SEVENTY-SECOND
ANNUAL MEETING
OF THE
UNITED STATES LIVESTOCK SANITARY ASSOCIATION
JUNG HOTEL
New Orleans, Louisiana
October 6, 7, 8, 9, 10, 11, 1968
PROCEEDINGS

SEVENTY-SECOND
ANNUAL MEETING

of the
UNITED STATES
LIVESTOCK SANITARY
ASSOCIATION

JUNG HOTEL
New Orleans, Louisiana
October 6, 7, 8, 9, 10, 11, 1968
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<tr>
<td>H. B. Stoenner</td>
<td>Hamilton, Montana</td>
</tr>
<tr>
<td>M. J. Twiehaus</td>
<td>Lincoln, Nebraska</td>
</tr>
</tbody>
</table>

**Committee on Livestock Markets and Transportation**

Dr. G. C. Stiles, Chairman, Jefferson City, Missouri

<table>
<thead>
<tr>
<th>Name</th>
<th>State/City</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. N. Butler, Jr.</td>
<td>Phoenix, Arizona</td>
</tr>
<tr>
<td>J. H. Brashear</td>
<td>Oklahoma City, Oklahoma</td>
</tr>
<tr>
<td>F. S. Lee</td>
<td>Brownlee, Nebraska</td>
</tr>
<tr>
<td>J. N. McDuffie</td>
<td>Atlanta, Georgia</td>
</tr>
<tr>
<td>M. D. Mitchell</td>
<td>South Dakota</td>
</tr>
<tr>
<td>E. E. Montgomery</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>F. W. Peterson</td>
<td>Omaha, Nebraska</td>
</tr>
<tr>
<td>A. G. Pickett</td>
<td>Topeka, Kansas</td>
</tr>
<tr>
<td>C. T. Sanders</td>
<td>Kansas City, Missouri</td>
</tr>
<tr>
<td>A. P. Schneider</td>
<td>Boise, Idaho</td>
</tr>
<tr>
<td>R. Schnell</td>
<td>Dickinson, North Dakota</td>
</tr>
<tr>
<td>F. J. Schoenfeld</td>
<td>Salt Lake City, Utah</td>
</tr>
<tr>
<td>D. H. Spangler</td>
<td>Olympia, Washington</td>
</tr>
<tr>
<td>Ingvard Svarre</td>
<td>Sidney, Montana</td>
</tr>
</tbody>
</table>

**Committee on Mastitis – 1969**

Dr. K. J. Peterson, Chairman, Corvallis, Oregon

<table>
<thead>
<tr>
<th>Name</th>
<th>State/City</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. S. Bryan</td>
<td>Urbana, Illinois</td>
</tr>
<tr>
<td>Dr. J. C. Davidson</td>
<td>Hyattsville, Maryland</td>
</tr>
<tr>
<td>R. J. Farnsworth</td>
<td>St. Paul, Minnesota</td>
</tr>
<tr>
<td>R. S. Guthrie</td>
<td>Ithaca, New York</td>
</tr>
<tr>
<td>N. B. Haynes</td>
<td>Ithaca, New York</td>
</tr>
<tr>
<td>E. L. Henkel</td>
<td>Salem, Oregon</td>
</tr>
<tr>
<td>R. I. Hostetler</td>
<td>Pullman, Washington</td>
</tr>
<tr>
<td>H. G. Hodges</td>
<td>Poughkeepsie, New York</td>
</tr>
<tr>
<td>E. J. Kersting</td>
<td>Storrs, Connecticut</td>
</tr>
<tr>
<td>John McDonald</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>D. S. Postle</td>
<td>Madison, Wisconsin</td>
</tr>
<tr>
<td>R. J. Schroeder</td>
<td>South Gate, California</td>
</tr>
<tr>
<td>J. V. Smith</td>
<td>Hartford, Connecticut</td>
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</tbody>
</table>
### Committee on Meat and Poultry Hygiene - 1969

**Dr. W. E. Jennings, Chairman, Auburn, Alabama**  
**Dr. G. B. Estes, Co-Chairman, Richmond, Virginia**  

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
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<tbody>
<tr>
<td>A. Dewey</td>
<td>Chicago, Illinois</td>
</tr>
<tr>
<td>M. Sonnenberg</td>
<td>Sterling, Colorado</td>
</tr>
<tr>
<td>J. A. Killick</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>R. L. Maderia</td>
<td>Elizabethville, Pennsylvania</td>
</tr>
<tr>
<td>J. S. Stein</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>J. K. Payne</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>L. J. Rafoth</td>
<td>Chicago, Illinois</td>
</tr>
<tr>
<td>L. H. Burkert</td>
<td>St. Paul, Minnesota</td>
</tr>
<tr>
<td>M. R. Humphrey</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>E. D. Baker</td>
<td>Madison, Wisconsin</td>
</tr>
<tr>
<td>D. H. Spangler</td>
<td>Olympia, Washington</td>
</tr>
<tr>
<td>W. W. Sechnor</td>
<td>Little Rock, Arkansas</td>
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<tr>
<td>W. W. Sadler</td>
<td>Davis, California</td>
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<tr>
<td>J. A. Libby</td>
<td>St. Paul, Minnesota</td>
</tr>
<tr>
<td>D. C. Kelley</td>
<td>Manhattan, Kansas</td>
</tr>
<tr>
<td>V. L. Dahl</td>
<td>Columbus, Ohio</td>
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### Committee on Nominations, Resolutions and Internal Affairs - 1969

**Dr. John F. Quinn, Chairman, Lansing, Michigan**  

<table>
<thead>
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<tr>
<td>C. L. Campbell</td>
<td>Tallahassee, Florida</td>
</tr>
<tr>
<td>G. J. Grennan, Jr.</td>
<td>Providence, Rhode Island</td>
</tr>
<tr>
<td>Grant S. Kaley</td>
<td>Albany, New York</td>
</tr>
<tr>
<td>J. W. Safford</td>
<td>Helena, Montana</td>
</tr>
<tr>
<td>A. P. Schneider</td>
<td>Boise, Idaho</td>
</tr>
<tr>
<td>K. F. Wells</td>
<td>Ottawa, Ontario, Canada</td>
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</tbody>
</table>

### Committee on Parasitic Diseases and Parasiticides

**Dr. W. C. Tobin, Chairman, Denver, Colorado**  

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
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<tbody>
<tr>
<td>D. W. Baker</td>
<td>Albuquerque, New Mexico</td>
</tr>
<tr>
<td>J. H. Brashear</td>
<td>Oklahoma City, Oklahoma</td>
</tr>
<tr>
<td>George L. Crenshaw</td>
<td>Davis, California</td>
</tr>
<tr>
<td>J. H. Hourigan</td>
<td>Hyattsville, Maryland</td>
</tr>
<tr>
<td>J. E. Kleck</td>
<td>Albuquerque, New Mexico</td>
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<tr>
<td>F. R. Koutz</td>
<td>Columbus, Ohio</td>
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<tr>
<td>H. B. McGrath</td>
<td>Kansas City, Missouri</td>
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<tr>
<td>M. D. Mitchell</td>
<td>Pierre, South Dakota</td>
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<tr>
<td>A. C. Newman</td>
<td>Opelika, Alabama</td>
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<tr>
<td>R. D. Radeleff</td>
<td>College Station, Texas</td>
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<tr>
<td>I. H. Roberts</td>
<td>Albuquerque, New Mexico</td>
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<tr>
<td>R. H. Singer</td>
<td>Bryan, Texas</td>
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<tr>
<td>D. E. Zinter</td>
<td>Beltsville, Maryland</td>
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### Committee on Pharmaceuticals - 1969

**Dr. G. T. Edds, Chairman, Gainesville, Florida**  

<table>
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<tr>
<td>D. J. Anderson</td>
<td>Ft. Worth, Texas</td>
</tr>
<tr>
<td>A. Freeman</td>
<td>Chicago, Illinois</td>
</tr>
<tr>
<td>K. L. Gabriel</td>
<td>Philadelphia, Pennsylvania</td>
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<tr>
<td>R. Gessart</td>
<td>Kalamazoo, Michigan</td>
</tr>
<tr>
<td>H. E. Gouge</td>
<td>St. Joseph, Missouri</td>
</tr>
<tr>
<td>D. F. Green</td>
<td>Rahway, New Jersey</td>
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<tr>
<td>H. L. Jones</td>
<td>Somerset, New Jersey</td>
</tr>
<tr>
<td>Fred Kingma</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>K. Mayer</td>
<td>Chicago, Illinois</td>
</tr>
<tr>
<td>R. D. Radleff</td>
<td>College Station, Texas</td>
</tr>
<tr>
<td>S. F. Scheidy</td>
<td>Philadelphia, Pennsylvania</td>
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### Committee on Public Relations and Local Arrangements - 1969

**Dr. W. E. Lyle, Chairman, Madison, Wisconsin**  

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
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<tbody>
<tr>
<td>W. L. Bendix</td>
<td>Richmond, Virginia</td>
</tr>
<tr>
<td>J. H. Brashear</td>
<td>Oklahoma City, Oklahoma</td>
</tr>
<tr>
<td>E. M. Ellis</td>
<td>Ames, Iowa</td>
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<tr>
<td>A. A. Erdmann</td>
<td>Madison, Wisconsin</td>
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<tr>
<td>Howard Obenchain</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>Norman Powers</td>
<td>Lake Luzerne, New York</td>
</tr>
<tr>
<td>J. C. Shook</td>
<td>Harrisburg, Pennsylvania</td>
</tr>
</tbody>
</table>

### Committee on Transmissible Diseases of Poultry - 1969

**Dr. H. E. Goldstein, Chairman, Columbus, Ohio**  

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
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<tbody>
<tr>
<td>Raleigh Allen</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>R. A. Bankowski</td>
<td>Davis, California</td>
</tr>
<tr>
<td>F. G. Buzzell</td>
<td>Augusta, Maine</td>
</tr>
<tr>
<td>L. C. Grumbles</td>
<td>College Station, Texas</td>
</tr>
<tr>
<td>J. E. Hanley</td>
<td>Dade City, Florida</td>
</tr>
<tr>
<td>R. Hogue</td>
<td>Lafayette, Indiana</td>
</tr>
<tr>
<td>A. E. Janawicz</td>
<td>Montpelier, Vermont</td>
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<tr>
<td>T. L. Landers</td>
<td>Chamblee, Georgia</td>
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<tr>
<td>S. A. Moore</td>
<td>Washington, D. C.</td>
</tr>
<tr>
<td>H. E. Nadler</td>
<td>Albany, New York</td>
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<tr>
<td>B. S. Pomeroy</td>
<td>St. Paul, Minnesota</td>
</tr>
<tr>
<td>W. Schofield</td>
<td>St. Louis, Missouri</td>
</tr>
<tr>
<td>J. B. Thomas</td>
<td>Columbia, South Carolina</td>
</tr>
<tr>
<td>J. W. Walker</td>
<td>Washington, D. C.</td>
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<tr>
<td>C. W. Wilder</td>
<td>Augusta, Maine</td>
</tr>
</tbody>
</table>
### Committee on Public Health and Radiological Fallout

- **Chairman**: Dr. Richard L. Parker, Atlanta, Georgia
- Members:
  - R. Fagan, Westchester, Pennsylvania
  - N. L. Meyer, Hyattsville, Maryland
  - A. B. Park, Washington, D.C.
  - I. M. Saturne, Ames, Iowa
  - Calvin Schwabe, Davis, California
  - J. H. Steele, Atlanta, Georgia
  - J. H. Stewart, Washington, D.C.
  - E. E. Wedman, Ames, Iowa
  - R. D. Wenger, Alexandria, Virginia
  - Robert Singer, College Station, Texas

### Committee on Rabies – 1969

- **Chairman**: Dr. J. C. Shook, Harrisburg, Pennsylvania
- Members:
  - L. N. Butler, Jr., Phoenix, Arizona
  - E. A. Cabrey, Ames, Iowa
  - D. W. Dressen, Atlanta, Georgia
  - O. D. Dye, Atlanta, Georgia
  - Robert Singer, College Station, Texas
  - D. E. Lundholm, Reno, Nevada
  - E. R. Quortrup, San Diego, California
  - F. B. Schnurrenberger, Springfield, Illinois
  - K. Sikes, Lawrenceville, Georgia

