Figure 13 shows the numbers of submissions of thoracic granulomas from 107 federally inspected slaughter establishments each slaughtering over 20,000 cows annually. There were no submissions from 34 of these establishments this year, from 1 to 5 submissions from 55 of the establishments, and 6 to 98 cases from 18 establishments.

There has been a steady increase in the use of the comparative-cervical tuberculin test as an aid in classifying suspects and deviators to the caudal fold test. The committee on tuberculosis and Johne's Disease of this organization has been strongly urged to adopt the
comparative-cervical test as the only official retest for suspects and deviators to the caudal fold test. There are now 464 State and federally employed regulatory veterinarians trained and approved to conduct the test (Figure 14). Every State, except New Mexico, and Rhode Island has at least one trained and approved veterinarian.

In conclusion, we as members of the cooperative State-Federal team committed to the task of eradication of bovine tuberculosis shall have to rededicate ourselves to renewed and accelerated efforts if we are to succeed in eradication of this disease.
Tuberculosis Eradication

BOVINE TUBERCULOSIS AREA STATUS

(June 30, 1975)

*NO M. BOVIS REACTORS FOR OVER 5 YEARS

US. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE

47 TUBERCULOUS HERDS BY LOCATION

(Fiscal Year 1975)

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
**METHODS OF LOCATING TUBERCULOUS HERDS INITIALLY DETECTED IN FISCAL YEAR 1975**

- **Traceback of Regular Kill Slaughter Animals** (14)
- **Tracing Exposed Cattle from Affected Herds** (20)
- **Traceback from Affected Herd** (1)
- **Area Testing** (1)
- **Sale Test** (1)

**DETECTING HERDS WITH TB INFECTION**

- **All Other Tuberculin Testing**
- **Epidemiologic Tracing**


Numbers: 141, 111, 86, 74, 70, 67, 52, 38, 30, 29, 47, 35
Tuberculosis Eradication

TUBERCULOUS HERDS
(by Fiscal Year and Type Cattle)


DAIRY BEEF

1971: 35 dairy, 76 beef
1972: 32 dairy, 26 beef
1973: 14 dairy, 24 beef
1974: 13 dairy, 17 beef
1975: 39 dairy, 12 beef
1976: 47 dairy, 17 beef

US DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Tuberculosis Eradication

PROPORTION OF TUBERCULOUS HERDS DEPOPULATED
(Fiscal Year 1975)

29 Herds Depopulated Tuberculous Herds

U S DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
STATE-FEDERAL TUBERCULOSIS

Tuberculosis Eradication

PROPORTION OF TUBERCULOUS HERDS
FY 1965 THROUGH 1975 AND THOSE DEPOPULATED

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<td>141</td>
<td>111</td>
<td>86</td>
<td>74</td>
<td>70</td>
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<td>Number Depopulated</td>
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<td>14</td>
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US DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Tuberculosis Eradication

TUBERCULOUS HERDS DEPOPULATED
(Type of Cattle by Fiscal Year)

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<td>17</td>
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<tr>
<td>Beef</td>
<td>14</td>
<td>16</td>
<td>9</td>
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U.S. DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Tuberculosis Eradication

195 TUBERCULOSIS CASES (Regular Kill)
ANIMALS IDENTIFIED AND UNIDENTIFIED
(Fiscal Year 1975)

59 WITH IDENTIFICATION
40% UNSUCCESSFUL
60% SUCCESSFUL

136 NO IDENTIFICATION
76% UNSUCCESSFUL
24% SUCCESSFUL

US DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Tuberculosis Eradication

195 TUBERCULOSIS CASES (Regular Kill)
BY SLAUGHTER CLASS
(Fiscal Year 1975)

FEEDERS
82% UNSUCCESSFUL
18% SUCCESSFUL

ADULTS
41% UNSUCCESSFUL
59% SUCCESSFUL

US DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Tuberculosis Eradication

TUBERCULOSIS TRACEBACK INVESTIGATIONS (Regular Kill)

- CASES NOT TUBERCULOSIS
- CASES OF TUBERCULOSIS

<table>
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<tr>
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<tr>
<td>1970</td>
<td>1025</td>
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<tr>
<td>1971</td>
<td>922</td>
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<td>1972</td>
<td>879</td>
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<td>1973</td>
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<td>1974</td>
<td>1042</td>
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<tr>
<td>1975</td>
<td>1487</td>
</tr>
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<td>1976</td>
<td>1682</td>
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U.S. Department of Agriculture, Veterinary Services, Animal and Plant Health Inspection Service

GRANULOMAS SUBMITTED FROM 94 FEDERAL ESTABLISHMENTS SLAUGHTERING OVER 100,000 CATTLE*

(FY 1975)

- 32 Establishments
- 41 Establishments
- 21 Establishments

*Excluding Calves

U.S. Department of Agriculture, Veterinary Services, Animal and Plant Health Inspection Service
Tuberculosis Eradication

GRANULOMAS SUBMITTED FROM 107 FEDERAL
ESTABLISHMENTS SLAUGHTERING OVER 20,000 COWS
(FY 1975)

VETERINARY SERVICES LABORATORIES, AMES, IOWA

NONE

1-5 CASES

6-98 CASES

34 ESTABLISHMENTS

55 ESTABLISHMENTS

18 ESTABLISHMENTS

U.S. DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Tuberculosis Eradication

VETERINARIANS APPROVED*
TO CONDUCT COMPARATIVE-CERVICAL TEST
(As of July 1, 1975)

*TOTAL APPROVED - 464

U.S. DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
REPORT OF THE COMMITTEE ON TUBERCULOSIS
AND JOHNE'S DISEASE

Chairman: P. L. Smith, Sacramento, California
Co-Chairman: A. R. McLaughlin, Madison, Wisc.


The Committee on Tuberculosis and Johne's Disease met on Wednesday and Thursday afternoon. The following reports were made:

1. Symposium on Swine Mycobacterial Infections, Madison, Wisconsin, September 30 to October 1, 1975—Dr. A. R. McLaughlin, Dr. W. Prichard, and Mr. N. Black.


3. T.B. Outbreaks in Illinois and Georgia—Dr. P. L. Spencer and Dr. Darrell Johnson.

4. Problems of T.B. in Non-Human Primates and Other Exotic Animals in Zoological Parks—Dr. M. S. Silberman and Dr. R. K. Sikes.

5. In Vitro Lymphocyte Immunostimulation as an Aid in the Diagnosis of Tuberculosis—Dr. Donald W. Johnson.

6. Johne's Disease Problems—Dr. J. Dick, Dr. Weldy, and Dr. A. B. Larsen.

Committee discussion covered these presentations as well as other topics concerning tuberculosis in swine, cattle, and exotic animals.

I. Tuberculosis in Swine

The major findings presented at the symposium on swine mycobacterial infections were reviewed as well as some recommendations generated by the symposium. One consideration is the stigma associated with the term "swine tuberculosis". Substitute nomenclature such as "swine mycobacteriosis" has been suggested. The committee feels further justification is necessary to warrant a name change from swine tuberculosis to swine mycobacteriosis.

The recommendations of the Committee on Food Animal Hygiene and Inspection and the Committee on Transmissible Diseases of Swine Relative to Swine Tuberculosis were reviewed. This committee feels that further studies on the etiology, diagnosis, pathogenesis, epidemiology, and public health significance of the disease complex
are needed. Sound scientific evidence must be accumulated before attempting to secure relief of the economic burden to the swine industry caused by meat inspection regulations. The committee recommends that Veterinary Services explore the possibility of securing a national authority to assemble all available information on the subject of swine tuberculosis. It is the committee's understanding that Livestock Conservation, Inc. would publish this information.

II. Tuberculosis in Exotic Animals

Tuberculosis has apparently increased in exotic ruminants and non-human primates, especially those in zoos and game parks. The committee recommends that the Cattle Diseases Staff, Veterinary Services, accumulate and distribute available information on the subject. It further recommends that states seek authority restricting movement of T. B. infected or exposed animals in situations where there is a potential human or livestock health hazard.

III. Tuberculosis in Cattle

A report on tuberculosis in California feedlot animals was given. In the past two and three-quarters years, 25 cases which yielded *M. bovis* originated from Mexico. Import requirements were discussed. Dr. Del Rio of Mexico reported on the present status of T.B. eradication in Mexico.

The nation-wide problem of disposal of tuberculin reactors was discussed, but the problem was not resolved. It was recommended that the USDA conduct or sponsor studies to determine thermal death points of the mycobacterial organisms found in livestock at the time of slaughter. Presently, meat inspection requires meat from carcasses passed for cooking (PFC) to be heated to a temperature of 170 degrees F. for one-half hour; a flexible time-temperature relationship could allow more outlets for meat from PFC carcasses.

Uniform Methods and Rules (UMR) were discussed, two amendments were considered which were eventually tabled.

It was suggested that under tuberculin test interpretation the deviator classification be dropped and any animal showing a response to tuberculin be classified a suspect. At the present time, it is the committee's opinion that the deviator classification is needed to assure adequate reporting of tuberculin test responses.

Consideration was given to mandatorily requiring the comparative cervical (C-C) test on the retest of all suspects. The committee recognized this as desirable. However, before considering a change in the UMR, a study should be made to determine the position of all state animal health officials and the availability of trained personnel. Also, if the Veterinary Services training requirement is maintained, an assurance must be secured that the training can be provided at the
state level and in a reasonable and timely manner. Dr. J. Dick, Chairman, and Dr. C. S. Duncan were appointed to a subcommittee to make a feasibility study of making the C-C test a mandatory retest requirement. The subcommittee was instructed to report their findings and recommendations at the 1976 annual meeting.

The committee continues to strongly recommend the C-C test as the method of choice for retesting animals demonstrating sensitivity to the tuberculin test.

The subcommittee to study the utilization of paramedical personnel in surveillance tuberculin testing reported on their findings. There were 35 states in favor of the proposal; 11 were not in favor; three were neutral; and one state did not reply. The subcommittee study and recommendations were accepted by the committee and forwarded with this report to the Executive Committee for their consideration. In the event the Executive Committee approves the study report, the subcommittee will formulate uniform training procedures and guidelines for state-federal cooperative agreements.
THE NEWCASTLE DISEASE EPIDEMIC IN SOUTHERN CALIFORNIA, 1971-1973: DESCRIPTIVE EPIDEMIOLOGY AND EFFECTS OF VACCINATION ON THE ERADICATION PROGRAM

M. J. Burridge, B.V.M.&S., M.P.V.M.a
H. P. Riemann, D.V.M., Ph.D.a
W. W. Utterback, D.V.M., M.P.V.M.b
E. C. Sharman, D.V.M.c

INTRODUCTION

A major epidemic of velogenic viscerotropic Newcastle disease (VVND) occurred in southern California between 1971 and 1973. A cooperative state-federal task force was established in March 1972 to eradicate the disease and details of the regulatory aspects of the eradication program have been published.1 During the eradication program, the task force collected extensive data on the domestic avian flocks in the affected areas of southern California, and preliminary epidemiological analyses of some of these data have been described.4,5,6 Following the successful conclusion to the VVND eradication program, the complete data were made available to the University of California at Davis for epidemiological analysis. Results of the analysis to determine the methods of spread of VVND virus in the epidemic have been described elsewhere,1 and this report gives a summary of the descriptive epidemiology of the epidemic and of the effects of vaccination on the eradication program.