### Committee on Regulatory Veterinary Medical Resources – 1969

- **Chairman**: Dr. J. G. Milligan, Montgomery, Alabama
- Members:
  - C. E. Boyd, Columbia, South Carolina
  - E. L. Brower, Trenton, New Jersey
  - J. R. Hay, Chicago, Illinois
  - D. L. Houston, Washington, D.C.
  - T. A. Ladson, College Park, Maryland
  - M. D. Mitchell, Pierre, South Dakota
  - F. J. Mulhern, Hyattsville, Maryland
  - J. F. Quinn, Lansing, Michigan
  - Glenn B. Rea, Salem, Oregon
  - K. F. Wells, Ottawa, Ontario, Canada
  - F. B. Wheeler, Baton Rouge, Louisiana

### Committee on Diseases of Sheep and Goats – 1969

- **Chairman**: Dr. M. D. Mitchell, Pierre, South Dakota
- **Co-Chairman**: Dr. J. L. Hourrigan, Hyattsville, Maryland
- Members:
  - C. C. Beck, East Lansing, Michigan
  - G. L. Crenshaw, Davis, California
  - W. J. Hadlow, Hamilton, Montana
  - W. A. Hickman, Pierre, South Dakota
  - Blaine McGowan, Davis, California
  - R. E. Simmons, Boise, Idaho
  - Matt Sutton, Onida, South Dakota
  - O. H. Timm, Dixon, California
  - Ward Van Horn, Buffalo, South Dakota
  - S. B. Walker, Austin, Texas

### Committee on Salmonellosis – 1969

- **Chairman**: Dr. A. A. Erdmann, Madison, Wisconsin
- Members:
  - C. E. Boyd, Columbia, South Carolina
  - L. Boyd, Chicago, Illinois
  - William Dubbert, Washington, D.C.
  - W. C. Farrell, Hartford, Connecticut
  - J. R. Hay, Chicago, Illinois
  - W. E. Lyle, Madison, Wisconsin
  - J. G. Miller, Tifton, Georgia
  - J. E. Porter, Washington, D.C.
  - O. M. Ray, Washington, D.C.
  - J. H. Steele, Atlanta, Georgia
  - J. H. Stewart, Washington, D.C.
  - J. C. Wetzler, Phoenix, Arizona
  - S. T. Wilson, Hyattsville, Maryland

### Committee on State-Federal Relations – 1969

- **Chairman**: Dr. Frank B. Wheeler, Baton Rouge, Louisiana
- Members:
  - W. L. Bendix, Richmond, Virginia
  - J. R. Hay, Chicago, Illinois
  - T. A. Ladson, College Park, Maryland
  - M. D. Mitchell, Pierre, South Dakota
  - John L. O’Harra, Reno, Nevada
  - Glen B. Rea, Salem, Oregon
  - J. C. Shook, Harrisburg, Pennsylvania
  - J. V. Smith, Hartford, Connecticut
OFFICERS AND COMMITTEES

Committee on Transmissible Diseases of Swine – 1969
Dr. D. P. Gustafson, Chairman, West Lafayette, Indiana
R. A. Bankowski, Davis, California
M. J. Barta, Chicago, Illinois
E. H. Bohl, Wooster, Ohio
H. W. Dunne, State College Pennsylvania
E. M. Dwyer, Boston, Massachusetts
James E. Fox, Ashland, Ohio
D. A. Fuller, Des Moines, Iowa
E. O. Haeltgerman, Lafayette, Indiana
Robert Hall, Madison, Wisconsin
J. B. Nance, Alamo, Tennessee
E. I. Pilchard, Urbana, Illinois
M. Ristic, Urbana, Illinois
N. E. Schulz, Hyattsville, Maryland
J. P. Torrey, Ames, Iowa

Committee on Tuberculosis and Paratuberculosis – 1969
Dr. Grant S. Kaley, Chairman, Albany, New York
V. H. Berry, Washington, D.C.
C. E. Boyd, Columbia, South Carolina
E. L. Brower, Trenton, New Jersey
Jack G. Frink, St. Paul, Minnesota
O. J. Halverson, Salem, Oregon
C. L. Henderson, East Lansing, Michigan
M. G. Hynes, Dublin, Ireland
D. S. Ingraham, Harrisburg, Pennsylvania
C. E. Kord, Nashville, Tennessee
A. B. Larsen, Ames, Iowa
H. Leikowitz, Buffalo, New York
A. E. Lewis, Ottawa, Ontario, Canada
A. R. McLaughlin, Madison, Wisconsin
W. L. Mallman, East Lansing, Michigan
Rolland Paul, Des Moines, Iowa
A. R. Ranney, Hyattsville, Maryland
A. P. Schneider, Boise, Idaho
R. M. Scott, Montpelier, Vermont
P. L. Smith, Sacramento, California
R. E. Sneddon, Denver, Colorado
W. D. Yoder, Ames, Iowa

Committee on Vesicular Diseases – 1969
Dr. E. C. Sharmar, Chairman, Bowie, Maryland
J. J. Callis, Greenport, L.I., New York
J. H. Graves, Greenport, L.I., New York
R. P. Hanson, Madison, Wisconsin
E. W. Jenney, Ames, Iowa
N. L. Meyer, Hyattsville, Maryland
L. O. Mott, Ames, Iowa
Frank B. Wheeler, Baton Rouge, Louisiana
R. J. Yedloutzchng, Greenport, L.I., New York

Committee on Import-Export
Dr. John F. Quinn, Chairman, Lansing, Michigan
Subcommittee on Domestic Animals and Birds:
Dr. H. G. Wixom, Chairman
Dr. C. L. Campbell, Tallahassee, Florida
Dr. Paul C. DeLay, Beltsville, Maryland
Dr. Frank Harding, Geneva, Illinois
Dr. James R. Hay, Chicago, Illinois
Dr. James B. Henderson, Austin, Texas
Dr. John R. Landridge, Hyattsville, Maryland
Mr. Robert Rumler, Brattleboro, Vermont

Subcommittee on Animal Products and Byproducts:
Dr. J. C. Shook, Chairman, Harrisburg, Pennsylvania
Dr. E. L. Brower, Trenton, New Jersey
Dr. J. J. Callis, Greenport, L.I., New York
Dr. Grant S. Kaley, Albany, New York
Dr. W. W. McMichael, Hyattsville, Maryland
Dr. A. R. Miller, Falls Church, Virginia
Dr. W. L. Sulzbacher, Beltsville, Maryland

Subcommittee on Wild and Endangered Species of Animals and Birds:
Dr. T. H. Reed, Chairman, Washington, D.C.
Dr. R. A. Bankowski, Davis, California
Dr. George E. Cottral, Greenport, L.I., N.Y.
Dr. L. J. Goss, Cleveland, Ohio
Mr. Gus Griswold, Philadelphia, Pennsylvania
Mr. R. E. Omohundro, Arlington, Virginia
Dr. John Richardson, Atlanta, Georgia
Dr. Charles C. Schroeder, San Diego, California
Dr. C. L. Smith, Hyattsville, Maryland
Dr. Robert Willson, Royal Oak, Michigan
PROPOSED AMENDMENT TO THE CONSTITUTION
AND BY-LAWS OF THE ASSOCIATION

The Board of Directors, as instructed by the Executive Committee, makes the following proposal for amendment to the constitution and by-laws:

Add, at the proper line following the individual membership provision, these words:

“Any individual member who has maintained membership in this association for 35 years, or if such member is at the point of retirement, for 25 years, may be elected to life membership by the Executive Committee. Such life membership shall carry with it all the rights and privileges of regular individual membership, including receipt of the Annual Proceedings of this Association. Such life membership shall be exempt from the payment of dues or any other assessments. All past presidents shall automatically become life members.

“Members of the Executive Committee will be eligible for such life membership; but for such member, the requirements for maintaining individual membership will be waived. But the period of time for such membership will be as herein provided.

“The Executive Committee may, at its discretion, confer honorary individual memberships. Such memberships shall be exempt from the payment of dues or other assessments and may be withdrawn at the discretion of the Executive Committee”. 
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sept. 27-28, 1897†</td>
<td>Fort Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Fort Worth, Texas</td>
</tr>
<tr>
<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, N. Y.</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>25. Nov. 28-29-30, 1921</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. F. Crewe, Bismarck, N. D.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>26. Dec. 6-7-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>27. Dec. 5-6-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Mont.</td>
<td>*Dr. O. E. Dyson, Kansas City, Mo.</td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Person</td>
<td></td>
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<tr>
<td>Dec. 2-3-4, 1925</td>
<td>Chicago, Ill.</td>
<td>Dr. J. H. McNeil, Trenton, N. J.</td>
<td></td>
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<tr>
<td>Nov. 30-Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>Dr. L. Van Es, Lincoln, Neb.</td>
<td></td>
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<tr>
<td>Dec. 5-6-7, 1928</td>
<td>Chicago, Ill.</td>
<td>Dr. C. A. Cary, Auburn, Ala.</td>
<td></td>
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<tr>
<td>Dec. 4-5-6, 1929</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 3-4-5, 1930</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 2-3-4, 1931</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 6-7-8, 1933</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 5-6-7, 1934</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 4-5-6, 1935</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 2-3-4, 1936</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 6-7-8, 1939</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
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<tr>
<td>Dec. 4-5-6, 1940</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
</tr>
<tr>
<td>Dec. 3-4-5, 1941</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
</tr>
<tr>
<td>Dec. 2-3-4, 1942</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
</tr>
<tr>
<td>Dec. 6-7-8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
</tr>
<tr>
<td>Dec. 5-6-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
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<tr>
<td>Dec. 4-5-6, 1946</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
</tr>
<tr>
<td>Dec. 3-4-5, 1947</td>
<td>Chicago, Ill.</td>
<td>Dr. O. E. Dyson, Wichita, Kan.</td>
<td></td>
</tr>
<tr>
<td>Oct. 12-13-14, 1949</td>
<td>Columbus, Ohio</td>
<td>Dr. Jean V. Knapp, Tallahassee, Fla.</td>
<td></td>
</tr>
<tr>
<td>Nov. 1-2-3, 1950</td>
<td>Phoenix, Ariz.</td>
<td>Dr. T. O. Brandenburg, Bismarck, N.D.</td>
<td></td>
</tr>
<tr>
<td>Sept. 23-24-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>Dr. Ralph L. West, St. Paul, Minn.</td>
<td></td>
</tr>
<tr>
<td>Nov. 10-11-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. Childs, Ottawa, Canada.</td>
<td></td>
</tr>
<tr>
<td>Nov. 28-29-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. H. F. Wilkins, Helena, Mont.</td>
<td></td>
</tr>
<tr>
<td>Nov. 13-14-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dr. G. H. Good, Cheyenne, Wyo.</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. 4-5-6, 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. 15-16-17-18, 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>65. Oct. 3-Nov. 1-2-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>Dr. R. A. Hendershott, Trenton, N. J.</td>
</tr>
</tbody>
</table>

§This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
INVOCATION

HARRY E. GOLDSTEIN, D.V.M.

Almighty God, our Heavenly Father, we beseech Thee to be present at this Annual Meeting of the United States Livestock Sanitary Association, assembled in Thy name and presence.

We desire to commence and to continue and to conclude our discussions and deliberations in the remembrance of Thine own existence.

We beseech Thee, also, to inspire all the members of the Association with right and true ideals of the commendable work in which they are engaged. Direct our Association in all its deliberation and help them to succeed in all their efforts for the good of their country and welfare of their fellow-man.

We beseech Thee to direct us in all our doings with Thy gracious favor, that we may glorify Thy Holy name in transacting the business of the Seventy-Second Convention of this Association.

Amen.
MEMORIAL SERVICE

H. E. Goldstein
Columbus, Ohio

President Quinn, Distinguished Guests
Ladies and Gentlemen -

We are assembled in this session to pay tribute to the memory of those members of this association who have endeared themselves to this association, and have passed on since our last meeting.

Mark Anthony, in his funeral oration for Julius Caesar, said “The evil that men do lives after them; the good is oft interred with their bones.” That is not true, my friends, I am happy to say. If it were, the world would be chaos today, because evil is destructive. It is the good which lives forever, and the evil which is forgotten. It is true the Scriptures tell us that “the sins of the father shall be visited upon his children even unto the third and fourth generation,” but they also say that “goodness and mercy endureth forever.”

No evil endures long; even the horrors of war are forgotten in a generation. But the good the men, such as the splendid men we are honoring today, accomplished in this world can never die.

And now let us go forth resolved that their lives have not been in vain; that the high principles which actuated them shall henceforth have an uplifting influence upon us so that the good which they did may endure forever.

“Let us not mourn their going;
Let us rejoice to know
That—earthly labors finished—
They were prepared to go

“Let us emulate their virtues,
That men of us may say:
The world is brighter, better,
Because they passed this way.”

So that the memory of our departed members may be recorded, let the record show:


MEMORIAL SERVICE


L. M. HURT — (Iowa State University - 1904) Died - October 2, 1967.


GRAYDON S. McKEE — (Ohio State University - 1933) Died - April 11, 1968.


JOSEPH E. SALSBURY — (Kansas City University - 1914) Died - December 1, 1967.


J. D. RAY — (Kansas City Veterinary - 1914) Died - October 5, 1968.

May we bow our heads for a moment of silent prayer. . . . . . . Amen

The only thing I can say in concluding is to repeat the words of Philip James Bailey:

“We live in deeds, not years; in thoughts, not breath;
In feelings, not in figures on a dial,
We should count time by heart-throbs.
He most lives who thinks most, feels the noblest,
acts the best.”
President Elect’s Message to the 72nd Annual Meeting of the United States Livestock Sanitary Association - New Orleans, Louisiana - October 9, 1968

John L. O’Harra, D.V.M.

President Quinn, Commissioner Pearce, Distinguished Guests, Ladies and Gentlemen...

I am pleased and greatly honored to have this opportunity as President Elect to address you in attendance at the 72nd annual meeting of this distinguished organization. We assure you good people of New Orleans and the Pelican State of Louisiana that your welcome has proved most sincere and we are happy to return and enjoy more of your southern hospitality that was so in evidence when the U.S.L.S.A. met here thirteen years ago in 1955. We thank you for the second time for being host to our meeting. I am also aware you have a sanctuary for the Ivory-billed woodpecker - it is good to know your heart is “for the birds”. We want you to know we are glad you were included in the Eleven Million Dollar deal we made back in 1803 when we bought you—you were worth it. Coming from the desert State of Nevada I also think you are very wasteful with water in allowing the Mississippi River to flow uninhibited into the Gulf of Mexico.

President Quinn, in behalf of the membership, I would like to commend you and thank you for the fine job you have done as President of this Association during the past year. You and the Presidents who have preceded you have set examples of leadership that are a real challenge to a President Elect to continue the high level of dedicated leadership in the U.S.L.S.A. I assure you the Committees, Officers and Membership will cooperate to the fullest extent to make this, your meeting, an outstanding one.

I would like to report to you that the Association is in good condition. Internal affairs associated with the change of the Secretary’s office after 25 years and other association matters have caused some confusion and concern but have been met and resolved to the best of the ability of those responsible.

This is the 72nd year of the U.S.L.S.A. About the time this association celebrates its centennial we will be turning the corner into the 21st Century. All agricultural practices will probably be beyond our dreams of today. Dynamic technological changes are imminent in all areas in which this association is concerned. We are moving in this direction rapidly at the present time but are only on the threshold. We must take advantage of all the new tools and techniques as rapidly as they are made available and encourage research at all times to give us new developments. It is past time we develop atomic age procedures to cope with the more complex problems resulting from inter-continental travel, physical inspection procedures, morbidity and mortality reporting, animal identification and the many other responsibilities that are ours. As a challenge—who will develop some means of “tagging” a herd where identification will be positive and instant by use of an electronic device.
DIAGNOSTIC LABORATORIES:

A vitally important group associated with this organization—The Conference of Veterinary Laboratory Diagnosticians have just completed their eleventh annual meeting. Their committee deliberations will come forth with recommendations for upgrading and setting minimum standards for all veterinary diagnostic laboratories, including physical requirements and personnel. I recommend the USLSA assist in devising and adoption of an official procedure for accreditation or registration of these laboratories in the near future. Certification of their capability should be mandatory in all categories and particularly where diagnostic determinations are a pre-requisite to interstate movement. I fear that too many laboratory determinations on animal specimens are questionable due to status, staff and physical facilities—particularly in our private laboratories.

We need your assistance, Dr. Saulmon, in a properly developed Animal Health Division reference laboratory or laboratories to "back up" and supplement our State agencies in the special categories we face occasionally and cannot be geared to handle. Reference assistance should also include specially trained personnel to help cope with emergencies on a State or Regional level and facilities for special training of staff. Further reference to this problem will be forthcoming in the report of the State-Federal Relations Committee.

MANPOWER:

Some two years ago Dr. Mulhern, then serving as Director of the Animal Health Division of the Agricultural Research Service, seriously predicted that within twenty to twenty-five years some twenty and perhaps twenty-one major communicable diseases of cattle, swine, sheep and poultry will be eradicated. With the present rapid advances in preventive procedures and therapeutic measures, it looks like this goal will be crossed well before the target dates. We have Brucellosis about licked and will soon be on a maintenance basis and searching for the last infected animal.

We should have some research breakthroughs soon that will enable us to reach the point of total eradication of tuberculosis without a prolonged program. Perhaps this will be assisted with the adopted and proposed stepped up procedures in our present program.

Hog Cholera is on the way out and probably will be eradicated on schedule with the continuing support of the swine industry.

In the very near future it appears doubtful, with the exception of emergency or exotic disease outbreaks, that we will have any long drawn out and detailed cooperative State-Federal programs similar to the ones we are now in the areas of completing.

I would recommend the committee on Regulatory Veterinary Medical Resources be continued and they continue to survey and gather information upon which to base recommendations involving the utilization of veterinary manpower and services. I would charge this committee to be aware that consumer protection is the trend of the times. The consumer protection services will be making increasing demands for professional manpower, many of whom should be veterinarians. Animal products inspection must remain under veterinary supervision in order to
insure true protection to the consumer, if such is the desire and demand. I would charge the committee to become exceedingly knowledgeable in the need for manpower in research, in import surveillance and quarantine activities, welfare of Laboratory Animals and in all health categories. I believe we are in full agreement that we must make the most judicious use of the veterinarians available, particularly those who are oriented toward the programs mentioned and plan toward utilization of this limited number to the greatest advantage. The committee could well work toward recommendations involving training of a higher percentage of our veterinarians to carry on in the many requirements of scientific endeavor, other than small animal practice, and to utilize further the dwindling numbers of our large animal practitioners.

In addition, I would charge this committee with the responsibility of making recommendations leading to the upgrading of the many position categories where it is so sorely needed; a look at minimum qualifications for certain positions and recommended ways for adequate compensation for professional personnel in these responsible categories.

BOARD CERTIFICATION OF REGULATORY VETERINARIANS:

The regulatory veterinarian, I believe you will agree, is engaged in a specific discipline within the profession. This specialty, in the opinion of many, has reached a degree of responsibility demanding recognition and requiring guidelines for measurement of proficiency leading to certification by a responsible Board. I propose to appoint an ad hoc committee charged with the responsibility of developing proposals and guidelines for establishment of The American College of Regulatory Veterinary Practice.

This committee shall work closely with the Council on Public Health and Regulatory Veterinary Medicine, the Council on Education and the Advisory Board on Veterinary Specialties of the American Veterinary Medical Association. The American Veterinary Medical Association must be the parent organization of this college, but the USLSA must be the instigator. I recommend the Executive Committee grant carte blanche authority to this small group in developing this proposal. The committee shall work closely with the Board of Directors and will report fully to the Executive Committee of the USLSA. I believe time is in the essence and it is a responsibility of the USLSA to expedite the establishment of The American College of Regulatory Veterinary Practice. I further recommend that the committee be retained until its function is completed, then be dismissed.

MEMBERSHIP:

Members of this Association, I would like to refer you to page 15 of the Report of the 70th Annual Meeting of the United States Livestock Sanitary Association where our Past President, Grant Kaley, referred to the hypothetical situation of Joseph L. Doaks and the United States Livestock Sanitary Association. Gentlemen, poor old Joe is still with us and he is in no better shape today than he was in 1966. There are potentially thousands of these Joe Doaks in the many endeavors that are too numerous to mention, all of which you people know. The individual members are the backbone of our financial structure and our organization’s strength.
Particularly so when they attend our Annual Meetings and pay their registration fees. Inspiration and information should come from the Secretary’s office, numerous times throughout the year. Our annual meeting, one of the finest in existence for industry and scientific people to meet on a common level, should receive promotional activities immediately following the close of the preceding meeting and continue throughout the year. The invitations to attend should be warm and welcome and the highlights of the meeting should be brought to attention numerous times throughout the year. The social activities in the various areas that we have all learned to enjoy, should be highlighted and the ladies by all means, issued a warm invitation to attend with their husbands. I urge the Committee on Public Relations to actively engage in a sincere campaign to increase the membership of this association by all means that are practical.

I firmly believe that unless the membership attitude of this association is improved, the future of the United States Livestock Sanitary Association could be in doubt.

CONSTITUTION AND BY-LAWS CHANGE:

At the 71st Annual Meeting of this Association held last year in Phoenix, Arizona, the proposal was initiated for changing the name of this association to the United States Animal Health Association. I urge that we move on into greater challenges as the United States Animal Health Association, which denotes being sound in body and free from diseases by any means.

CONCLUSION:

Many of the sound policy recommendations of this association will appear in the State-Federal Relations Committee report to be given later in the meeting. I would urge special attention be given to the remarks on import-export procedures, National Animal Diagnostic Reference Laboratory, welfare of laboratory animals program, a meaningful disease reporting system, research, veterinary biologics and meat inspection, as setforth in this committee report.

The record of accomplishments of this association have been achieved by the exchange of ideas and cooperation of the state and federal animal health agencies, veterinary practitioners, veterinary educators, agricultural extension personnel, research workers in all levels, our diagnostic laboratories, the industry, whether it be producer, processor or marketing agency and a long line of etc., etc., etc. The programs and policies of this association must have the cooperation and support of all these groups to move forward.

I look forward to this next year with humility. I hope that I can satisfactorily discharge the responsibility that goes with the office of President of this Association. Thank you.
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

REPORT OF THE SECRETARY-TREASURER FOR 1968

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

I present here the first annual report of the incumbent Secretary and Treasurer. This will constitute mainly the Treasurer's report and a statement of our financial condition for the fiscal year prepared by our auditor, Mr. Henry H. Budd.

Following the close of the 1967 meeting in Phoenix, the new Secretary journeyed first to Baton Rouge, Louisiana, and then to New Orleans, in company with Doctor Wheeler, to begin arrangements for the 1968 meeting here at the Jung during this week. As the Secretary was on vacation and he had intended to visit some friends in Florida for some fishing anyway, this was accomplished at no cost to the Association. Early in December of 1967, the President and the Secretary met with Doctor Hendershott in Trenton, where the liquid assets of the Association were turned over to the new Treasurer and the President. At that time also, the Addressograph plates that are the property of the Association were received by the Secretary, along with an IBM typewriter, a small adding machine, and a tape recorder. The new Secretary also received a set of bound books in which the previous Treasurer had been keeping receipts and disbursements. No other correspondence or records of the Association were made available. The Addressograph machine itself was stated to be the property of the previous Secretary and therefore was not transferred.

The Association's offices were transferred to Richmond and for a brief period occupied quarters in the same suite occupied by the Secretary as State Veterinarian of Virginia, at 1444 East Main Street. Early this year, the offices were moved to a more commodious two-room suite in the same building but entirely separate and to itself, and the Association still maintains the same mailing address. At first it was thought that the Association would negotiate a lease with the State government, which owns the building; but as this building is slated for removal to make way for a new east-west expressway within the city, the Division of Engineering and Buildings of the State government kindly permits the Association to use these offices rent-free until such time as the building is demolished, which should be within the next year or two. Pending the selection of a permanent office, we have refrained from purchasing any furniture and are now using desks, chairs, etc., that are the property of the State. We have, however, purchased one table for the sum of $12 and a Remington electric typewriter. The IBM typewriter received from the previous Secretary was not in working condition when it was received, and the IBM people stated that it was not worth repairing. We switched to the Remington machine because it does everything the IBM would do and was roughly $90 cheaper.