MATERIALS AND METHODS

The area of southern California studied is shown in Fig. 1. There were 38,410,479 domestic birds in 16,460 flocks in the epidemic area, and the vast majority (98.6%) of those birds was in 844 commercial flocks. Flocks were classified as commercial (1,000 or more birds per flock) or backyard (less than 1,000 birds per flock). The commercial chicken industry in that area consisted of almost exclusively layer or layer-related operations.

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bU.S. Department of Agriculture, Animal and Plant Health Inspection Service (Veterinary Services), 702 Colorado Street, Austin, Texas 78701.
cChief, Technical Support, Emergency Programs, U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Veterinary Services), Hyattsville, Maryland 20782.
Research supported by a contract (USDA 12-16-4-15) with the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture.
During the epidemic, 1,297 flocks were depopulated, 391 due to infection with VVND and 906 due to exposure to virus. Positive VVND diagnosis was made either during field investigation on the basis of symptomatology and/or necropsy lesions, or by virus isolation. Flocks were depopulated due to exposure following known or suspected movement of birds into the flocks from infected premises. Only flocks depopulated due to field diagnosis or virus isolation were considered infected with VVND in this study.

Among the data collected from each infected flock, the following were used in this study: location, number of birds by species, class of poultry, Newcastle disease (ND) vaccination status, date and species of first bird sick, clinical signs and necropsy lesions in affected chickens, method of VVND diagnosis, and date depopulation completed. Similar information was collected from uninfected flocks that were investigated during the epidemic.

The data were analyzed on a flock basis by consideration of the following:

1. flock attack rates, classifying flocks according to species and, with chickens, by size and type of operation;
2. epidemic and depopulation curves;
3. incidence studies, by species affected.

The flock was the unit of concern in this study. Sickness of a single bird was defined as a case, whereas disease in a flock was an outbreak.

To investigate the effect of ND vaccination on the course of the epidemic and on the eradication program, VVND was studied in chickens in 2 areas. The areas, designated A and B, are shown in Fig. 2; they had similar types of poultry operations and management practices. Area A, containing 1,705 flocks and a lightly vaccinated chicken population, was the area of initial spread of disease south and west from Fontana, the original focus of the epidemic. During that early period of spread, from November 1971 to May 1972, VVND was introduced into a chicken population that had been exposed to little vaccination. The majority of the commercial chicken flocks in that area had received only one round of vaccination prior to infection or investigation, and the backyard chickens were mainly unvaccinated. Area B, containing 590 flocks and a heavily vaccinated chicken population, was not infected with VVND until later in the epidemic and the disease spread through chicken flocks in which the officially supervised vaccination program had been completed. All commercial flocks in area B had been vaccinated several times before exposure to virus and many of the backyard flocks had been vaccinated at least once.

The data on VVND in chicken flocks in areas A and B were analyzed and compared by consideration of the following: attack rates, depopulation rates (proportion of flocks depopulated due to either infection or exposure to virus), morbidity and mortality rates, and methods of VVND diagnosis.
It is realized that areas A and B are not ideal for the above comparison since additional factors, such as time and phases in the eradication program, differed between the 2 areas. However, it was the best approach possible and, in analyzing the results, consideration was given also to the other variables.

RESULTS AND DISCUSSION

**Flock Attack Rates**

VVND initially became established in the chicken population of Fontana, and later spread to turkeys, pigeons, game birds, and ducks and geese. During the epidemic, VVND was diagnosed in 391 flocks. The affected species were chickens in 337 flocks (86.2%), exotic birds in 26 (6.6%), pigeons in 10 (2.6%), game birds in 8 (2.0%), turkeys in 7 (1.8%), and ducks or geese in 3 (0.8%). The flock attack rates for the epidemic area are given in Table 1, showing that the highest attack rates were found in commercial chicken and turkey flocks. Within mixed backyard flocks, chickens were the affected species in the majority (90.5%) of outbreaks.

The attack rate in commercial chicken flocks was markedly higher than that in similar backyard flocks. Chickens were infected throughout the epidemic, but the pattern of spread of VVND within this species differed between flock types with respect to time. In the first half of the epidemic, up to the end of August 1972, the majority (62.9%) of infected chicken flocks were of the backyard type, whereas in the second half of the epidemic most new outbreaks (65.4%) were in commercial operations. These findings indicate that commercial chicken flocks were more directly involved in the maintenance of the epidemic. Furthermore, the attack rate increased with size of chicken flock (Table 2), with the highest rate of 24.4% found in the large commercial operations of over 100,000 birds, indicating the effect of intensification of poultry production on the spread of VVND.

The epidemic in commercial chicken flocks was essentially limited to those involved in the layer industry, with 145 (98.0%) of the infected commercial flocks being pullet or layer operations (Table 3). While dissemination of VVND virus was widespread among those flocks, there was no spread of disease to broiler flocks, either in the epidemic area or north across the mountains into the extensive broiler industry of the high desert area of Los Angeles and San Bernardino counties.

Twenty-six outbreaks of VVND were diagnosed in exotic birds. From late 1971 to August 1972, 14 separate isolations of VVND virus were made from recently imported flocks, with infection spreading into 8 aviaries and 4 mixed backyard flocks. All infected imported birds were diagnosed positive on the basis of virus isolation, with few showing macroscopic signs of VVND, emphasizing the inherent dangers of spread of ND through international trade in exotic birds.
**Epidemic and Depopulation Curves**

The incidence of VVND in flocks, by month of first sickness, is shown in the form of an epidemic curve in Fig. 3, together with the depopulation curve for infected flocks by month of completion of flock depopulation. There was a distinct peak in the epidemic curve in February 1972, and a smaller peak in December 1972. Depopulation of the flocks involved in the first and major peak was markedly delayed, whereas depopulation of those infected later in the epidemic was relatively rapid.

The 2 peaks of the epidemic curve occurred in the winter, with decreased incidence in the hot dry summer months. Interpretation of these data, with respect to the seasons of the year, must be guarded. Undoubtedly one of the most important factors contributing towards the first peak incidence of disease was the lack of eradication activity between the end of January and mid-March 1972. During that period, there was no depopulation of newly infected flocks, allowing a build-up of infection to occur. The second peak in late 1972 and early 1973 was associated, in part, with intensified surveillance following the declaration of an extraordinary emergency in November 1972. Therefore, the seasonal incidence of VVND in this epidemic was related in most probability not only to prevailing climatic conditions, but also to the different phases in the eradication program. Other factors, such as an increase in disease security by poultrymen, may also have contributed to the reduction in incidence during the summer months of 1972.

**Clinical Signs and Necropsy Lesions in Chickens**

The most common clinical sign in chickens was respiratory distress, followed by severe depression. Another prominent sign of VVND in chickens was edematous swelling of the head, a feature not commonly recorded in ND. Diarrhea and paralysis were also seen in some infected birds.

The most prevalent necropsy lesions in chickens were those in organs and tissues of the respiratory and gastro-intestinal tracts, particularly in the trachea, proventriculus and the lymphoid tissues of the intestine, indicating the viscerotropic nature of the virus. In heavily vaccinated flocks, such as those in area B, affected birds (mainly the young poorly immunized pullets) exhibited similar clinical signs and necropsy lesions to those in unvaccinated flocks.

**Effects of Vaccination on the Eradication Program**

While vaccination reduced losses in infected flocks, it did not prevent infection with VVND virus, 234 (59.8%) of infected flocks having been vaccinated prior to VVND diagnosis.

In area A there was quick reporting and diagnosis of VVND, with
high morbidity and mortality rates (50-100% and 15-100% respectively) in infected chicken flocks, many of which showed typical signs and lesions of the disease. All outbreaks were diagnosed through examination of sick birds, either on clinical observations or by virus isolation. In contrast, most infected chicken flocks in area B showed lower morbidity and mortality rates (1-50% and <1-15% respectively), and 70% of the outbreaks in the heavily vaccinated commercial flocks of area B were diagnosed through the surveillance procedures of either virus isolation from dead chickens submitted during the routine necropsy surveillance program or infection of sentinel birds. These results indicate that vaccination tended to make the disease subclinical. Typical clinical disease was eventually seen in some of these flocks when young birds that could not be adequately immunized, were exposed to virus. In order to secure diagnosis in the other flocks, expensive and laborious techniques had to be introduced because of the subclinical infections. These techniques were time consuming, allowing some infected vaccinated flocks to act as foci of infection for further spread of the disease.1

The VVND attack and depopulation rates are compared by flock type and by area in Table 4. The epidemic in area A lasted for 24 weeks, with the incidence rate rapidly dropping to zero following the inception of the cooperative state-federal task force in March 1972 and their subsequent depopulation of all infected and exposed flocks. In contrast, the epidemic in area B lasted for 63 weeks, despite a substantially smaller avian population. Undoubtedly one of the factors that prolonged the epidemic in the latter area was the intensive vaccination program in its commercial chicken flocks. The persistence of infection in area B led to an attack rate of 38.7% in commercial chicken flocks in that area, which was nearly twice the overall attack rate of 20.0% for that flock type, and allowed secondary spread of disease to areas that had been free of VVND for several months.1

The flock depopulation rates were markedly higher in area B than in area A (Table 4). This difference is a reflection of the intimate contact between flocks in the former area as well as an indication of the more drastic measures that had to be implemented to ensure successful eradication of VVND from a heavily vaccinated population.

It has been shown that VVND virus was mechanically disseminated by vaccination crews in this epidemic.1 Since a vast number of birds had to be immunized rapidly, vaccination was often accomplished by inexperienced people who had little concept of the security precautions necessary to prevent disease spread. Furthermore, since infected vaccinated flocks in this epidemic shed virus for up to 4 months,5 vaccination crews could have become contaminated at the time of revaccination and spread virus mechanically to new premises. These findings amply demonstrate the importance of professional supervision of ND vaccination programs during an epidemic, with
vaccination of only healthy flocks and strict adherence to hygienic precautions necessary to prevent disease spread.

SUMMARY AND CONCLUSIONS

The VVND flock attack rate was highest in commercial chickens, which were almost exclusively involved in the layer industry. With chicken flocks, the attack rate increased with size of flock, from 1.0% for those containing less than 1,000 birds to 24.4% for the large commercial operations of over 100,000 birds. All 391 infected flocks were depopulated, together with 906 flocks which had been exposed to infected birds. The 2 peaks of the epidemic curve occurred in the winter, with decreased incidence in the hot dry summer months, and this seasonal incidence of VVND was related not only to prevailing climatic conditions, but also to other factors including the phases in the eradication program.

Vaccination reduced both morbidity and mortality rates in flocks exposed to VVND virus, but did not prevent infection with that virus, 59.8% of infected flocks having been vaccinated prior to VVND diagnosis. However, 2 undesirable effects of vaccination were the tendency to make disease subclinical and the mechanical spread of virus by vaccination crews. It is evident, therefore, that careful consideration must be given to the value of vaccination before it is implemented as a control measure in a VVND eradication program. It is clear that the use of vaccination in such a program must be accompanied by adequate hygienic precautions to prevent disease spread and by sophisticated techniques, supported by an efficient laboratory network, to diagnose subclinical infections.

ACKNOWLEDGMENTS

The authors acknowledge the State-Federal VVND Task Force at Riverside for making the data available, and thank Dr. F. M. Patterson of the State of California Department of Food and Agriculture (Bureau of Animal Health) and Dr. J. I. Moulthrop of the Animal and Plant Health Inspection Service of the U. S. Department of Agriculture for their assistance.
Fig. 1. VVND epidemic area of southern California, with shaded areas indicating significant mountain ranges north of San Diego County.

Fig. 2. Areas A and B of southern California studied to investigate effects of ND vaccination on the VVND eradication program.
Fig. 3. Flock incidence of VVND, and time of total depopulation of infected flocks, by month in southern California, 1971-1973.

TABLE 1 - VVND Attack Rates by Type of Flock in Southern California, 1971-1973

<table>
<thead>
<tr>
<th>Flock type</th>
<th>Total</th>
<th>VVND-positive</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial chicken</td>
<td>739</td>
<td>148</td>
<td>20.0</td>
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<tr>
<td>Commercial turkey</td>
<td>40</td>
<td>6</td>
<td>15.0</td>
</tr>
<tr>
<td>Game bird</td>
<td>122</td>
<td>6</td>
<td>4.9</td>
</tr>
<tr>
<td>Exotic bird</td>
<td>500</td>
<td>22</td>
<td>4.4</td>
</tr>
<tr>
<td>Backyard turkey</td>
<td>27</td>
<td>1</td>
<td>3.7</td>
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<tr>
<td>Mixed backyard</td>
<td>5,271</td>
<td>116</td>
<td>2.2</td>
</tr>
<tr>
<td>Pigeon</td>
<td>568</td>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>Backyard chicken</td>
<td>8,795</td>
<td>84</td>
<td>1.0</td>
</tr>
<tr>
<td>Duck &amp; goose</td>
<td>398</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>All flocks</td>
<td>16,460</td>
<td>391</td>
<td>2.4</td>
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### TABLE 2 - VVND Attack Rates by Size of Chicken Flocks in Southern California, 1971-1973

<table>
<thead>
<tr>
<th>Flock size</th>
<th>Number of flocks</th>
<th>VVND-positive</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1,000</td>
<td>8,795*</td>
<td>84</td>
<td>1.0</td>
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<tr>
<td>1,000 - 10,000</td>
<td>133</td>
<td>21</td>
<td>15.8</td>
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<tr>
<td>10,001 - 100,000</td>
<td>524</td>
<td>107</td>
<td>20.4</td>
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<tr>
<td>&gt;100,000</td>
<td>82</td>
<td>20</td>
<td>24.4</td>
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* Excluding mixed backyard flocks

### TABLE 3 - VVND Attack Rates by Type of Commercial Chicken Operation in Southern California, 1971-1973

<table>
<thead>
<tr>
<th>Flock type</th>
<th>Number of flocks</th>
<th>VVND-positive</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pullets</td>
<td>57</td>
<td>20</td>
<td>35.1</td>
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<tr>
<td>Layers</td>
<td>620</td>
<td>125</td>
<td>20.2</td>
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<tr>
<td>Hatcheries</td>
<td>20</td>
<td>2</td>
<td>10.0</td>
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<tr>
<td>Breeders</td>
<td>32</td>
<td>1</td>
<td>3.1</td>
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<tr>
<td>Broilers</td>
<td>10</td>
<td>0</td>
<td>0</td>
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</table>
TABLE 4 - VVND Attack Rates and Depopulation Rates of Different Flock Types by Area of Southern California, 1971-1973

<table>
<thead>
<tr>
<th>Flock type</th>
<th>Total number of flocks</th>
<th>Attack rate (%)</th>
<th>Depopulation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area A</td>
<td>Area B</td>
<td>Area A</td>
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<tr>
<td>Commercial chicken</td>
<td>186</td>
<td>119</td>
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<td>Backyard chicken</td>
<td>1388</td>
<td>429</td>
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<td>Non-chicken</td>
<td>131</td>
<td>42</td>
<td>10.7</td>
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<tr>
<td>All flocks</td>
<td>1705</td>
<td>590</td>
<td>8.6</td>
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REFERENCES

ANIMAL PROTEIN CONSERVATION REPORT

FORWARD

Dr. John Atwere, Chairman

It has been shown many times that — due to the currently accepted eradication procedures of depopulation of infected and exposed animals and disposal of the carcasses by burial, burning or rendering — foreign animal diseases can take a serious toll in edible animal protein. With the increasingly serious food shortage, questions have been raised about the loss of protein that would result from such eradication methods.

To help answer these questions, a multi-agency work group — sponsored by Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture — was formed in July of 1973. The primary concern of this group was to examine the feasibility of conserving animal protein for human or non-human (pet or livestock) use from animals exposed to certain diseases. Many factors — including regulatory controls, biosafety to man and animal populations, speed, cost, trade disruption, and the availability of facilities and manpower — were considered.

The members of the group represented primary levels of regulatory responsibility and technical expertise involved in producing, transporting, and processing animal protein.

Detailed reports dealing with technical and policy aspects of conserving animal protein were submitted by work group members. Summaries of those reports are included in this discussion.* Full texts of individual appendices are available on request from:

Emergency Programs, VS, APHIS, USDA
Federal Building, Room 748
Hyattsville, Maryland 20782

Work group members and all the other contributors are to be commended for their help in exploring the feasibility of conserving animal protein during foreign animal disease outbreaks.

*Because of length, the appendices referred to in the report are not included in this publication but are available upon request.
# Members of the Work Group on Conserving Animal Protein

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<tr>
<th>Name</th>
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</tr>
</thead>
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<tr>
<td>Dr. John W. Walker</td>
<td>Senior Staff Veterinarian, Poultry Diseases Staff, Veterinary Services</td>
</tr>
</tbody>
</table>
ANIMAL PROTEIN CONSERVATION
WORK GROUP REPORT

INTRODUCTION

Outbreaks of exotic animal diseases (diseases foreign to the United States) would have serious consequences for the consuming public, if a large portion of the U.S. livestock population were to be destroyed.

Import regulations are designed to prevent the introduction of such foreign animal diseases. But occasionally, destructive diseases do enter the country. When they do, the U.S. Department of Agriculture's (USDA) policy has been to eradicate the disease by destroying all animals infected or exposed to the disease. The carcasses are usually buried or burned on the affected premises or rendered into an inedible product. Also restrictive quarantines are placed on the areas surrounding the disease outbreak.

Experience has shown that this policy is the most effective method of stamping out animal diseases.

If an outbreak of an exotic disease should occur in a major livestock producing area with a high population density the subsequent loss of animal protein might seriously reduce the nation's food supply. In the event of such a catastrophe the work group studied possible alternative methods that could be used to stamp out disease outbreaks and yet conserve animal protein.

Animal protein conservation (APC) would involve processing the retrievable protein in such a way as to (1) make the finished product safe for human or non-human consumption, and (2) assure that the finished product could not transmit the disease to other susceptible animals.

Foot-and-mouth disease, considered to be the most devastating of the exotic diseases, was selected as the model to relate to during the deliberations of the work group.

After extensive study, the work group is of the opinion that the existing facilities for transportation, slaughter, processing, rendering and storage are not adequate to make conserving animal protein a realistic economic alternative to the current methods of disposal of exposed animals. Many factors were enumerated that indicated the need for further technological development. These deliberations should be periodically reviewed in the light of possible changes in the national and world food supply, economics, and the future development of the new technology that this report recommends. Many of these technological developments are reasonably attainable and future prospects for protein conservation appear feasible.

The following is a brief discussion of the major topics covered by the work group in reaching this decision about a highly virulent, easily transmitted disease. These points are discussed more fully in the appendices referred to in each section.
FACTORS AFFECTING APC

There are several major factors which would influence a decision to conserve protein; political considerations, public opinion, prevailing food availability, and monetary pressures. These factors should be strongly in support of an APC effort before such a program is even considered.

The following aspects will be important, when considering an APC program.
1. Safety of the finished product.
2. Economics.
3. Physical facilities to transport and process the exposed animals.
4. Public acceptance of the finished product.
5. Public health aspects.
6. Biological security.

SAFETY OF THE FINISHED PRODUCT

Conserving animal protein would be accomplished by slaughtering and special processing only animals not showing symptoms of the disease. All products resulting from APC must be safe not only for human or non-human consumption, but must also be incapable of spreading the disease (See Appendix D).

ECONOMICS

Production of animal protein in the United States has operated for years on fluctuating (but usually narrow) margins of profit, and any limitations or restrictions on production could upset the delicate balance. Economics effects, in addition to losses to owners, would include income losses to processors, handlers, transporters, etc., higher prices to consumers, indemnity and other compensation claims paid by the government, and program costs.

The economic feasibility of an APC effort, therefore, would depend on:

1. The economic impact of the animal disease eradication program—Extensive destruction of animals would have an immediate local impact on the farmers involved as well as a broad effect on consumer animal protein supplies and prices. This initial impact would be followed by long range effects involving losses to producers, feed suppliers, livestock handlers and processors, and the effects within the local areas of reduced income.

2. The economic benefits and costs of an APC program—The degree to which a feasible APC program could reduce losses to affected groups would have to be weighed against program costs and budget limitations.

3. Public concern—The public concern for losses and other economic impacts of an animal disease eradication situation in an
area of concentrated large-scale cattle feeding would, no doubt, be
more intense than the impacts of the situation on the total meat price
and supply to consumers. But it should also be realized that, as the
size of the affected area increases (thus including a greater number
of Congressional districts or States) public concern over potential
losses would also significantly increase. Thus public concern would
have a direct effect on any APC efforts. (See Appendices Q and R).

**PHYSICAL FACILITIES**

The physical facilities for conserving protein include: vehicles
for transporting the animals to slaughter; facilities for slaughter;
processing, freezing, boning, cooking, and canning. The work group
concluded that present capacity in these areas would be insufficient
to handle the amounts of meat that would be generated during a large
scale protein conservation effort.