During the middle of December, Mrs. Robert D. Blanton was employed as office secretary. She and the Secretary-Treasurer set out to try to understand the workings of the Association and the operations of the office of its Secretary and

xxv
Treasurer. We know we have made many mistakes, but we are learning, and we have certainly tried.

It will be seen from the financial statement at the end of this report that $3,295.59 was spent in traveling. As our Association has indicated it wished, it has been represented at various meetings by our President or someone selected by the President to represent us. The Secretary wishes to compliment our President for his energy and the time he has given to representing the Association at these meetings. There is no question that this has improved not only our image but our working relationship with these organizations. The Secretary also wishes to compliment the other officers for their willingness to move in at short notice to represent us where the President found he was unable to do so. They have, without exception, accepted these assignments unhesitatingly.

As nearly as the Secretary can figure from the Addressograph plates and the tape list furnished by the previous Secretary, we have 1,166 members. Of these, 860 have paid their 1968 dues; 306 have not. Presently pending are applications for membership for 39 individuals, who have paid their $10 annual dues; and we have two applications for allied organization membership as provided by the new Constitution. The first one is from the American Veterinary Medical Association, which has indicated that it wishes to have Dr. James R. Hay represent it on our Executive Committee. The second application is from the Competitive Livestock Markets Council, and they have indicated that they wish Mr. C. T. Sanders to represent them on our Executive Committee. All of these applications are in order, and it is hoped the Executive Committee will accept them into membership during this meeting.

The Secretary has this year departed from the usual format for our printed program, and he hopes the change meets with your approval. He will welcome any comments for further improvement or further change as you may wish. As the Association broadens the base of its influence and operations, more and more organizations devoted to the same ends that we are find their interests paralleling ours to an extent that it is to everyone's advantage for meetings to be held either together or at the same time and place. You will note on this year's program that in addition to our affiliate, the Conference of Veterinary Laboratory Diagnosticians, there are seven other organizations meeting here with us this week. In the future, there will be others. We already have a request from the Administrator of the Consumer and Marketing Service of the United States Department of Agriculture that this agency be provided a seat on our Executive Committee, and it has been suggested that they schedule the annual meeting of their meat inspection people in conjunction with this Association's annual meeting, rather than elsewhere. A further change in the format of our week-long proceedings certainly is inevitable. With the approval of the Board of Directors, we have made a beginning in trying a somewhat different approach to our literary program. We have not achieved this to any material degree at this meeting, but we hope to at the next. This change involves permitting each committee one literary paper on our general program in addition to the committee report, and a program progress report if necessary, with the proviso that the committee may have an additional literary paper presented at the committee meeting here, and then if it is considered worthy by the committee itself, it will be published in our annual Proceedings. As stated, we are making a
start in this direction this year, and we hope to extend it if it proves practical.

It is hoped that you will study the financial statement of the Association at the end of this report with care. Any member of the Association who wishes a copy of this may receive one at any time after the close of this meeting, and prior to the receipt of the printed Proceedings of the meeting, by requesting it from the Secretary.

The Secretary wishes to express his appreciation for the understanding and patience shown him during this, his first year in the office, and his especial appreciation for the courtesy and cooperation extended him by the other officers.

UNITED STATES LIVESTOCK SANITARY ASSOCIATION
1444 EAST MAIN STREET
RICHMOND, VIRGINIA 23219

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR PERIOD OCTOBER 1, 1967 THROUGH SEPTEMBER 30, 1968

CASH BALANCE, OCTOBER 1, 1967

<table>
<thead>
<tr>
<th>Bank</th>
<th>Location</th>
<th>Amount</th>
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</thead>
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<tr>
<td>First Trenton National Bank</td>
<td>Trenton, New Jersey</td>
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<tr>
<td>Trevose Savings and Loan Association</td>
<td>Morrisville, Pennsylvania</td>
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<tr>
<td>Michigan National Bank</td>
<td>Lansing, Michigan</td>
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<td>Sandia Savings and Loan Association</td>
<td>Albuquerque, New Mexico</td>
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<td>First Trenton National Bank</td>
<td>Trenton, New Jersey</td>
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$10,458.30

INCREASED BY CASH RECEIPTS:

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<td>Individual Dues</td>
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<td>Official Dues</td>
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<tr>
<td>Proceedings</td>
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<tr>
<td>Reprints</td>
<td>$920.50</td>
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<tr>
<td>Registration Fees</td>
<td>$6,495.00</td>
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<tr>
<td>Foreign Animal Disease Handbooks</td>
<td>$236.00</td>
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<tr>
<td>Interest Income</td>
<td>$1,259.14</td>
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<tr>
<td>Cost of Tours at Annual Meeting at Phoenix, Arizona</td>
<td>$3,310.00</td>
</tr>
<tr>
<td>Miscellaneous Revenue</td>
<td>$447.29</td>
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$27,836.83

TOTAL BEGINNING BALANCE AND RECEIPTS $38,295.13
### UNITED STATES LIVESTOCK SANITARY ASSOCIATION
1444 EAST MAIN STREET
RICHMOND, VIRGINIA 23219

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR PERIOD OCTOBER 1, 1967 THROUGH SEPTEMBER 30, 1968

**DECREASED BY CASH EXPENDITURES:**

<table>
<thead>
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<td>Annual Meetings</td>
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<td>Social Security Tax</td>
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<td>Communications</td>
<td>$1,126.73</td>
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<tr>
<td>Travel:</td>
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<tr>
<td>Dr. John F. Quinn, President</td>
<td>$1,252.84</td>
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<tr>
<td>Dr. John L. O'Hara, President-Elect</td>
<td>$417.52</td>
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<tr>
<td>Dr. Frank B. Wheeler, First Vice-President</td>
<td>$363.82</td>
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<tr>
<td>Dr. Jean V. Smith, Second Vice-President</td>
<td>$57.42</td>
</tr>
<tr>
<td>Dr. W. L. Bendix, Secretary-Treasurer</td>
<td>$200.14</td>
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<td>Dr. R. A. Hendershott</td>
<td>$786.54</td>
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<tr>
<td>Dr. J. G. Flint</td>
<td>$102.77</td>
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<td>Ella R. Blanton, Office Secretary</td>
<td>$114.54</td>
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**$32,249.94**

**CASH BALANCE - SEPTEMBER 30, 1968**

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<td>Southern Bank and Trust Company</td>
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<tr>
<td>Richmond, Virginia (savings)</td>
<td>$5,266.98</td>
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<tr>
<td>Southern Bank and Trust Company</td>
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</tr>
<tr>
<td>Richmond, Virginia (checking)</td>
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<tr>
<td>Morrisville, Pennsylvania</td>
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</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td></td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td>$1.00</td>
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</tbody>
</table>

**$6,045.19**
UNITED STATES LIVESTOCK SANITARY ASSOCIATION
1444 EAST MAIN STREET
RICHMOND, VIRGINIA 23219

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1967 THROUGH SEPTEMBER 30, 1968

REVENUE:
Total Cash Receipts ........................................... $27,836.83
Add - Accounts Receivable ................................... 7,563.20
Total Revenue (Accrual Basis) .............................. $35,400.03
Less - Expenditures .......................................... 32,249.94
Net Revenue Over Expenditures ............................. $ 3,150.09

NET WORTH - SEPTEMBER 30, 1968
Accounts Receivable ........................................ 7,563.20
Balance - Southern Bank and Trust Company Richmond, Virginia
  Checking .................................................... 776.21
  Savings Account .......................................... 5,266.98
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 ..................... 520.25
U. S. Treasury Bonds ....................................... 20,000.00
Furniture and Fixtures ..................................... 882.00
Net Worth, September 30, 1968 ............................. $35,135.64

ANALYSIS OF CHANGE IN NET WORTH
Net Worth, September 30, 1967 ............................ 31,985.55
Increased by:
  Net Revenue from Operations
  for Fiscal Year Ended September 30, 1968 ............. 3,150.09
Net Worth, September 30, 1968 ............................. $35,135.64

Henry H. Reed
Accountant

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WELCOME TO LOUISIANA

(Honorable Dave L. Pearce, Commissioner of Agriculture of Louisiana made a very warm and cordial speech welcoming the United States Livestock Sanitary Association to New Orleans, but at his request his speech was not published.)

RESPONSE TO WELCOME

A. A. Erdmann

It is a pleasure to respond to those fine words of welcome. There has been a pattern developing in the USLSA where the response to the welcome has been made by a representative from an opposite part of the country. When we met in Buffalo, New York, the response was from California. When we met in Lansing, Michigan, it was from New Mexico. Thirteen years ago we met in Louisiana and were welcomed by Commissioner Pearce. The response was from Ohio. It is therefore logical for me, from Wisconsin, a state at the far end of the Mississippi, to respond to the welcome of the fine state of Louisiana, and this beautiful city of New Orleans.

New Orleans – the name itself brings to mind the notes of Dixieland Jazz – Mardi Gras – and the spicy aroma of fine food and strong coffee. Basin Street and Rampart Street will long live in the memories of the entertainment world of this great country. The Louisiana Purchase of 1803 was probably the most important single event making the United States the world power it is today. With the purchase of the Louisiana territory, the geographical limits of the United States were doubled, and our boundaries extended from ocean to ocean.

"On one of the sight-seeing tours here, the guide likes to relate the history of the Civil War in eloquent words, such as, 'Here a platoon of brave Southern lads knocked the stuffing out of ten thousand Yankees'; or 'Here three regiments of New York infantry were routed'; or "This is the spot where a handful of Men in Gray captured 15,000 Yanks." After about so much of this, a proper Boston lady asked if the North didn’t win at least one battle during the war. Our guide replied, "No Ma’am, not while I’m driving this bus, they didn’t."

The Civil War is over – and a part of the past. The country is united and we work together to achieve whatever goals we establish for ourselves. There is no more united organization in the United States than the USLSA. We have one goal – and that is, to establish a livestock population in this country which is free of disease, and an asset to the entire economy. To do this, we must be united.

I know that Commissioner Pearce has had a long and illustrious career in the field of agriculture. He is a native of Louisiana, and has spent his lifetime in this work. He is now serving his fourth term as Commissioner of Agriculture. His interests are in all phases, from soil conservation to livestock marketing. He has been active in the legislature of this state, where he served for 8 years. Four of these were as Chairman of the Committee on Agriculture. He was selected by
Progressive Farmers as Man of the Year in 1954, and received an Honorary State Farmer Degree from Louisiana Future Farmers, in 1953. His guidance and leadership in agriculture have made Louisiana a garden spot. Rapid progress in the field of livestock disease eradication and control has been made in this state, even though located in a most difficult geographical area in which to carry out such work.

Commissioner Pearce has found time, amid his busy career in agriculture, to enjoy many other activities. He has reigned as King of the Cotton Festival, Dairy Festival, and Yambilee Festival. He studied music as a young man, and I'm sure he has contributed to the musical heritage of Louisiana; in fact, he has served as President of the State Singing Convention. He is an active leader of his church and the Gideons Society.

Commissioner Pearce, it is indeed a pleasure for us, the members of the USLSA, to meet in New Orleans. We gladly accept your welcome and offer of hospitality. Should we return to your fair city and great state in another 13 years, we will expect to be again welcomed by you.
The Animal Welfare Committee met at 8:00 P.M. Sunday evening. All members were present except Mr. M. L. Morse representing the Humane Society of the United States.

President Quinn again charged the Committee with its present and future responsibilities. In the present year the committee was instructed to investigate the need for filling gaps in the present Federal legislation which does not ensure healthy and humanely treated animals to be provided the public. Public Law 89-544 appears to have certain limitation which prevents proper enforcement of care of animals under certain conditions. Three areas of concern were noted by Dr. Quinn as of first priority.

1. Pet Shops
2. Pounds
3. Animal Shelters

In future years the Committee may wish to extend its interests to other areas of animal welfare.