1. **Transporting facilities**—Vehicles for transporting exposed
animals or their products must be biologically secure. Refrigerated
trailers could be modified to assure biological security, but it is ques-
tionable whether an adequate number of these units could be obtained
if many animals were involved. Refrigerated vehicles would also be
in great demand for both refrigerated storage and as carriers of the
dressed meat from exposed animals for further processing. (See Ap-
pendices B and C).

2. **Slaughter facilities**—The development of emergency slaugh-
ter facilities appears to be impractical. (See Appendices E, H, and I).
The actual slaughter capability in case of an outbreak could not be
expected to exceed three times the existing capacity in an area (in-
crease to three shifts). If a three-fold increase could be accomplished,
it would necessitate a nine-fold increase in existing cold storage (for
cooling out and holding for three days prior to boning). Present cold
storage and freezer capacity does not exist to meet this increased
need. (See Appendix J).

3. **Processing facilities**—Commercial and military freezer space
is limited, therefore, storage relief would probably come from renting
refrigerated freight cars and/or ocean-going refrigerated van con-
tainers, etc. Boning facilities would be the greatest limiting factor as
they currently equal only about one-fifth of the existing slaughter
capacity. These factors will need to be resolved when large concen-
trated livestock populations are to be depopulated by slaughter in a
short period of time. (See Appendices G and P).

4. **Rendering capability**—Existing rendering capability could
be expected to be increased about 50 percent. This would be a mere
token effort, if a large feedlot was to be depopulated and rendered.
(See Appendix K).
VACCINATION

Many European countries follow a policy of containment and slaughter post-outbreak, i.e., quarantining, vaccinating, and holding exposed animals until the outbreak is over and then slaughtering these animals at a normal rate when facilities become available. (See Appendix N). This alternative would require facilities or arrangements in the United States for production or purchase of the vaccines necessary to treat exposed animals during an outbreak. At the present time, there are no provisions for the production or purchase of vaccines for the foreign diseases that might be involved during protein conservation measures. Facilities for stockpiling such vaccine would also be necessary. (See Appendices M and O).

PUBLIC ACCEPTANCE OF THE PRODUCT

Public acceptance of the product might be difficult to obtain. Consumers could form strong psychological objections to such a product which would make it difficult to sell, thereby defeating the purpose of the APC efforts. The same might hold true for product prepared for non-human (pet) use since people usually have strong feelings about their pets' welfare.

The "public relations" of an APC effort must be carefully studied, whenever such measures are being considered, and steps must be taken to ensure a cooperative and receptive public. (See Appendix A.

PUBLIC HEALTH ASPECTS

The public health factor would influence protein conservation efforts. Certain animal disease producing agents pose no threat to human health. Other agents pose a threat and must receive special consideration. (See Appendices T and U). Also the nutritive value of the product must be considered. (See Appendix F).

BIOLOGICAL SECURITY

Maintaining biological security during transport of exposed animals to slaughter would be difficult. Refrigerated trucks would be most easily modified to assure biological security. Other trucks could be modified, but more expense and time would be involved. (See Appendix C).

There would be a need to modify facilities and conduct close surveillance at processing plants to ensure that workers who handle exposed animals and/or their products would not come into contact with the finished product or susceptible animals. All liquid and solid wastes leaving the plant must be treated in a manner to ensure biological security. (See Appendices D, M, N, O, and V).
SUMMARY:

A. Objectives of animal protein conservation (APC).
   1. Retrieve wholesome, edible protein for human and/or non-human consumption from animals exposed to foreign animal diseases.
   2. Conserve animal protein that might otherwise be lost during foreign animal disease eradication programs.

B. Criteria for considering APC.
   1. When an eradication program becomes so extensive that it threatens the supply of animal protein to consumers.
   2. When the magnitude of the outbreak is such that conservation appears to be economically and/or psychologically appropriate.

C. "Musts" for APC.
   1. Sound eradication procedures must not be violated.
   2. Product must be from animals free of signs and lesions of disease.
   3. USDA inspection standards must not be violated.
   4. APC operations must be compatible with sound public health principles.
   5. Wholesomeness and nutritive value of product must be maintained.
   6. The final product must be one the public can accept.

D. Procedure for APC.
   1. Establish quarantine area around infection.
   2. Restrict all movement of susceptible animals and potentially contaminated products.
   3. Dispose—by burial, burning, or rendering—of clinically ill animals.
   4. Conserve protein only from recovered, exposed, and vaccinated animals.
   5. Move animals from infected premises to slaughter under official seal and (to the extent possible) in biologically secure vehicles.
   6. Move carcasses or animal products to processing plants, or storage facilities under official seal.
   7. Cook or otherwise treat protein by established procedures to render the end products safe for distribution through normal channels.
   8. Clean and disinfect, under supervision, all equipment and vehicles used in handling potentially contaminated animals and/or their products.
   9. Slaughter and/or process according to APC procedures any animal or product handled by a slaughter plant, cold storage
facility, boning and cooking facility that handles APC animals or their products.

10. Clean and disinfect, under supervision, all such facilities and equipment used to handle exposed animals or products before they may return to normal production.

11. Maintain control over personnel who may come in contact with APC animals and their products to prevent disease spread.

12. Dispose of effluents and solid wastes in a biologically safe manner and under official supervision.

**RECOMMENDATIONS**

To make protein conservation a viable alternative to burial, burning or rendering, the committee recommended that immediate action be taken to develop technology in the following areas:

1. Modification of vehicles for hauling animals in a biologically secure manner;
2. Development and expansion of cold storage facilities for post-slaughter use;
3. Development of means for holding animals until they could be normally slaughtered;
4. Development of biologically secure methods of disposal of effluents and solid wastes;
5. Planning of slaughterhouse and processing plant procedures for protein conservation;
6. Development of an inexpensive and efficient method to slaughter on site.

With increased potential in these areas, animal protein conservation on a large scale could be considered as a safe, effective means of retrieving consumable protein during an outbreak of a highly transmissible foreign animal disease. However, APC may not be the most economical method for carcass disposal.
The Ad Hoc Epizootic Attack Committee met on November 3, 1975 and after preliminary discussion including one agenda item the Committee voted to adjourn and reconvene at 7 a.m. on November 4, 1975. Twenty-two members and guests were present.

A bibliography on Foot and Mouth Disease produced by Emergency Programs of Veterinary Services, Animal and Plant Health Inspection Services, was reviewed by the Committee. Approximately 3,000 entries are included in the bibliography, which covers over 58% of all English publications on FMD. Some foreign language articles have been translated into English and are included in the listing. During the next few years all foreign language articles on FMD will be translated into English and added to the bibliography. Emergency Programs is building a data bank on some 30 exotic diseases which pose a threat to the livestock and poultry industries of the United States. A complete bibliography will be produced on each disease after they have been added to the data bank. Currently bibliographies are available on Swine Vesicular Disease, African Swine Fever, Foot and Mouth Disease and Newcastle Disease.

A draft of a handbook on the control and eradication of Foot and Mouth Disease was reviewed by the Committee. Concern was expressed about the involvement of wildlife, the disposal of milk, and the disposal of carcasses. Several members were appointed to review and make recommendations on these subjects. Emergency Programs plans to submit this draft for publication in early 1976. In final form the handbook will consist of approximately 35 pages and will contain all essential information for field personnel to carry out an eradication program.

Three other handbooks are being developed for African Swine Fever, Newcastle Disease and Hog Cholera.

The Committee heard a report made by Dr. Jack Hyde from USDA Agricultural Research Service on current incomplete studies on the survival of Foot and Mouth Disease virus (FMDV) in milk and milk products from cows with active Foot and Mouth viral infection. Portions of the work that has been completed indicates that FMDV will live through all types of recognized pasteurization procedures and will survive temperatures within a few degrees of the
boiling point. Survival of FMDV was readily demonstrated on inoculation into susceptible bovines but not on cell culture.

Because of these preliminary FMDV results this Committee is greatly concerned about getting these results considered on a timely basis into the planning for handling potential FMD outbreaks.

The Committee wishes to commend USDA-ARS Plum Island Animal Disease Center for initiating the FMDV survival studies in milk and milk products and urges USDA to complete them as soon as possible.

The Committee received an excellent preliminary report by Dr. E. H. McCauley on a study of The Economic Impact of Foot and Mouth Disease and its control in the U. S.

The objectives of this study are to determine and measure the factor of the cost benefit relationship of various strategies against FMD, determine the cost of allowing FMD to become endemic and provide guidelines for indemnification payments.

If FMD is allowed to spread through the U. S. without control procedures being applied we estimate that in the first year that direct losses to producers and loss of imports will amount to $4.15 billion. The loss of secondary industries (packers, feed suppliers, transportation, etc.) would be some multiple of this. So the direct and indirect loss is estimated to be as much as ten billion. Because of the nature of the demand for meat and milk, it is estimated that the transfer of the direct producer loss to consumers would result in price increases of 10, 8, and 27 percent for beef, pork, and milk respectively. The economic impact for subsequent years is estimated to be considerably less because of increased immunity, public and private vaccination and quarantine procedures and shifting of resource use to accommodate the changed supply situation.

In the conduct of a FMD eradication program it is conceivable that decisions will have to be made on continuing the strict slaughter policy or changing to another strategy. To assist in this decision a data analysis and epidemiologic prediction system (EPIC) has been designed. The idea of this system is to provide decision makers with rapid and accurate reports about the technical success, cost and logistics of the eradication program. The results of this data analysis would be used along with data on herds at risk in the quarantine area and cost of eradication estimate to project the cost of continuing the strict eradication policy for the next time period. We are working on identifying and quantifying other factors which could predictably affect the projected estimates. Presently APHIS is investigating the feasibility of incorporating the EPIC system into their administrative and operational framework.

The cost of continuing the eradication approach must be weighed against the benefits of avoiding the cost of other strategies or of turning the disease loose. The principle intermediate strategy being
considered is to contain a large area of the U. S. in which FMD is controlled by vaccination and selected slaughter and premise quarantine. The cost and economic impact of such a regionalization program are being estimated.

Also in the course of this study certain sub-studies have developed which bear on the main theme. The estimate of impact of disease on feed efficiency, the quantification of physical events which alter disease predications and the feasibility of alternative disposal techniques are some of these sub-studies.

Recommendations:

1. The Committee recommends that APHIS continue to support the FMD economic studies in cooperation with the University of Minnesota.

2. The Committee recommends that APHIS initiate action in conjunction with the several veterinary schools to develop graduate studies on the economic aspects of animals diseases. Further, APHIS and the universities should explore the possibility of funding for these projects by the Agency for International Development and Food and Agriculture Organization of the United Nations to enable participants to study the socio-economic aspects in countries where the diseases exist.

The Committee discussed an Animal Protein Conservation report which is being offered for publication in these proceedings.

This Committee recommends that USDA study the feasibility of developing plans on standards that may be used for the construction of new plants or in major remodeling of rendering plants designed to ensure the biological security of rendered end products in the face of an exotic disease outbreak.

This Committee concurred with the Wild and Marine Life Disease Committee in submitting the resolution concerning the environmental impact study for the proposed construction of the Pan American Highway through the Darien Gap area in Panama and Columbia.

We are submitting for publication in the proceedings an article titled “The Newcastle Disease Epidemic in Southern California, 1971-1973: Descriptive Epidemiology and Effects of Vaccination on the Eradication Program.”
REOVIRUS AND ROTAVIRUS INFECTIONS*

C. A. Mebus, D.V.M., Ph.D.**

The Reovirus Study Group of the Vertebrate Virus Subcommittee of the International Commission for the Nomenclature of Viruses recommended the establishment of several genera within the family Reoviridae. These are: 1) Orthoreovirus (host range - vertebrates), 2) Orbivirus (host range - insects and vertebrates), 3) A genus comprising wound tumor virus and rice dwarf virus (host range - plants), and 4) A genus comprising cytoplasmic polyhedrosis viruses (host range - insects).

In the above proposed classification, the Nebraska calf diarrhea virus (also known as calf diarrheal reovirus-like agent), was listed as a species; epizootic diarrhea of infant mice (EDIM) was listed as a probable species in the genus Orthoreovirus. The calf agent was also listed as a probable species in the genus Orbivirus. In the 1975 Western Hemisphere Committee on Animal Virus Characterization catalog, the Nebraska calf diarrhea virus was placed in the genus Orbivirus.

Recent reports of a reovirus-like agent (also referred to as an orbivirus, orbivirus-like agent, rotavirus and duovirus) being an etiologic agent in acute gastroenteritis of children in many parts of the world has stimulated considerable study of reovirus-like agents. As a result of these studies, the consensus of the groups working with the reovirus-like agents is that the characteristics of these agents are sufficiently different from other viruses in the genera Orthoreovirus and Orbivirus to warrant the establishment of a new genus in the family Reoviridae. This paper will review the work pertaining to the classification of the reovirus-like agents.

Reovirus-like agents have been demonstrated to be etiological agents for or found to be associated with the following diseases: 1) epizootic diarrhea of infant mice (EDIM), 2) neonatal calf diarrhea, 3) acute non-bacterial gastroenteritis of children, 4) diarrhea in piglets and 5) diarrhea in foals. Reovirus-like particles have also been found in the feces of lambs and goats. In addition, there are two reovirus-like agents not associated with enteritis: the simian virus S.A. 11 and the "related" O agents from sheep and cattle (isolated from abattoir offal of sheep and cattle in South Africa).

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Of the above agents, the calf diarrhea virus, which has been adapted to cell culture, has been more fully characterized. The recommendation that the calf agent be placed in the family Reoviridae was based on similarities in the following characteristics to those of recognized members of the family: general morphology, cytopathogenicity, resistance to lipid solvents, stability at pH3, relatively high thermostability, and double stranded RNA.

The recommendation that the virus causing acute gastroenteritis of children be placed in the family Reoviridae was based on similarities in morphology and morphogenesis of the virions to known species in the family. Specifically, double-shelled particles resembled reovirions, single-shelled particles resembled orbivirus particles, and the areas of “viroplasm” or “virus factories” in the infected cells were similar to those observed in reovirus and orbivirus infected cells.

Morphologically all the reovirus-like agents are similar. Typical virions are shown in Fig. 1. The particles consist of a hexagonal-shaped core having a diameter ranging from 30-36 nm from which radiate short cylindrical capsomeres. Reported diameters of these single-shelled virions range from 56-67 nm. Attached to the ends of the cylindrical capsomeres is a second or outer layer of capsomere which gives the virion a sharp outline. Reported diameters of these double-shelled virions vary from 73-87 nm. This outer layer of capsomeres is easily detached especially in sucrose gradients, thus the wide range of virion diameters in the literature may have resulted in part from measuring single or double-shelled particles.

Serologically, the reovirus-like agents are related. The calf diarrhea and human diarrhea virus have been shown to be antigenically related by the serum neutralization test, immune electron microscopy (IEM), direct and indirect immunofluorescence, and complement-fixation. By IEM, convalescent calf serum agglutinated single-shelled but not double-shelled human diarrhea virions, whereas convalescent human serum agglutinated both type particles. These results suggest that the outer capsid layer of the human diarrhea virus contains host specific antigen and the inner layer has antigen in common with the calf diarrhea virus. The S.A. 11 virus and “O” agents and EDIM by complement-fixation are antigenically related to the calf and human viruses. The porcine reovirus-like agent was shown to be antigenically related to the calf agent by immunofluorescence and neutralization tests.

Reovirus-like agents differ from the accepted species in the genera Orthoreovirus and Orbivirus in fine morphological structure, morphogenesis of the virions, serology, and in biochemical characteristics of the nucleic acids.

Morphologically, the double capsid reovirus-like virions have a more sharply defined outline than reovirions. The inner layer of capsomeres in the reovirus-like virions are elongated and promi-
REOVIRUS AND ROTAVIRUS INFECTIONS

whereas those on the reovirions are smaller and more spherical.1

The morphogenesis of the human diarrhea virus, EDIM, and S.A. 11 differs from the classical reovirus in that both naked and enveloped particles accumulate in rough endoplasmic reticulum vesicles.10,13

Serologically, the calf agent is unrelated to reovirus 1, 2, and 37,11,24 and to 20 orbiviruses11 including bluetongue virus.7,8,11

The calf diarrhea reovirus-like agent and reovirus differ by polyacrylamide gel electrophoretic analysis in the number and banding patterns of the RNA segments in the genomes. The calf diarrhea genome consists of 11 segments of double stranded RNA whereas the reovirus genome consists of 10 segments.1,19

In light of the close relationship of the calf diarrhea virus, human diarrhea virus, porcine diarrhea virus, EDIM, S.A. 11 virus, and the “O” agents, and the above differences between the reovirus-like agents and viruses in the genera Orthoreovirus and Orbivirus, the establishment of a new genus for the reovirus-like agents has been recommended. Rotavirus8 was proposed by the English workers to emphasize the wheel like appearance of the virion. The Australian workers have proposed the name Duovirus4 to describe the double-shelled capsid structure and to emphasize involvement in enteric infection (duodenum). The Australian group has recently stated that they will support the name Rotavirus. Rotavirus has historical precedence, therefore, its adoption would be in order. Designation of a genus for the reovirus-like agents is desirable so that references to these agents in the literature can be standarized.
Fig. 1. Calf diarrheal reovirus-like particles. Notice the hexagonal-shaped core, radiating cylindrical capsomeres and sharp outline of the virions.

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Bluetongue (BT) is an insect-borne virus disease of domestic and wild ruminants. The disease was first described in 1881 in South Africa by Hutcheon\textsuperscript{26} who called it “epizootic catarrh”. Spruell suggested in 1902 that the disease was caused by an intracorpusscular parasite or plasmodium\textsuperscript{46} but indicated in 1905 that the causative agent was a virus.\textsuperscript{47} Theiler\textsuperscript{52} in 1906 reported that the infectious agent was probably a virus when he demonstrated that the infectivity from infected sheep blood could be passed through a Berkefeld filter. The disease was thought to be confined to sheep until Bekker \textit{et al.}\textsuperscript{2} in 1934 in South Africa isolated BT virus from the blood of infected cattle with signs similar to those for foot-and-mouth disease. BT disease was known only in South Africa until the 1940's when it was recognized in Cyprus, Palestine, Syria, Turkey and the United States and by the 1950's in Spain, Portugal and West Pakistan.\textsuperscript{18}

BT virus was first isolated in the U.S. in 1952 by McKercher \textit{et al.}\textsuperscript{17} from sheep in California although the disease was thought to exist at least as early as 1948 in Texas.\textsuperscript{16} The first isolation of BT virus in the U.S. from cattle was made in 1959 by McCrory and Foster (unpublished) at the Denver Animal Disease Research Laboratory from a blood sample submitted from Oregon. BT virus has been isolated from sheep or cattle from most states west of the Mississippi River and from several states east of the Mississippi River. Serological evidence for BT virus antibody in ruminants has been reported for all but 4 states in the U.S.

The role of wild ruminants in the epizootiology of BT is uncertain. A summarization by Hourrigan and Klingsporn\textsuperscript{17} stated that individuals of certain species in the U.S., including bighorn sheep, white-tailed deer, mule deer and exotic zoo animals such as Reeve’s muntjac and greater kudu, have shown mild to severe signs of natural clinical BT accompanied by virus isolations. Other wild ruminants, including white-tailed deer, elk and pronghorn antelope in the U.S. and buffalo, blesbok and hog deer in Africa, have developed inapparent to mild to severe BT disease and viremia after experimental infection. In addition, serum antibody to BT virus has been detected for many species of wild and zoo ruminants. Although it is well documented that wild ruminants serve as recipient hosts in nature, it remains to be demonstrated that they can, in turn, serve as infected donors in the spread of BT disease.

\textit{Insect Vector.} Du Toit\textsuperscript{9} in 1944 in South Africa incriminated small biting flies of the genus \textit{Culicoides} (Diptera: Ceratopogonidae) as vectors of BT disease of sheep. Price and Hardy\textsuperscript{44} in 1954 collected females of \textit{C. variipennis} (Coquillett) in Texas, ground them, and inoculated two sheep that subsequently developed clinical BT. Foster
et al.\textsuperscript{14} in 1963 proved that \textit{C. variipennis} was a biologic vector of BT virus in sheep. Luedke \textit{et al.}\textsuperscript{13} in 1967 extended the role of \textit{C. variipennis} as a vector by showing biologic transmission of BT virus in cattle. Foster \textit{et al.}\textsuperscript{13} reported that attenuated BT virus (commercial vaccine, chicken embryo origin) was transmitted from vaccinated to nonvaccinated sheep by \textit{C. variipennis}. Serial transmission of the vaccinal virus from sheep to sheep was also shown; i.e., the recipient host in each test was used as the infecting donor of the next test, and so forth. The bite of a single infected fly was sufficient to transmit the virus. One infected fly can develop $10^{6}$-$10^{7}$ infectious BT virus particles within its body.\textsuperscript{15} Although other insects such as the sheep ked, \textit{Melophagus ovinus} (L.), may play a role in the transmission of BT,\textsuperscript{12} \textit{Culicoides} flies must be considered the principal, if not the only, vector. All members of the orbivirus genus, of which BT virus is the type species, multiply in arthropods as well as in vertebrates (Table 1).