The Committee believes that there is a need for the various states to supplement and extend coverage or protection beyond the present Laboratory Animal Welfare Act. Some of these areas lend themselves well to control by the States through enactment of enabling legislation to permit responsible officials to issue regulations pertaining to the problem. The legislation would better not concern itself with details, better controlled by regulations more easily changed as need arises. Such legislation would permit flexibility and aid in uniform establishment of health and humane standards to better protect the laboratory animals.

The Committee recognized the areas designated by President Quinn are the most pressing and in need of correction as they are not touched upon by PL 89-544.

1. PET SHOPS
Humane Standards of care and treatment are not required in most States. In the populous areas of the country thousands of animals are marketed annually without regard for the welfare or health of the animal. Buyers have little or no protection from possible loss of their newly acquired pet from disease, malnutrition and parasites. Many of these animals have traveled hundreds of miles with little or no regard for their health or welfare.

2. POUNDS
Establishment of Pounds in the urban areas is a necessary part of urban life. Lost, strayed or unwanted animals present problems and hazards to the human population. Few, if any States have established standards of humane care and treatment of these animals. We believe that uniform standards for management, care and treatment of the animals, should be established. Present laws on this
subject are directed mainly to retention of the animals for varying time periods and to their disposal after that period.

3. **ANIMAL SHELTERS**
In considering this problem the Committee recognizes that some Shelters are well run and give ample protection to the animal. It is also recognized that there are no uniform standards for the operation of Shelters and extreme abuses occur. Conditions have been observed that point out that there is need for controls to require proper care for these animals, especially the sick and injured. Animal Shelters vary greatly in ownership, facilities offered, and services performed. The Committee believes that the States should set standard rules and regulations for the care and treatment in animal shelters regardless of their ownership.

The Committee was further charged with evaluation of The Laboratory Animal Welfare Act and the recognized need for controls by the State dealers not covered by PL 89-544 such as bunchers or collectors of small numbers of dogs or cats, auctions, trade days and boarding kennels. This Association should enlist the active support of all groups concerned with production, acquisition, transportation and utilization of animals found in these areas.

It is proposed that the various Committee members evaluate the proposals for improvement of prevalent conditions presented and make recommendations for the viewpoint of the group they represent. These recommendations would then be used to draft a model law for consideration by the States.

The Committee proposes to meet periodically to evaluate and complete the charge given to it as soon as possible.
In order to refresh your memory and to put this report in proper perspective, I am taking the liberty of reading a portion of last year’s report.

After a lengthy discussion dealing with the need for specifically approved markets, inspection and releasing of livestock from federally inspected stockyards and the need for uniformity of requirements the committee requests the United States Livestock Sanitary Association make the following recommendations to the United States Department of Agriculture:

1. For animal health purposes, a single set of standards covering all species, for livestock markets be developed and the inspection of such markets be a joint cooperative State-Federal effort, and that prior to release all livestock meet the interstate and state of destination requirements.

The above can best be handled by a Memorandum of Understanding between the State and Federal animal health officials and the livestock market management in the respective states. If the above recommendations are carried out, it will replace the need for specifically approved markets, and will place all livestock markets meeting the standards on an equal basis.

The committee felt last year’s recommendation could best be accomplished by defining a Livestock Market, and a Livestock Dealer, and by developing minimum Animal Health Standards relating to the facilities utilized in the business.

The committee defines a Livestock Market as:

**LIVESTOCK MARKET**

A livestock market is hereby defined in relation to animal health as a business conducted in facilities as necessary for the receiving, handling and care of livestock consigned to it for sale and sold competitively by those merchandising services it renders, and where the required veterinary inspection of such livestock is maintained.

The committee defines a Livestock Dealer as:

**LIVESTOCK DEALER**

A livestock dealer is hereby defined in relation to animal health for the purposes of these regulations as a business conducted in facilities as necessary for the receiving, handling and care of livestock purchased by it for its own account for resale, or for the account of any principal for delivery to him, exclusive of livestock purchased for direct slaughter at a slaughtering plant location, and where the required veterinary inspection of such purchased livestock is maintained.

The committee proposes the following Animal Health Standards:

**A. GENERAL STANDARDS**

1. PROVIDE DESIGNATED INSPECTORS FREE ACCESS TO PREMISES
2. MAKE DOCK TICKETS, BILL-OUT SLIPS, IDENTIFICATION
RECORDS AND OTHER SIMILAR MARKET RECORDS AVAILABLE TO INSPECTORS AT REASONABLE HOURS.

3. ISSUE NO RELEASE FOR LIVESTOCK TO BE REMOVED FROM THE MARKET UNTIL INSPECTED BY THE DESIGNATED INSPECTOR AND CERTIFIED IN ACCORDANCE WITH STATE OF DESTINATION AND FEDERAL INTERSTATE REGULATIONS.

4. COOPERATE IN IDENTIFICATION PROCEDURES OF LIVESTOCK HANDLED IN THE MARKET AND MAINTAIN RECORDS OF SUCH IDENTIFICATION.

5. PROVIDE WELL DRAINED AND CONSTRUCTED PENS AND LIVESTOCK-HANDLING FACILITIES WHICH ARE MAINTAINED IN CLEAN AND SANITARY CONDITION.

6. PROVIDE DISTINCTLY MARKED QUARANTINE PENS FOR THE SEGREGATION OF ANIMALS SHOWING SIGNS OF OR EXPOSED TO A COMMUNICABLE DISEASE. SUCH PENS SHALL BE LOCATED AND CONSTRUCTED SO AS TO PREVENT CONTACT WITH OTHER ANIMALS, AND TO PREVENT DRAINAGE FROM CONTAMINATING OTHER AREAS.

7. PROVIDE RESTRAINING CHUTES, EXTERNAL PARASITE TREATMENT, AND VACCINATION FACILITIES, SO THAT THE MARKET IS CAPABLE OF HANDLING LIVESTOCK WHEN NECESSARY TO COMPLY WITH REGULATIONS APPLICABLE TO THE SPECIES BEING HANDLED.

8. PROVIDE FOLLOWING CLEANING AND DISINFECTANT FACILITIES:
   a. A WATERING SYSTEM CARRYING AT LEAST 40 POUNDS PRESSURE WITH SUFFICIENT HOSES, NOZZLES, ETC., TO REACH PENS AND ALLEYS AS NECESSARY.
   b. AN AMPLE SUPPLY OF APPROVED DISINFECTANT.
   c. POWER-SPRAY EQUIPMENT IN WORKABLE CONDITION CAPABLE OF 200 POUNDS PRESSURE PER SQUARE INCH.
   d. ADEQUATE FACILITIES AND SERVICE AT NOMINAL COST FOR CLEANING AND DISINFECTING CARS, TRUCKS AND OTHER VEHICLES AS REQUIRED BY REGULATIONS.

9. PROMPTLY CLEAN AND DISINFECT PENS WHICH HAVE CONTAINED LIVESTOCK AFFECTED WITH A COMMUNICABLE DISEASE AS DIRECTED BY THE INSPECTOR.

10. PROVIDE FOR PROMPT REMOVAL OF WASTE MATERIAL, MANURE, USED BEDDING, AND DEAD ANIMALS IN A MANNER APPROVED BY THE DESIGNATED INSPECTOR.

B. **SPECIAL STANDARDS**

1. SWINE HANDLING FACILITIES
   a. PROVIDE COVERED PENS FLOORED WITH AN IMPERVIOUS MATERIAL FOR HANDLING FEEDER AND BREEDING SWINE. CLEAN ALL PENS AFTER EACH DAY'S USE.
   b. MAINTAIN FEEDER PIGS AND BREEDING SWINE SEPARATE FROM SLAUGHTER HOGS. IF YARDED IN ADJOINING PENS,
THEY ARE TO BE SEPARATED BY SOLID PARTITIONS.

2. CATTLE HANDLING FACILITIES
   a. PROVIDE SEPARATE CLEAN PENS (OR A DIVISION) FOR YARDING ANIMALS FROM MODIFIED CERTIFIED OR BRUCELLOSIS FREE AREAS SEPARATE FROM CATTLE OF UNKNOWN ORIGIN, AND THOSE CONSIGNED TO SLAUGHTER.

3. SHEEP HANDLING FACILITIES
   a. PROVIDE SEPARATE CLEAN PENS (OR A DIVISION) FOR YARDING ANIMALS ORIGINATING IN SHEEP SCABIES FREE AREAS FROM THOSE OF UNKNOWN ORIGIN.
   b. PROVIDE A SUPPLY OF APPROVED DIPS FOR TREATING SCABIES INFECTED AND EXPOSED SHEEP.

*MARKETS NEED PROVIDE SPECIAL FACILITIES ONLY FOR SPECIES HANDLED.
The Committee on Regulatory Veterinary Medical Resources has continued to study the problem of how we can best accomplish the job that is before us with the resources that we have at hand. In their report last year, the Committee reported visiting a great number of the states and reported their findings after a study of the organizational setup of each of these states visited.

This year the Committee has made a thorough study of recommendations that have been made in the past by the president of the organization and have made a conscientious effort to evaluate these recommendations as they pertain to the present setup.

The recommendations made by President Brandenburg in 1949 were directed at underpaid, understaffed state livestock sanitary organizations. From the interpretation of the remarks of Dr. Brandenburg, it would seem that he was well pleased with all of the programs of the Bureau of Animal Industry and with their ability to carry out efficient disease control programs. He was very much concerned about the salaries of the various state livestock sanitary officials and their inability to cope with disease control problems due to the interference of local politics.

Dr. Brueckner again voiced concern about the inability of various state organizations in 1956. The Committee feels that it was Dr. Brueckner’s opinion that states should become more active in support of laboratory facilities in the field of poultry and livestock disease so it would not be necessary for the federal government to take over these phases of disease control.

Dr. Milligan’s remarks in 1958 were directed at the combining of the offices of the State Veterinarian and Veterinarian in Charge, thus turning the whole disease control organization over to centralized government in Washington.

Dr. Safford’s remarks in 1965 suggested that the federal monies be turned over to the various states and that the federal government maintain regional offices to supervise all the work that was being done so that federal monies could be withdrawn immediately when the work did not come up to the standards of the U. S. Department of Agriculture.

The Committee has given careful consideration to the remarks that have been made by the past Presidents and attempted to fit these remarks into the effects that they have had and might have on our present and future efforts in livestock disease control. The conditions that existed when Dr. Brandenburg made his remarks in 1949 have, to a great extent, been corrected. The state livestock sanitary official in most cases draws what must be considered a responsible salary. The salary of the State officials, according to our survey, indicate that, as a whole, theirs are
somewhat lower than salaries paid to federal veterinarians, but the state livestock sanitary official enjoys certain privileges that federal officials do not find granted to them. In most instances, the state official is now placed under civil service and is no longer subjected to the political pressures that were true a few years back.

The matters referred to in Dr. Brueckner's address in 1956 have been corrected mainly because laboratories have been established throughout the various states which have been staffed with well trained personnel so that it has not been necessary for the federal government to take over all the laboratory work within the various states.

The remarks made by Dr. Milligan in 1958 seem to have gotten results at that time because since that address there has been no more combining of the offices of the State Veterinarian and Veterinarian in Charge.

Careful consideration has been given to the proposal made by Dr. Safford. It is the opinion of this Committee that the establishment of four (4) regional offices to supervise the disease control programs within the various states would lend itself to a complete federal take-over of the supervision of all state funds in the field of disease control and would leave the various state officials subject to the whims and fancies of centralized government. Anytime the regional office was in disagreement with the programs of the various states all that would be necessary to whip these states into line would be to cut off the federal funds and tell the states to do it our way or there will be no more federal funds available. In the report of this Committee last year, we stated that the present dual setup of having a Veterinarian in Charge and a State Veterinarian in each state served the purpose of being a check and balance between state and federal authorities. We reported that in most of the states that were surveyed we found good working relationships between state and federal authorities and that in almost every instance where there were differences, these differences were due to the lack of the state to assume its part of the responsibility of disease control efforts. We find that during the past year there has been an effort on the part of most states to further assume more of the responsibility of the disease control effort and that in a few states where the inadequacies of the state's ability to meet its responsibility were so glaring, efforts are now being made to bring these states up in line with the Nation.