\textbf{Stability.} The summarization in Table 2 illustrates that BT virus, which has been considered very stable when in blood,\textsuperscript{4,39} loses much of its stability when there is less extraneous protein to serve as a protectant. The stability of BT virus in blood $>$ in embryo supernate $>$ in partially purified embryo supernate $>$ in cell culture fluids + stabilizers $>$ in cell culture fluids $>$ in purified preparations from which all extraneous protein is removed. This relationship supports Svehag's work\textsuperscript{50} with BT virus-infected embryo supernates purified to different degrees in that rate constants calculated for the second phase of each two-component inactivation curve at 56°C were inversely related to the nitrogen content. The disparity of the results in Table 2 for the stability of BT virus to slow freezing may not be an anomaly because conditions involved for temperature and BT virus-infected preparations were different. Svehag\textsuperscript{50} further found that inactivation at higher temperatures (46-56°C) was associated with marked changes in enthalpy and entropy compatible with protein inactivation, whereas the thermodynamic data at a lower temperature range (37-46°C) suggested RNA inactivation.

Several authors have reported on the solvent resistance of orbiviruses, e.g. BT,\textsuperscript{4,48,51} EHD\textsuperscript{3} and Ibaraki\textsuperscript{26} and on their lability in acid.\textsuperscript{3,26,40,51} The summary in Table 3, after the work of Borden \textit{et al.},\textsuperscript{3} shows that BT, EDH and Ibaraki viruses, in contrast to the toga-viruses but similar to the reoviruses, are resistant to lipid solvents. Although most authors are in agreement that inactivation of BT virus occurs at pH $< 6$, lability at the higher pH values is less defined. Svehag \textit{et al.}\textsuperscript{51} reported that BT virus was stable in Michaelis buffer of pH 6 to 8 but was directly inactivated outside of this pH range. Verwoerd\textsuperscript{56} found that BT virus was most stable at a pH of 9.0 in a low salt concentration, 0.002 M Tris-HCl. Verwoerd also found that much of the resistance to lipid solvents including deoxycholate is reduced when BT virus is purified, although the resistance
is still relatively high when compared with that of the "typical"
arboviruses (togaviruses).

**Morphology.** In 1966, Bowne and Jones,\(^5\) Owen and Munz\(^4\) and
Studert *et al.*\(^9\) published electron micrographs of BT virus. Owen
and Munz, who observed primary lamb kidney (PLK) cell-adapted
virus in negative stain, found virions either with or without a distinct
envelope with an average diameter of 100 and 60 nm, respectively.
They further presented evidence for an icosahedral shape. Studert
*et al.* estimated BT virus to have a diameter of 53 nm in negative
stain and observed that the virus was hexagonal, presumably icosa-
hedral, in shape; morphological features resembled those of reo-
viruses. Bowne and Jones observed BT virus in the salivary gland
of the insect vector, *C. variipennis*, and found nonenveloped virions
within lighter staining discrete masses in the cytoplasm and mature-
appearing enveloped virus at the periphery of the mass.

Ritchie and Bowne\(^6\) examined BT virus grown in PLK cells and
concluded that the virus architecture was more complex than pre-
viously suspected. In thin section, they found two characteristic types
of particles. Core particles with diameters of about 55 nm and sharp
hexagonal transverse profile were the most frequently encountered
and apparently corresponded to the particles described by Studdert
*et al.*\(^9\) The second characteristic particle type was > 70 nm and con-
sisted of the core particle surrounded by a peripheral shell and outer
envelope. These "mature" particles were noted in the extra-cellular
space or at or near the plasma membrane. Ritchie and Bowne\(^6\) via
negative stain found particles variable in size and scattering patterns.
They recognized hollow pentagonal morphological units and reovirus
symmetry on particles < ca. 55 nm. Those particles with diameters
of 55-65 nm often showed fine surface projections that represented
structure units of the virion's outer shell. Particles 60-65 nm in
diameter were generally amorphous, presumably due to enmeshing
of their numerous surface projections. Those > ca. 65 nm were rarely
seen, and they appeared to be enveloped in a distensible membrane
covering whose surface was usually smooth contoured, but occasional-
ly bore sparse, uniform projections about 12-13 nm long. Ritchie and
Bowne believed that these large forms corresponded to "mature
virions" seen in thin sections. They further found that the larger
characteristic particles were not present in preparations previously
treated with fluorocarbons.

Lecatsas,\(^11\) in a study on the formation of BT virus in BHK 21
cells, stated that the virus essentially had no envelope. He found
evidence for the liberation of particles through breaks in the cell
membrane but none to indicate that budding of the cell membrane
liberated virus particles.

Els and Verwoerd\(^11\) found that negatively stained purified BT
virus consisted of a single layer of 32 capsomeres arranged in 5:3:2
symmetry; the size of the virion was ca. 54 nm and it had no envelope, although occasionally cellular membranes, termed "pseudo-envelopes", were found wrapped around one or more particles.

Bowne and Ritchie, working with BT virus grown in PLK cells, corroborated the complex architectural design of BT virions. They stated that BT virions existed as two principal types; unenveloped particles and the more complex membrane-bound forms. The reovirus-like core particles generally had an additional layer of fine surface projections that may in turn be covered by a membrane derived from the host cell. The diameter of the smallest unenveloped forms with typical reovirus symmetry was 45-55 nm, that of the most common particles resembling cockleburs and with indistinct reovirus symmetry was 60-70 nm, and that of the largest unenveloped forms with no reovirus symmetry was 70-80 nm. These authors stated that envelopment occurred by budding of the virus particles into the interior of a cytoplasmic vesicle or out through the plasma membrane. The membranous envelope had the structure of a trimolecular unit membrane; no evidence was obtained that these membranous envelopes were altered.

Murphy et al., in a comparative study on the orbivirus genus concluded that BT virus propagated in BHK 21 cells was nonenveloped and had a mean diameter of 68 nm. Cromack et al. found via negative stain and in thin section that BT virus from Madin-Darby bovine kidney cells had a diameter of 65 nm and had no envelope. They further observed that BT virus appeared to be extruded from the cell as it was made and that there was no evidence for virus release by budding from the plasma membrane.

Martin and Zweerink isolated two distinct types of particles during purification of BT virus obtained via L cells. The first type, BTV L (light), had the greater specific infectivity and had a diameter of 69 nm with a poorly defined surface. This first type corresponded to Bowne and Ritchie's most common particle (Table 4). Their second type, BTV D (dense), with a lower specific infectivity had a diameter of 63 nm and 32 capsomeres clearly visible on the surface. This type corresponded to Bowne and Ritchie's uncommon particle (Table 4).

Tsai and Karstad determined the diameter of EHD virus in thin section to be 58-62 nm. Murphy et al. and Thomas and Miller found EHD virus to be indistinguishable morphologically from BT virus and to be nonenveloped. Thomas and Miller found EHD virus via negative stain to have a diameter of 53 nm and Murphy et al. found in thin section a diameter of 62 nm. Thomas and Miller via thin section observed virus to bud into cytoplasmic vacuoles or extracellular spaces with the retention of a closely applied host cell membrane component.

Ito et al. via negative stain found that Ibaraki virus was ca. 55
nm in diameter, had no envelope, but had icosahedral configuration and a capsid consisting of 32 capsomeres arranged in 5:3:2 symmetry. Occasionally, one or more virions in a pseudo-envelope were observed. In thin section, they observed unenveloped core particles and occasionally more complex membrane-bound forms. They found that a limiting unit membrane envelope around the virus was acquired during release by budding from cell membranes.

**Physical parameters.** Polson made the first measurements on the virus particle size in 1948. He determined by methods of ultrafiltration that both virulent virus from sheep and egg-adapted virus had diameters of 100-150 nm. Ultracentrifugal methods gave a diameter of 108-133 nm for the virulent virus and 128 nm for the egg-adapted virus. Polson gave 1.15 as the buoyant density in sucrose and 1.11 in serum albumin. These measurements infer that Polson's viruses were enveloped or membraned.

Verwoerd reported that 80% of infective virus remained attached to the cell fraction that, on extractions with fluorocarbon (Frigen 113, Hoechst Chemical Co.) followed by isopycnic banding in a CsCl density gradient, yielded two bands with densities of 1.18 and 1.26. He found that the band with a density of 1.26 contained most of the infectivity, which when negatively stained and examined in the electron microscope consisted of impure virus associated with cellular material. Verwoerd found that treatment of the 1.26 band of virus with Tween 80 and ether converted the infectivity to a density of 1.38. This change in density indicated that the contaminating material was lipid in nature. Analytical ultracentrifugation revealed heterogeneity; however, by the method of Polson and van Regenmortel, Verwoerd found a sedimentation coefficient (s-rate) of 650 S for the faster moving component. Verwoerd et al. after purification of BT virus by extractions with fluorocarbon, Tween 80 and ether followed by moving zone procedures, found two infectivity bands with buoyant densities of 1.38 and 1.39 in CsCl density gradients. They calculated according to the method of McEwen that the 1.39 and 1.38 density bands of virus had s-rates of 520 S and 750 S, respectively.

Martin and Zweerink purified BT virus from Freon-extracted cell homogenates by moving zone centrifugation in sucrose gradients followed by isopycnic banding in CsCl gradients. They found two infectivity bands at densities of 1.38 (BTV D: s-rate = 470 S) and 1.36 (BTV L: s-rate = 550 S). They found the specific activity of BTV L, which corresponds to Bowne and Ritchie's most common particle (Table 4), to be 10- to 100-fold higher than the core particle, BTV D. A comparison of these two virus types is given in Table 5.

**Virus structure.** BT virus consists of ca. 80% protein and 20% ds RNA. Electron microscopy by Els of BT virus in protein monolayers spread on urea showed that each virion liberated an average
of about six fragments although he occasionally observed up to 10 fragments. Els found a large variation of filament lengths with an average of 3.5 nm total composite length and with a maximum of 6.5 nm to give an estimated RNA molecular weight (MW) of $13.6 \times 10^6$. In addition, Tsai and Karstad$^{55}$ obtained evidence that EHD virus contained linear filaments of dsRNA with a total MW of $12.2-15.1 \times 10^6$.

Verwoerd$^{56}$ reported that nucleic acid extracted from purified BT virus yielded three peaks (10S, 12S, 14-16S) representing three size classes identical to reovirus RNA in moving zone centrifugation through sucrose. Verwoerd et al.$^{60}$ with improved resolution found five peaks of 8.5, 10, 12, 14 and 15.5 S in sucrose gradients corresponding to MW's of 0.5, 0.8, 1.5, 2.3 and 3.0 $\times 10^4$, respectively. These authors resolved BT virus RNA into 10 components by polyacrylamide gel electrophoresis (PAGE) in which the fractionation pattern was similar, but not identical, to that obtained for reovirus (Table 6). The ten peaks are not as clearly grouped into three size classes as reovirus RNA but correspond more closely to the five components in sucrose gradients.$^{60}$ The MW's of BT virus RNA segments one through 10 obtained by Martin and Zweerink$^{35}$ and Verwoerd et al.$^{58}$ via PAGE are given in Table 6; their results were equivalent, with the possible exception of genome segment 1. Verwoerd et al.$^{59}$ estimated a MW of $15 \times 10^6$ for the viral nucleic acid. Verwoerd$^{56}$ found a marked similarity between the base compositions of BT and reoviruses. This similarity between BT virus and reovirus and two other dsRNA viruses, one of plant origin and the other of insect, is shown in Table 7. The 42-44% guanine + cytosine content of these RNA viruses is similar to the 40-44% found for DNA of all chordates.

Martin et al.$^{34}$, Martin and Zweerink$^{35}$ and Verwoerd et al.$^{58}$ via PAGE described seven polypeptides corresponding to seven of the 10 dsRNA genome segments (Table 6). These authors found five polypeptides associated with the core particle and two comprising the outer projections that form the outer diffuse layer (Table 8). De Villiers$^8$ compared polypeptide MW's of different BT virus serotypes and found the results in agreement with those of molecular hybridization studies of the genome segments; these results suggested that the main determinants of serological specificity reside in the two polypeptides that form the outer coat of the virus.

_Macromolecular synthesis._ Huismans$^{21}$ found that both virus-specific ssRNA and dsRNA were synthesized during infection and that ssRNA was formed in large excess over the dsRNA. The rate of RNA synthesis was maximum between 10 and 13 hours after infection. He found that the ssRNA was in association with the polyribosomes and consisted of components with s-rates varying between 12 and 22 S. Hybridization of ssRNA with dsRNA indicated that the ssRNA was probably mRNA.
Huismans\textsuperscript{22} found no inhibition of cellular RNA before 7 hours after infection of L cells with BT virus. The length of the lag phase before inhibition of protein synthesis was initiated was dependent upon the number of infecting particles; i.e., an increase in the multiplicity of infection resulted in a decrease in the length of the lag phase.

Martin and Zweerink\textsuperscript{15} and Verwoerd and Huismans\textsuperscript{29} determined that BT virus, converted to the high density form by removal of the two polypeptides that form the outer diffuse layer, had RNA-dependent RNA polymerase (transcriptase) activity. These authors elicited no transcriptase activity \textit{in vitro} with the lighter, more common particle that contained all seven polypeptides. This enzyme, which can be assayed by its ability to incorporate nucleoside triphosphates into RNA in an \textit{in vitro} system, produced 10 ssRNA segments that were hybridized with dsRNA isolated from BT virus.\textsuperscript{59} These authors showed that the hybridization product, analyzed by PAGE, was indistinguishable from a hybrid obtained with BT virus mRNA isolated from infected cells. They deduced that the BT virus genome was transcribed fully \textit{in vitro} and \textit{in vivo} by an enzyme present in the virus capsid. Huismans and Verwoerd,\textsuperscript{29} by means of hybridization experiments, determined that the relative frequency of transcription of the respective 10 genome segments remained constant during the course of infection. These authors indicated that specific regulation was at the level of transcription. The similarity in molar ratios for mRNA synthesized \textit{in vitro} and \textit{in vivo} (Table 9) indicated that this control was a property of the virus itself.

Huismans and Howell\textsuperscript{23} hybridized \textsuperscript{3}H-labeled mRNA of different BT virus serotypes with saturating amounts of \textsuperscript{32}P-labeled dsRNA for each serotype. Analysis of the cross-hybridization products by PAGE indicated relatively large differences between the various serotypes. These authors found complete homology in only a few of the genome segments in the different serotypes. They found that each strain gave a unique hybridization pattern and that each pattern showed different degrees of homology between the genomes of the different serotypes. Huismans and Howell further showed that the immunological specificity of the serotypes was determined mainly by genome segment two of the virus, whereas segment six appeared to have a secondary influence. Cross-hybridization between virulent and attenuated strains of the same serotype also indicated small differences. Genome segments two and six code for the two polypeptides found in the outer diffuse layer of the virus (Table 8).

\textit{Soluble antigen.} Several investigators have reported on the serotype specificity of the virus-serum neutralization test and on the group specificity of the complement fixation, gel precipitin and fluorescent antibody tests in which an antigenic component is common to all strains of BT virus; e.g., see reviews by Bowne,\textsuperscript{4} and Howell
and Verwoerd.\textsuperscript{19} Kipps,\textsuperscript{30} utilizing centrifugation and diffusion followed by assay via complement fixation titers, found that the non-infective soluble antigen extracted from BT virus-infected suckling mouse brains behaved as spherical particles of about 8 nm in diameter. Jochim and Chow,\textsuperscript{32} in immunodiffusion tests, determined that the noninfective soluble antigen isolated from BT virus-infected PLK cell cultures had a MW of 160,000 to 200,000. Wang et al.\textsuperscript{61} estimated the soluble antigen to have a MW of 208,000 and to be immunologically identical to one component of the virus particle.

<table>
<thead>
<tr>
<th>Serological subgroup</th>
<th>Host</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluetongue</td>
<td>Ruminants</td>
<td>Culicoides (punkies, no-see-ums, and sand flies [not to be confused with the phlebotomine sand flies])</td>
</tr>
<tr>
<td>Epizootic hemorrhagic disease of deer</td>
<td>Ruminants</td>
<td>Culicoides</td>
</tr>
<tr>
<td>African horse sickness</td>
<td>Horses</td>
<td>Culicoides</td>
</tr>
<tr>
<td>Palyam</td>
<td>Mammals</td>
<td>Mosquitoes and Culicoides</td>
</tr>
<tr>
<td>Eubenangee</td>
<td>Mammals</td>
<td>Mosquitoes</td>
</tr>
<tr>
<td>Corriparta</td>
<td>Mammals, birds</td>
<td>Mosquitoes</td>
</tr>
<tr>
<td>Chanquinola</td>
<td>Mammals</td>
<td>Phlebotomus (sand flies)</td>
</tr>
<tr>
<td>Kemerovo</td>
<td>Mammals</td>
<td>Argasidae (soft-bodied) and Ixodidae (hard-bodied) ticks</td>
</tr>
<tr>
<td>Colorado tick fever</td>
<td>Rodents and man</td>
<td>Dermacentor (a hard-bodied tick)</td>
</tr>
<tr>
<td>Ibaraki</td>
<td>Cattle</td>
<td>Unknown</td>
</tr>
<tr>
<td>Wound tumor</td>
<td>Sweet clover, etc.</td>
<td>Grasshoppers</td>
</tr>
<tr>
<td>Rice dwarf</td>
<td>Rice plant</td>
<td>Leafhoppers</td>
</tr>
</tbody>
</table>

Adapted from Fenner et al.\textsuperscript{12} and Verwoerd\textsuperscript{57}
<table>
<thead>
<tr>
<th>BTV-infected preparation</th>
<th>Storage</th>
<th>Infectivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Room temperature for 25 years</td>
<td>Remained viable</td>
<td>(39)</td>
</tr>
<tr>
<td>Blood</td>
<td>4 C for 1 year</td>
<td>No appreciable loss of titer</td>
<td>(4)</td>
</tr>
<tr>
<td>Blood</td>
<td>4 C for several years</td>
<td>Stable: ~50% loss of titer</td>
<td></td>
</tr>
<tr>
<td>Chicken embryo supernate diluted 1:1 in phosphate buffer, pH 7</td>
<td>5 C for 1 month</td>
<td>~50% loss of titer</td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td>5 C for 3 years</td>
<td>~100% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 C for 1 month</td>
<td>~40% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 C for 3 years</td>
<td>99% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~27 C for 1 month</td>
<td>&lt;50% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~27 C for 3 years</td>
<td>95% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~70 C for 1 month</td>
<td>&lt;50% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~70 C for 3 years</td>
<td>~50% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~70 C for 1 year</td>
<td>~95% loss of titer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~70 C for 3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken embryo supernate (CES)</td>
<td>4 C for up to 1 year</td>
<td>Relatively stable</td>
<td>(4)</td>
</tr>
<tr>
<td>CES partially purified by treatment with fluorocarbon</td>
<td>4 C for up to 1 year</td>
<td>Relatively unstable</td>
<td></td>
</tr>
<tr>
<td>Cell culture</td>
<td>37C, —20C, &amp; —70C for 25 days</td>
<td>Extremely unstable</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td>4 C for 25 days</td>
<td>Relatively stable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Room temperature for 25 days</td>
<td>Intermediately stable</td>
<td></td>
</tr>
<tr>
<td>Cell culture + proteins</td>
<td>Above temperatures for 25 days</td>
<td>Improved stability</td>
<td></td>
</tr>
<tr>
<td>Purified from cell culture</td>
<td>&quot;None&quot;</td>
<td>Loss of stability</td>
<td>(56)</td>
</tr>
<tr>
<td>Blood, egg, cell culture, suckling mouse brain</td>
<td>Slow freezing at —10 to —20C</td>
<td>Unstable</td>
<td>(1,20,29)</td>
</tr>
<tr>
<td>Chicken embryo supernate</td>
<td>Slow freezing at —27 C</td>
<td>Stable</td>
<td>(50)</td>
</tr>
</tbody>
</table>
TABLE 3. Resistance of infectivity of selected arboviruses and reoviruses to some standard chemicals.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Orbiviruses</th>
<th>Caliciviruses</th>
<th>Rhabdoviruses</th>
<th>Reoviruses</th>
<th>Togaviruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether (lipid solvent)</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Chloroform (lipid solvent)</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Deoxycholate (detergent)</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Acid: pH 3</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Acid: pH 5-6</td>
<td>Susceptible</td>
<td>Susceptible</td>
<td>Susceptible</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Adapted from Borden et al.3
### TABLE 4. Bluetongue virus (BTV) morphology