We do not feel that there is a great waste of regulatory veterinary medical resources at the present time. The job is so great that there is not enough manpower available to accomplish all of the results that the various states would like to see accomplished and both federal and state personnel in the majority of the states are gearing all of their efforts in an attempt to accomplish the desired results.

We have discussed with federal officials the necessity of a complete integration of state and federal forces in order to accomplish the job ahead. We have been assured by these officials in Washington that they are most interested in accomplishing the very best possible job and that they are not interested in taking over the job of dictating to the states the methods and procedures that must be followed to carry out the job of disease control and eradication in the various states.

The Committee wants to commend the ARS on its training programs to regulatory personnel that has been open to state as well as federal employees. We ask that each state take advantage of these opportunities and that they continue to
support the efforts. We ask that training programs for vesicular diagnosticians be reopened and that state and federal veterinarians be included in the programs.

The Committee supports the concept of specialty boards in the field of regulatory veterinary medicine and request the United States Livestock Sanitary Association lend its support to the establishment of such specialty boards.

The Committee commends the American Veterinary Medical Association for their efforts in behalf of Regulatory Veterinary Medical salaries. We ask that they continue their efforts toward correcting the inequities that still exist.

The Committee has heard the charge given it by the president-elect in his address of this morning. They pledge their wholehearted support to him during his term as president of this Association.
REPORT OF THE COMMITTEE ON
PARASITIC DISEASES AND PARASITICIDES

United States Livestock Sanitary Association
Seventy-Second Annual Meeting
October 7-11, 1968

W. C. Tobin, Denver, Colorado, Chairman; Donald W. Baker, Albuquerque, New Mexico; J. H. Brashear, Oklahoma City, Oklahoma; George L. Crenshaw, Davis, California; J. H. Hourrigan, Hyattsville, Maryland; J. E. Kleck, Albuquerque, New Mexico; F. R. Koutz, Columbus, Ohio; H. B. McGrath, Kansas City, Missouri; M. D. Mitchell, Pierre, South Dakota; A. C. Newman, Jr., Opelika, Alabama; R. D. Radeleff, College Station, Texas; I. H. Roberts, Albuquerque, New Mexico.

Your committee reviewed the report submitted by Dr. J. H. Hourrigan concerning the recent disclosures of cattle fever ticks outside the Buffer Zone in Texas, with great interest. The committee requests ANH, USDA to immediately provide a recommended protocol for the management of livestock which are being or have been moved from tick infested premises.

The committee discussed at length the problems resulting from the regulation which requires that certain chemicals used against mite infestation must be registered as drugs. The committee is submitting a resolution through the USLSA directed to the Director of Health, Education, and Welfare, requesting that all compounds used against ectoparasites be registered as pesticides rather than as drugs.

Attention of ANH, USDA is again directed to the resolution of this committee instigated in 1966, which requested that the classification of the condition caused by choriopites, sarcopites, and psoregates species of mites be classified as Scabies rather than Mange, and urge that ANH take the steps necessary to implement the requested change in classification.

The committee recognizes and commends the progress made by Livestock Conservation, Incorporated in the area of Cattle Grub Control. Further, it is recommended that area control programs be established in order that further reduction of incidence of cattle grubs can be accomplished. A plan for this program is currently being drawn up by the committee.

The committee reviewed the current Sheep Scabies situation and wishes to express its concern as six scabies infested flocks were disclosed during fiscal year 1968 and one to date in the current fiscal year. The committee urges all regulatory officials to re-double their effort to continue a most active program until sheep scabies is completely eradicated.

It is recommended by the committee that import regulations be established requiring that exotic and zoo animals be treated for all types of parasites at the origin of their shipment rather than at destination.

The summaries of this fiscal year’s activities in sheep scabies eradication, cattle
scabies eradication, tick eradication, and screwworm eradication programs are being submitted for inclusion in the published proceedings of the United States Livestock Sanitary Association.

**SHEEP SCABIES**

There were six outbreaks of psoroptic sheep scabies in fiscal year 1968 as compared to two in fiscal year 1967. The outbreaks occurred in Hopkins (July 1967) and Christian (May 1968) Counties, Kentucky; in Clarke (January 1968) and Culpeper (March 1968) Counties, Virginia; and in Dauphin (April 1968) and Chester (April 1968) Counties, Pennsylvania.

Part 74 was amended placing each of the infected counties in the Infected-Eradication Area following disclosure of infection. Hopkins County, Kentucky, was returned to the Sheep Scabies Free Area on March 26, 1968; the other five counties remain in the Infected-Eradication Area. The State Farm Show Building, Harrisburg, Pennsylvania, was returned to the Sheep Scabies Free Area on July 12, 1968, in order to allow special sheep sales and shows to be held without requiring sheep to be dipped upon leaving the show.

During fiscal year 1969, special emphasis will be placed on eradication efforts in the areas in which scabies was found during fiscal year 1968. The program provides for intensive application of all eradication measures which have been found to be of value previously. Measures of particular value are: (1) Two area inspections per season of all sheep by trained inspectors; (2) Extra inspections at concentration points; such as markets, dealers, feedlots, and at slaughter; (3) Development of reporting of suspicious flocks by shearers, 4-H, FFA, and others. This may be stimulated if local groups can be influenced to offer rewards. It is imperative that, in all States, more pressure must be placed against the sheep scabies mites if we are, in fact, to eradicate the disease.

**CATTLE SCABIES**

Psoroptic cattle scabies was reported in three Washington feedlots — one each in Yakima, Benton, and Grant Counties. The Yakima and Benton County outbreaks were confirmed in December 1967 from scrapings taken at their respective feedlots. The Grant County outbreak was disclosed in January 1968 from scrapings taken from cattle presented for slaughter.

Part 73 was amended placing Yakima, Benton, and Klickitat Counties under Federal quarantine effective December 29, 1967, and Grant County effective January 23, 1968. The quarantine was released from Klickitat County on January 24, 1968, and from Yakima, Benton, and Grant Counties on February 20, 1968.

A fourth outbreak was disclosed in a Miller County, Arkansas, herd from scrapings taken at the Texarkana Stockyards, Texas, from a bull consigned for slaughter.

All herds in Vermont were inspected for scabies in an active eradication effort. Of 6,705 herds, 1,126 were found infected with chorioptic mites and 13 with sarcoptic mites. Heated lime-sulphur, in spray-dip machines, is being used for treatment.
PARASITIC DISEASES

TICK ERADICATION

Prevention—keeping the ticks out of the United States—is a major part of the effort against cattle fever ticks. A quarantine zone is maintained along the international boundary and the lower Rio Grande River in eight Texas counties as adjacent areas in Mexico are infested. Cattle from Mexico are carefully inspected for ticks at the border. They must be free of ticks and must be given a precautionary dipping before they can be imported.

Without these controls, cattle fever ticks would reinfest areas of the United States that have warm climates. In spite of continued efforts to keep out these parasites, they have reappeared from time to time, but vigilance and prompt eradication measures have eliminated the outbreaks.

Should the ticks gain a foothold, a piroplasm-carrying cattle imported from Mexico could furnish reservoirs leading to heavy losses in our cattle population.

Active program continued in Texas - During fiscal year 1968, in the Buffer Zone, 198 livestock illegally entering from Mexico were caught, of which 19 were tick-infested; 15 tick-infested Texas herds were found; and 51,718 lots of 1,330,160 livestock were inspected and 14,464 lots of 68,925 livestock were dipped.

Exotic ticks found - The usual number of exotic ticks were found on zoo animals imported or in the process of importation.

Ticks collected from cattle on St. Croix, U.S. Virgin Islands were identified on September 1, 1967, as Amblyomma variegatum. This was the first evidence that the tropical bont tick might establish itself on U.S. territory. This tick is an important pest in many areas of the world and a vector of diseases affecting animals and man. Immediate steps were taken to determine the extent of the infestation and to develop a plan to contain and eventually eradicate the parasite. The program includes dipping livestock in 0.125 percent Coumaphos (Co-Ral) at 7-day intervals and spraying the 6 infested premises with Carbaryl (Sevin) at 3-week intervals at the rate of 2 pounds active ingredient per acre. No A. variegatum ticks have been found since April.

SCREWWORM ERADICATION

During the winter of fiscal year 1968, the United States enjoyed a period of 101 days free of screwworm infestations. On March 26, 1968, the first case for 1968 was discovered in Starr County, Texas. Since that time the southwest and principally Texas is having the most severe screwworm outbreak since 1963.

The present screwworm situation in the southwest—particularly in Texas—is directly attributed to abnormal weather conditions that have existed for some months in northern Mexico and the southwestern United States. Persistent heavy rainfall has occurred in this region since Hurricane Beulah struck the Gulf Coast last year. Weather conditions—particularly rainfall and ground moisture—have been the most unusual in modern times, creating an environment extremely conducive to the natural propagation of screwworms. The southwestern ranchers say that there has not been a period for at least the last twenty years equal to last winter, spring, and early summer in the abundance of rainfall. Similar conditions have prevailed in northern Mexico. In the Big Bend country of Texas and the adjacent area of
Mexico, there has been more rainfall than in any year since 1914.

Because southwest livestock owners have been relieved of economic losses from screwworms in recent years, they have released many of the ranch hands who would normally be riding the range looking for screwworm-infested animals. Livestock surveillance decreased to a point where there was little routine inspection and treatment of animals by their owners. We recently discussed the current situation with key livestock producers in the southwest, and they are actively supporting an intensive drive to get animal owners to assist the program by resuming conventional methods for fighting screwworms, such as frequent inspections of animals, spraying of livestock, curtailing surgery, exercising care of livestock movements, etc.

For similar reasons, northern Mexican ranchers have also reduced their individual efforts to fight screwworms, and in addition, have taken advantage of the prolonged wet spell to move their animals northward into grazing areas along the border, which in average years would be almost devoid of vegetation. This has resulted in movement of screwworm-infested animals toward the barrier zone.

Due to the long period when screwworms were non-existent or when only sporadic cases were occurring, producers on both sides of the border became complacent and permitted calving, dehorning, castrating, shearing, and other wound-causing activities without using preventive medication or without confining these activities to those portions of the year when screwworms are normally at their lowest level. All of these factors have contributed to the situation that now exists in the States bordering Mexico.

The present funding for the screwworm program is based on a minimum amount required to conduct the program during normal years. There is no reserve for operating under extraordinary weather conditions such as we have had this year.

To combat this outbreak of screwworms it has been necessary to spend program funds at a greater rate than originally planned. The outbreak has been contained so far and as of September 11, 1968, there has been no eastward spread into East Texas or Louisiana. Additional funds will be needed in the spring of 1969 to finance operations designed to stop the usual spring buildup.

Screwworms are being reared on a media containing beef or pork lungs. During fiscal year 1968 there were 1666 laboratory-confirmed cases of screwworm in the United States. As of September 11, 1968, there were 2840 screwworm cases in calendar year 1968.

6,397,404,600 sterile screwworm flies were released during fiscal year 1968.

The ultimate solution for avoiding various degrees of seasonal infiltration of screwworms into the United States is to extend eradication into Mexico and establish a barrier of sterile screwworm flies at the Isthmus of Tehuantepec. Through a feasibility study directed and funded by the 89th Congress, the U. S. Department of Agriculture has determined that such a program would be both technically feasible and economically advantageous to this country.
COMMITTEE ON ANIMAL VIRUS CHARACTERIZATION

A. J. Kniazeff, Oakland, California, Chairman; J. H. Gillespie, Ithaca, New York, Co-Chairman; C. J. York, San Diego, California, Executive Secretary; F. R. Abinanti, Bethesda, Maryland; R. A. Bankowski, Davis, California; V. J. Cabasso, Berkeley, California; G. E. Cottral, Greenport, Long Island, New York; R. A. Crandell, Rio de Janeiro; H. W. Dunne, University Park, Pennsylvania; J. R. Gorham, Pullman, Washington; L. E. Hanson, Urbana, Illinois; W. R. Hinshaw, Frederick, Maryland; S. McConnell College Station, Texas; T. Moll, Pullman, Washington; R. C. Reisinger, Bethesda, Maryland; B. I. Wilner, Berkeley, California.

The work of the Committee on Characterization of Animal Viruses has continued to receive financial support from the National Institutes of Health. An important function of the committee has been to select specific reference virus strains to represent the various viruses. Work with many of the domestic animal viruses is far enough along to permit publication of the information. One or more articles will be released in the next few months. Viruses of all animals excluding man must be considered in order to provide maximum coverage. Accordingly, the Committee met for three days in San Diego in February, 1968 to discuss and initiate plans to include oncogenic viruses; viruses of laboratory animals, wild animals, and primates: chronic degenerative agents; as well as poikilothermic viruses. The Committee invited as consultants, experts in each field who provided much of the data for these recently included groups. It is anticipated that the reference virus data will be published as a catalogue for world wide distribution.

Much laboratory work remains to be done in order to ascertain the number of different viruses which exist for any particular group. In many instances it has not been possible to select reference viruses. It is becoming increasingly important to have an understanding of viruses on a world wide basis from both a basic research and a disease control point of view. In order to expedite the collection of such information a number of small working groups have been chosen in collaboration with the Eastern Hemisphere Committee on Animal Virus Characterization and WHO. These working groups are composed of active investigators who will be expected to exchange data and, where necessary and possible, viruses and antiserums, and to make recommendations to the parent committees. These working groups cover bovine and equine picornaviruses, porcine picornaviruses, feline picornaviruses, bovine, porcine, canine and avian adenoviruses, myxoviruses, paramyxoviruses, Rinderpest-measles group, avian leukosis, bovine, equine, porcine, canine, feline, and avian herpesviruses, poxviruses, hog cholera - swine fever - virus diarrhea group, and parvoviruses. This does not cover all viruses and other working groups will be established from time to time as needed.

The Committee is serving in an advisory capacity for several other agencies or organizations. These include the American Type Culture Collection in the selection
of viruses to be placed in repository, the National Cancer Institute of Allergy and Infectious Diseases regarding reference reagents, the U.S. Department of Agriculture- Agriculture Research Service in biohazard classification, and the National Library of Medicine in computerizing the library reference list for animal viruses.

Since it is recognized that the collection of data is of limited value unless made available to diagnostic and research laboratories, several steps are being taken to disseminate the information. One is the publication of articles in scientific journals as already mentioned; the second, is the completion of a data retrieval system; and the third, is the planning of an International Symposium on animal virus characterization. Two sub-committees have been organized for this purpose, and interest has already been expressed from one or more agencies for provision of the necessary financial support. It is hoped that this international symposium can be held sometime in 1969.

As implied throughout the Annual Reports of the Committee, new viruses are being discovered at a rapid rate. Much work remains to be done both at the cellular and molecular level to obtain information essential for classification. To keep this Association abreast of developments and to provide means of applying new information to problems of disease control requires the continued efforts of an active committee.
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

Report of the State-Federal Relations Committee
Washington, D. C.
April 30 - May 3, 1968


The State-Federal Relations Committee of the United States Livestock Sanitary Association met in Washington, D. C. on April 30 through May 3, 1968. At this meeting we were in conference with United States Department of Agriculture Officials and Directors and Staff of the Animal Health Division, Animal Disease and Parasite Research Division, Veterinary Biologics Division, Consumer and Marketing Service, Federal Extension Service and the Bureau of Veterinary Medicine of the Food and Drug Administration. 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 statements for mutual consideration and guidance.

Animal Health Division

This committee will not attempt to evaluate and comment on all of the many and varied programs and problems of the Animal Health Division, but will comment only on those areas where we believe special attention should be focused.

The recent outbreak of Foot and Mouth Disease in England has again re-emphasized the urgent need to modernize the methods and procedures to prevent the introduction of exotic diseases into this country. This cannot be corrected by merely increased finances and personnel, but the entire approach must be evaluated, even if outside professional scientific help is necessary to develop a new atomic age procedure to cope with the complex problems that are resulting from the increased inter-continental travel. Judging from the inadequate inspection service that is now being carried out, something must be done now. Certainly the modernization of the Import-Export procedures should have been initiated years ago. Further delays will prove disastrous.

Even though the threat of Foreign Diseases is foremost in our minds, we cannot overlook the grossly inadequate reporting system of diseases of domestic animals that plague the country. Not only is the early diagnosis and reporting of disease occurrences important to effective disease control, but the lack of knowledge as to the actual occurrence of diseases and the relationship to the economy and to the livestock industry makes it impossible for State and Federal disease control officials to determine which disease deserves the most urgent attention.
The importance of a National Animal Diagnostic reference laboratory cannot be over emphasized. The present diagnostic facilities at Ames are inadequate and space is needed for the expansion of the other facilities located at NADL. Serious consideration must be given to the establishment of a National Reference Laboratory which would assist in the upgrading of State Diagnostic Laboratories; would train both Federal and State personnel and would assist in the investigation of unusual disease conditions that may occur. In order to expedite the establishment of this much needed diagnostic facility, this committee recommends that a suitable location be selected and that leasing arrangements be finalized as soon as possible and in this way save time, money and unnecessary loss of life that could be prevented.

The committee recommends that One Million Dollars additional be provided by transfer from Brucellosis funds in order to accelerate the eradication of Tuberculosis by eliminating the last few foci of infection.

Since the present supply of Anaplasmosis Antigen will be exhausted by 1970, this committee recommends that Four Hundred Fifty Thousand Dollars ($450,000) be allocated for the purchase of an additional supply. We further recommend that feasibility studies be conducted to determine whether or not an Anaplasmosis eradication program is practical.

This committee wholeheartedly endorses the ANH in its amendments of its regulations to prohibit the interstate shipment of all Hog Cholera Vaccines as soon as it can be processed through legal channels.

At the present time the Welfare of Laboratory Animals Program is being financed from other funds not allocated for this purpose. This committee suggests that every effort be made to secure sufficient funds to adequately carry out the program even if funds are derived from other agencies than the Department of Agriculture.

In the face of the recent approval by the Conference on Interstate Milk Shippers of the Provision of the 1965 U.S.P.H.S. pasteurized milk ordinance requiring mandatory removal of all Mastitic cattle from milk supply by July 1970, it is essential that ANH develop and implement some acceptable form of cooperative program with the states to assist the dairy industry to meet these requirements. Every effort should be extended to develop and implement a uniform National Cooperative Program under ANH leadership. The alternative is 50 states going 50 separate ways which would be disastrous to the industry.

SUMMARY:
1. Need for modernization of Import-Export procedures.
2. Development of a meaningful disease reporting system.
3. Relocation and expansion of the National Diagnostic Laboratory; also to give reference services to State laboratories.
4. One Million Dollar transfer from Brucellosis to Tuberculosis to accelerate Tuberculosis eradication.
5. Four Hundred Fifty Thousand Dollars ($450,000) to replenish our supply of Anaplasmosis C. F. antigen.
STATE-FEDERAL RELATIONS

7. Welfare of Laboratory Animals program should get federal funding adequate to do the job.

8. Need for Animal Health leadership for development and implementation of a cooperative uniform mastitis control program.

Animal Disease and Parasite Research Division

On behalf of our association, our committee is very pleased to learn that final bids are now being considered for construction of the Southwest Veterinary Toxicology and Livestock Insects Research Laboratory at College Station, Texas.

We hope the building, equipping and staffing of this facility will go forward with dispatch and without interruption. Our association offers the department every assistance at our disposal to this end.

We cannot overly emphasize the importance to our economy and welfare we attach to this project. The continuing and rapid development of insecticides, chemosterilants, herbicides and a vast array of other powerful chemicals for widespread use throughout the entire broad field of Agriculture makes this program one of the most important assignments the Division now has.

We wish to place our association in full support of the Animal Disease and Parasite Research Division's efforts to bring the Beltsville Parasitological Laboratory up to its full potential. The work already accomplished at this facility is impressive and far reaching. The whole field of Veterinary Parasitology offers an enormous potential for good, far in excess of its cost, and should be developed so that its full potential may be reached without undue delay. Specifically, the most important need today at Beltsville is a central post mortem and incinerator facility. We hope that this can be provided in the 1970 budget.

The Division plans for future needs are modest when related to the great benefit to our economy that will result from their efforts. We wholeheartedly support these proposals for capital improvements and increased personnel in the disciplines of pathology, genetics, biochemistry, immunochemistry, physiology, physics, ecology, etc. Perhaps the general impression that parasites eat more than we do is true. If so, one of us must give way and we should do all we can to see that it is the parasites—not us.

We are concerned about the limitations placed upon the services provided by the National Animal Disease Laboratory at Ames, Iowa by space problems. We hope that the Biologics Division and the Diagnostic Services provided by the Animal Health Division can be moved to other quarters where quite frankly, such airtight security quarters are not needed. This would release much needed space for essential research, however it would not provide more than 50% of the current space needs. We recommend an early accomplishment of these moves and that plans go forward to expand the N. A. D. L. facilities to more adequately fulfill today's needs. The 1970 budget is the place to start.

A few words about Plum Island, perhaps the most critically important of all. The work and accomplishments at Plum Island Animal Disease Laboratory (PIADL) have been phenomenal. Space here is also a real problem. We are most heartened that Six Hundred Fifty Thousand ($650,000) Dollars is included in the 1969 fiscal year budget for planning added facilities. We urge sufficient funds be provided in
the 1970 fiscal year budget to construct and equip 70,000 feet of added space. This will not be cheap because this kind of facility at today's prices may run to One Hundred Dollars ($100.00) per foot, but can we afford not to?

Work of the division in foreign countries must not be allowed to lag. We are acutely aware of our balance of payments problem but we must not be "pound foolish".

We applaud the development at PIADL of a new Foot and Mouth Disease inactivated vaccine and the proposed field trials in Brazil and Holland, particularly in swine where both the need and danger are great.

Twenty two of 26 exotic diseases, each one of which could spell disaster to our livestock economy, exist in Africa. The potential for effective research on this Continent are enormous and must be actively supported. We pledge the full support of our association to this end and urge the department to do the same.

Recruitment of Scientific Personnel for research is becoming a real problem. Competition in this field is also increasing. This does not involve large numbers of men; only a few as a matter of fact. We most seriously urge the department not to permit the downgrading of requirements for these positions for reasons of money or competitive activity. This is too important for this kind of thinking—take steps to get the authority to pay this class of employee what it takes to get the best—tell us how we can help you to accomplish this. We promise our full support.

There is one more area of concern we would like to bring up—manpower, facility utilization and project priority.

Some years back, Chairman Whitten of the Agricultural Appropriations Sub-Committee had a staff investigation made on the utilization of the then available research facilities in the United States. We recall that the Association of Land Grant Colleges and Universities was also interested in this same subject. The USULA was also interested and very much still is.

We now request another review of this and ask that funds be provided to do a study in depth of our present facilities across-the-board and to project our future needs for as far in the future as we can see. We also would like some thought to be given to the question of government ownership versus leasing or a private contractual approach. We need some guidelines and some policy for the future. We not only urge the department to embark on this project but we ask that the USULA be made a part of it.

We are aware of the many calls on the department for research projects. Our own group is responsible for many of them. We are also becoming somewhat alarmed. We currently note research is being conducted by USDA on sixteen diseases of cattle and three types of parasitism that exist in this country; six diseases of swine plus parasitism; eight diseases of sheep and goats plus three types of parasitism; twelve diseases of poultry plus two types of parasitism; additional studies of parasitism over a broad area and in addition, Foreign Animal Diseases in cattle, swine, sheep, goats, poultry and horses.

Our manpower, our money and our facilities are not endless—we are greatly concerned that we may be spreading ourselves entirely too thin.

It is our opinion that this whole area needs review and study in depth. We must have a system of priorities for our research projects and the first priority must go to national problems with regional and local problems following.
We ask the department to establish and finance a task force to study this matter and make recommendations. We ask that the USLSA be allowed to participate in this study and report.

We further ask that a permanent group be set up to assign research projects and funds on a priority basis to best serve the National Interest.