<table>
<thead>
<tr>
<th>Source</th>
<th>Preparation</th>
<th>Description</th>
<th>Diameter</th>
<th>Membrane</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary LK cells</td>
<td>Negative stain</td>
<td>Icosahedral</td>
<td>60 nm</td>
<td>No</td>
<td>(41) 1966</td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Negative stain</td>
<td>Hexagonal &amp; icosahedral</td>
<td>100 nm</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><em>C. variipennis</em></td>
<td>Thin section</td>
<td>Less mature</td>
<td>53 nm</td>
<td>No</td>
<td>(49) 1966</td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>More mature</td>
<td>60 nm</td>
<td>No</td>
<td>(5) 1966</td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>Core particle</td>
<td>55 nm</td>
<td>No</td>
<td>(45) 1967</td>
</tr>
<tr>
<td><em>C. variipennis</em></td>
<td>Thin section</td>
<td>Core particle with a peripheral shell and outer envelope (mature particle)</td>
<td>&gt;70 nm</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>Core particle (reovirus symmetry)</td>
<td>&lt;55 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>Core particle with fine surface projections</td>
<td>60 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>Core particle with numerous surface projections</td>
<td>65 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>Core particle (uncommon)</td>
<td>65 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>BHK 21 cells</td>
<td>Thin section</td>
<td>Single layer of 32 capsomeres in 5:3:2 symmetry</td>
<td>54 nm</td>
<td>No</td>
<td>(31) 1968</td>
</tr>
<tr>
<td>BHK 21 &amp; L cells</td>
<td>Negative stain</td>
<td>Core particle</td>
<td>54 nm</td>
<td>No</td>
<td>(11) 1969</td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Thin section</td>
<td>Several core particles frequently found enclosed in a membrane</td>
<td>65 nm</td>
<td>No</td>
<td>(6) 1970</td>
</tr>
<tr>
<td>Primary LK cells</td>
<td>Negative stain</td>
<td>Core particle (uncommon)</td>
<td>65 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>BHK 21 cells</td>
<td>Negative stain</td>
<td>Core particle with a layer of fine surface projections (common)</td>
<td>65 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Bovine kidney cells</td>
<td>Thin section</td>
<td>Core particle with a thick overlay of fibrillar material (rare)</td>
<td>75 nm</td>
<td>No</td>
<td>&quot;Yes&quot;</td>
</tr>
<tr>
<td>(Madin-Darby)</td>
<td>Thin section</td>
<td>Core particles enclosed in a single membranous sac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L cells</td>
<td>Negative stain</td>
<td>Complex particle intrinsically enveloped</td>
<td>&gt;110 nm</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>BHK 21 cells</td>
<td>Negative stain</td>
<td>Particles identical to those described by Els and Verwoerd and Bowne and Ritchie</td>
<td>68 nm</td>
<td>No</td>
<td>(38) 1971</td>
</tr>
<tr>
<td>Bovine kidney cells</td>
<td>Thin section</td>
<td>Cubic symmetry</td>
<td>64 nm</td>
<td>No</td>
<td>(7) 1971</td>
</tr>
<tr>
<td>(Madin-Darby)</td>
<td>Thin section</td>
<td>Cubic symmetry</td>
<td>64 nm</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>L cells</td>
<td>Negative stain</td>
<td>BTV L (light) = Bowne &amp; Ritchie's most common particle</td>
<td>69 nm</td>
<td>No</td>
<td>(35) 1972</td>
</tr>
<tr>
<td>Bovine kidney cells</td>
<td>Negative stain</td>
<td>BTV D (dense) = Bowne &amp; Ritchie's uncommon particle</td>
<td>63 nm</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 5. Comparison of two types of purified nonmembraned bluetongue virus (BTV) particles.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Core particle with projections</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTV D (dense)</td>
<td>BTV L (light)</td>
<td></td>
</tr>
<tr>
<td>Band A</td>
<td>Band B</td>
<td></td>
</tr>
<tr>
<td>Density in CsCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.38</td>
<td>1.36</td>
<td>35</td>
</tr>
<tr>
<td>1.39</td>
<td>1.38</td>
<td>60</td>
</tr>
<tr>
<td>Sedimentation coefficient via moving zone centrifugation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>470 S</td>
<td>550 S</td>
<td>35</td>
</tr>
<tr>
<td>520 S</td>
<td>750 S</td>
<td>60</td>
</tr>
<tr>
<td>Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63 nm</td>
<td>69 nm</td>
<td>35</td>
</tr>
<tr>
<td>Specific infectivity ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

### TABLE 6. Number and molecular weights (MW's) of the RNA fragments and polypeptides of bluetongue (BT) and reoviruses (Major polypeptide components are underlined).

<table>
<thead>
<tr>
<th>Genome segment</th>
<th>RNA MW's x 10^-6</th>
<th>Polypeptide MW's</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT(58) BT(35) reo(58)</td>
<td>BT(58) BT(34,35) reo(58)</td>
</tr>
<tr>
<td>1 (L₁)</td>
<td>2.50 2.73 2.50</td>
<td>140,000 155,000 138,000</td>
</tr>
<tr>
<td>2 (L₂)</td>
<td>1.99 2.04 2.50</td>
<td>110,000 110,000</td>
</tr>
<tr>
<td>3 (L₃)</td>
<td>1.82 1.94 2.40</td>
<td>101,000 100,000 132,000</td>
</tr>
<tr>
<td>4 (M₁)</td>
<td>1.31 1.29 1.60</td>
<td>82,000 72,000 89,000</td>
</tr>
<tr>
<td>5 (M₂)</td>
<td>1.16 1.12 1.55</td>
<td>81,000</td>
</tr>
<tr>
<td>6 (M₃)</td>
<td>1.08 1.07 1.49</td>
<td>61,000 58,000</td>
</tr>
<tr>
<td>7 (S₁)</td>
<td>0.60 0.61 0.87</td>
<td>42,000 38,000 47,500</td>
</tr>
<tr>
<td>8 (S₂)</td>
<td>0.54 0.55 0.76</td>
<td>29,500 32,000 40,500</td>
</tr>
<tr>
<td>9 (S₃)</td>
<td>0.50 0.49 0.64</td>
<td></td>
</tr>
<tr>
<td>10 (S₄)</td>
<td>0.30 0.28 0.61</td>
<td>34,500</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Base</th>
<th>Bluetongue (Animal)</th>
<th>Reo (Human)</th>
<th>Polyhedrosis (Insect)</th>
<th>Rice dwarf (Plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>0.28</td>
<td>0.28</td>
<td>0.29</td>
<td>0.28</td>
</tr>
<tr>
<td>Guanine</td>
<td>0.22</td>
<td>0.22</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Cytosine</td>
<td>0.21</td>
<td>0.22</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Uridine</td>
<td>0.29</td>
<td>0.29</td>
<td>0.23</td>
<td>0.28</td>
</tr>
<tr>
<td>(A+G)/(U+C)</td>
<td>1.00</td>
<td>0.98</td>
<td>1.02</td>
<td>1.00</td>
</tr>
<tr>
<td>G+C</td>
<td>0.43</td>
<td>0.44</td>
<td>0.42</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Adapted from Verwoerd\textsuperscript{57}

TABLE 8. Location and relative amounts of each polypeptide in the bluetongue virion (BTV).

<table>
<thead>
<tr>
<th>Polypeptide (Equivalent genome segment)</th>
<th>Approx. molecular weight</th>
<th>Fraction of BTV protein</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>\textsuperscript{14}C(58)</td>
<td>Staining (34)</td>
</tr>
<tr>
<td>1</td>
<td>150,000</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>110,000</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td>3</td>
<td>100,000</td>
<td>0.16</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>80,000</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>60,000</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>7</td>
<td>40,000</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>8</td>
<td>30,000</td>
<td>0.35</td>
<td>0.29</td>
</tr>
</tbody>
</table>
TABLE 9. Molar ratios of BT virus mRNA synthesized *in vivo* (also mRNA isolated from polyribosomes in infected cells) and *in vitro*. (The relative frequency of transcription of the 10 genome segments remains constant during the time of infection).

<table>
<thead>
<tr>
<th>Genome segment MW x 10^-4</th>
<th>mRNA species</th>
<th>in vivo</th>
<th>in vitro</th>
<th>from polysomes</th>
<th>≈ integral rate of transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>1</td>
<td>0.040</td>
<td>0.040</td>
<td>0.044</td>
<td>1</td>
</tr>
<tr>
<td>1.99</td>
<td>2</td>
<td>0.052</td>
<td>0.042</td>
<td>0.052</td>
<td>1</td>
</tr>
<tr>
<td>1.82</td>
<td>3</td>
<td>0.045</td>
<td>0.038</td>
<td>0.047</td>
<td>1</td>
</tr>
<tr>
<td>1.31</td>
<td>4</td>
<td>0.075</td>
<td>0.066</td>
<td>0.063</td>
<td>2</td>
</tr>
<tr>
<td>1.16</td>
<td>5</td>
<td>0.173</td>
<td>0.182</td>
<td>0.157</td>
<td>5</td>
</tr>
<tr>
<td>1.08</td>
<td>6</td>
<td>0.073</td>
<td>0.070</td>
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Adapted from Huismans and Verwoerd24
REFERENCES


42. Polson, A. The particle size of bluetongue virus as determined by ultrafiltra-


46. Spruell, J. Report from veterinary surgeon Spruell on the result of his experiments with the malarial catarrhal fever of sheep. Agricultural Journal from the Cape of Good Hope. 20:469-477. 1902.


ANIMAL VIRUS CHARACTERIZATION COMMITTEE

Committee Members:
Chairman: S. McConnell
Co-Chairman: C. J. York

Members of the Committee on Animal Virus Characterization have actively participated in a number of activities during the past year. Several members attended the annual meeting of the World Health Organization - FAO Board of Comparative Virology to discuss virus taxonomy. Others met with the International Committee on Viral Taxonomy at the Virology Congress in Madrid, Spain, for similar purposes.

As a contribution of the committee members, a manuscript on animal reference viruses was published in the American Journal of Veterinary Research, July, 1975. The members express their appreciation to V. J. Cabasso for his efforts on this manuscript.

The committee sponsored, during this year's meeting, a program on the Reoviridae, a family of viruses currently of interest to the membership of the association because of their involvement in diarrheal diseases of a number of animal species and the production of bluetongue disease.

This unique family of viruses are double stranded RNA with a complex biophysical and biochemical character that requires extensive study. The subclassification of the family is unclear although several genera are tentatively established. These include the Orbiviruses of which bluetongue virus is the prototype, the Reoviruses which affect a broad range of animal species, and a closely related unnamed subgroup associated with virus diarrhea in calves, pigs, sheep, goats, and man.

The current information relating to the classification, ecology, epidemiology and characterization of this family of viruses will be appended to this report. We express deep appreciation to the participants whose expert knowledge contributed greatly to our committee efforts: Dr. Al Ritchie, Dr. C. A. Mebus, Dr. Neil Foster, Dr. B. Francy, and Dr. A. Dardiri.
1. Opening Remarks .................................................. S. McConnell

2. Introduction of Speakers ......................................... T. E. Walton
   a. Family Reoviridae ........................................... Al Ritchie
   b. Bluetongue Virus Characterization ....................... Neil M. Foster
   c. Colorado Tick Fever ......................................... Bruce Francy
   d. African Horse Sickness ...................................... A. Dardiri
   e. Reovirus and Rotavirus Infections ....................... C. A. Mebus

3. Summary .................................................................... C. J. York
80th ANNUAL MEETING
November 7-12, 1976
AMERICANA HOTEL
at Bal Harbour
Miami Beach, Florida

81st ANNUAL MEETING
October 16-21, 1977
RADISSON HOTEL, DOWNTOWN
Minneapolis, Minnesota

82nd ANNUAL MEETING
October 27-November 3, 1978
THE STATLER HILTON HOTEL
Buffalo, New York