SUMMARY:
1. Committee is happy the final bids on the Toxicology Laboratory at College Station, Texas are now being reviewed. This has been a generally neglected field.
2. Committee re-emphasizes the need to bring the Beltsville Parasite Laboratory operation to the full potential. This includes the capital improvements and personnel.
3. Committee hopes much needed additional space can be provided at NADL by moving diagnostic and biologic services to a new location. Further thought be given to more Research space at NADL in 1970 budget.
4. Committee hopes to see the 1970 budget contain planning and construction funds for new facilities at Plum Island. Delays here are entirely too costly.
5. Committee urges the department not to withdraw support for Foreign Research. This is the best protection we can develop against some twenty plus foreign diseases.
6. Committee is concerned about the downgrading of Research personnel. They must remain high and we must be competitive with other research groups.
7. Committee recognizes the need for a review in depth of the utilization of our research facilities and manpower to be sure we are making the best use of those we have. We request the USLSA be invited to participate in this review.
8. Committee urges an immediate and thorough study of the need for assigning priorities to research projects—both existing and new ones. We would like to participate in this study also. In this field the National Interest must take precedence over local interest.

Veterinary Biologics Division

The committee appreciated the opportunity to discuss with the Director and his staff the work of the Veterinary Biologics Division. From this discussion it is apparent that efforts to pick up the lag in the testing of Veterinary Biologics for sterility and potency is improving, but a great deal remains to be done to develop the proper facilities and personnel and bring the program of the division to a current status.

We have documented evidence from the State of Nebraska last year that serious problems are created for the livestock industry when inadequate testing procedures permit impotent and contaminated biologics products to enter interstate commerce. It was determined that inactivated Hog Cholera Vaccines which became involved in Hog Cholera breaks had not been subjected to any routine tests because of inadequate funding and personnel.
The need for adequate test animal facilities and testing laboratories is apparent. The present situation of operating with inadequate and divided animal and testing facilities at NADL, the City of Ames, Iowa and on the Iowa State University campus is inefficient. The need is obvious and to meet present requirements necessitates the expenditure of approximately Twenty six Million Dollars. We were further advised that Colorado State University has offered to provide adequate facilities, both animal and testing, to the Division for an approximate annual cost of Three Hundred Fifty Thousand Dollars. This proposal appears to the committee as the most economical means to provide a facility that will meet many present and future needs of the Division. It will also place the animal and testing resources in a community that offers access to a University environment whose resources will be available to the laboratory personnel as well as consolidate the program and permit expansion. A further advantage would be to provide a facility much faster than the government can construct a similar facility. The present space occupied at NADL by the Division could then be released at an earlier date.

Personnel of the Division, particularly in the laboratory section, are well trained and capable of being the nucleus for the larger staff which must be provided in order to permit the Division to accomplish its designated responsibility. Proper testing of Veterinary Biologics can not be the responsibility of the manufacturer and must be the responsibility of an unbiased agency. This dictates the immediate need for the Division to increase support and laboratory personnel over the next four or five years in order for the Division to meet its responsibilities. The committee supports the increase in appropriations for additional personnel during the next four years so that in 1972 the Division's program of testing Veterinary Biologics can be maintained and a program of research and development instituted.

SUMMARY:
1. The Committee supports the Division's interest in leasing adequate facilities at Colorado State University in order to provide more adequate animal facilities and testing laboratories in a consolidated operation rather than in the divided facilities in Ames, Iowa.
2. Committee supports the appropriation request for 1970 to provide the necessary support and laboratory personnel to provide sterility and potency testing on a current basis and recognizes the need to institute a program of research and development.

Consumer and Marketing Service

We were happy to hear Dr. R. K. Somers explain his conception of the new Wholesome Meat Act. It was a great departure from that which is presently being experienced by several of the States in their communications with certain of the Meat Inspection Field Staff in the implementation of their new legislation. Dr. Somers stated that "this is a whole new concept in meat inspection. For 60 years the Federal Service has operated without considering the possibility of anyone else participating in this responsibility. This new approach necessitates a re-indoctrination of Federal Staff which in itself is a major undertaking."

The committee was pleased to learn this was the interpretation of the new legislation by the Deputy Administrator of Consumer and Marketing Service and we
support and encourage any effort on his part to rapidly orient his field personnel to this philosophy.

Dr. Somer's offer to assist the states in establishing strong cooperative programs with money, technical help and training was most welcome. His statement that the Liaison Officer has authority over the Officer in Charge in the State clarifies this part of the chain of command. Several states have experienced confusion and uncertainty with regard to this point. Policies of this kind should be clearly and definitely communicated to the individuals involved. This is absolutely necessary for satisfactory and uniform operation at the state level.

The proposed reorganization which would combine the Red Meat and Poultry Inspection Services is strongly supported by the USLSA. This reorganization will provide for greater utilization of manpower with resultant economies.

We strongly recommend that the Meat Inspection Service immediately investigate the proposal that facilities, training aids and limited instructional personnel are available for training of both Veterinary and Lay Personnel at the Army facility located on Pershing Road, Chicago, Illinois. This would provide a saving to the Meat Inspection Division and eliminate the necessity for duplicating training aids and modules already available at slight cost through the Army.

Our committee recognizes the need for strong veterinary leadership as a means of improving efficiency and morale and recommends that competent, trained veterinarians be placed in key positions of management throughout the organization. We suggest a critical review of the classification of positions with a view towards upgrading such jobs so that the best talent available may be secured and retained. We strongly feel that with the greatly increased responsibility and extension of authority established by the Wholesome Meat Act, a much broader use of veterinary manpower will be necessary along with greatly increased authority for independent decision making at all levels in the program. For this reason we feel that to continue the recruitment of people at the G.S.-9 level is unrealistic and that the G.S.-11 range should be used for this purpose.

RULE OF REASON:
We agree with the basic principal of the "rule of reason". Dr. Somers has stated that the State and Federal Governments should be equal partners in this program, yet it appears that, if after discussion there is still a lack of agreement, the final decision on any given issue is still with the Federal Government. It seems to us that to make the "rule of reason" apply uniformly, a referee provision should be made in order that a compromise may be reached on those issues which cause an impasse between the State and Federal points of view.

This committee report is submitted with the hope that implementation of the suggestions made herein will be immediate and that our organization will be called upon to render our aid and assistance in so doing.
REPORT OF COMMITTEE ON RABIES


The committee wishes to give credit to the National Communicable Disease Center, Atlanta, Georgia for the statistical portion of this report which reviews the incidence of rabies in the United States for calendar year 1967.

In 1967, 4,609 laboratory-confirmed cases of rabies were reported to the National Communicable Disease Center; this is a 10 percent increase over 1966 and a 9 percent increase over the average for the last five years. The total includes 89 cases from Guam, which had not previously reported any cases. Forty-seven states reported animal rabies: only Connecticut, Delaware and Hawaii reported no rabies cases in 1967. Tennessee reported the largest number of cases with 559. The species accounting for the majority of laboratory-confirmed cases in 1967 were skunks (34%), foxes (21%), cattle (13%), dogs (9%), bats (9%), cats (6%), raccoons (3%) and equines (1%). Wild species accounted for 70% of the cases in animals. Only 412 rabid dogs were reported in the United States in 1967, the same number reported in 1965 and 1966. Two human rabies deaths occurred in the United States; both victims were United States citizens who had been exposed overseas. This was the first year since rabies case reporting was initiated that no human cases occurred from exposure within the continental United States.

INCIDENCE OF RABIES IN THE UNITED STATES BY TYPE OF ANIMAL 1953 - 1967*

<table>
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<tr>
<th>YEAR</th>
<th>DOGS</th>
<th>CATS</th>
<th>FARM ANIMALS</th>
<th>FOXES</th>
<th>SKUNKS</th>
<th>BATS</th>
<th>OTHER ANIMALS</th>
<th>MAN</th>
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<td>414</td>
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*Data prior to 1960 from USDA ARS Subsequent data from PHS, NCDC
**One human exposed in Guinea, Africa, the other in Cairo, Egypt
A total of 566,596 individual doses of human antirabies vaccine were produced and distributed by the two commercial manufacturers; state health departments in Illinois, Michigan, and Texas also produced human antirabies vaccine. The number of doses of human rabies vaccine distributed was approximately 400,000 less than in 1966.

Last year's committee reported a significant accomplishment in assisting in the organization of the National Rabies Advisory Council as recommended by the rabies committee in 1966 and that this held its initial meeting in Washington, D.C. in August 1967. This council had representation from the Departments of Agriculture, Interior, and Health, Education, and Welfare of the United States Government, along with representatives from the American Medical Association, the American Veterinary Medical Association, and the United States Livestock Sanitary Association.

Unfortunately, because of lack of financial support, there has been no follow-up to this initial meeting. The action suggested by the Council members at the initial meeting has not been instituted.

The Rabies Committee wishes to re-emphasize that if we are to achieve significant gains in the control of rabies we must have a nationally coordinated effort. We suggest that the National Rabies Advisory Council approach the National Research Council requesting their cooperation to sponsor and develop the system whereby the National Rabies Advisory Council could most effectively implement a nationwide rabies control effort.
Mr. Chairman, Members of the Association:

The Committee on Infectious Diseases of Sheep and Goats met and reviewed those diseases of sheep and goats which presently seem to plague the sheep and goat raisers most. Particular attention was given to the following diseases.

SHEEP FOOT ROT

Sheep Foot Rot continues to be a major obstacle to the economic production of sheep in much of the sheep rearing areas of the United States. Yet the Pilot Field Control Program being carried out in California, the programs being conducted by the State of Idaho and in other States have shown that proper application of standard foot rot control measures can eradicate the disease from well-managed flocks. If control measures are diligently pursued, the disease can be eradicated from entire States and areas. The committee was pleased that the Animal Disease and Parasite Research Division, U.S. Department of Agriculture, and the School of Veterinary Medicine, University of California, plan further research on the disease.

The Committee on Diseases of Sheep and Goats commends those States pursuing active foot rot control programs and recommends that State and Federal regulatory bodies give active support, including the necessary financing for continuation of the Pilot Project in California; the continuation of programs in those States actively working to control the disease; the initiation of inspection and control programs in other States; control movement of infected and exposed sheep; and the experimental testing of new chemicals to combat the organism of foot rot.

A sub-committee was appointed to collect information on losses caused by foot rot, costs of eradicating the disease, and cost/benefits which could be incurred for a foot rot program.

A resolution pertaining to the interstate movement of sheep in regard to foot rot was given to the Resolutions Committee.

BLUETONGUE

Bluetongue is recognized by sheep farmers, ranchers, and veterinarians as a costly disease to the sheep industry in many areas of the United States. It is also a major obstacle to the export of cattle and sheep to many countries of the world. During calendar year 1967, eleven isolations of bluetongue virus were made from sheep in the States of California, Colorado, Idaho, Indiana, Montana, New Mexico, New York, and Utah.
Oregon, and Utah. During the same period, eleven isolations of bluetongue virus have been made from cattle in the States of Colorado, Florida, Idaho, Indiana, Oregon, and Texas.

Bluetongue has long been recognized as a disease occurring in sheep in endemic areas of the western United States. However, it had not been reported east of the Mississippi until 1966 when it was isolated from cattle in Minnesota. It was also believed to be a disease that causes only inapparent infection in cattle. It is possible that the virus may be becoming more virulent for cattle. In many instances where the virus has been isolated from cattle or the disease has been reported in cattle, a clinical history is obtained of a typical syndrome appearing in the affected animals.

The Animal Disease and Parasite Research Division's Animal Disease Research Laboratory, Denver, Colorado, in cooperation with the Animal Health Division, is presently conducting a cooperative study to determine the reservoirs of the bluetongue virus, strains of virus present in the United States, modes of transmission of the disease, clinical syndromes in cattle and sheep, efficacy of the available vaccines, carrier state of affected cattle, sheep and/or other animals, and to develop a practical diagnostic test for the disease. It is hoped that the results of this study will provide the necessary information and tools whereby better control procedures can be developed to protect the cattle and sheep industries of the United States and to provide expansion of the foreign market for our cattle and sheep.

OVINE VIRAL ABORTION

This disease was first recognized in the United States in 1958. It is now widespread in the sheep producing areas and while the abortions caused by this disease are rarely of the explosive nature observed in Vibriosis, losses in infected flocks occur year after year and probably exceed those occurring from Vibro fetus.

Ovine Viral Abortion has been successfully controlled by vaccination in England, France, Germany, Italy, and Scotland and studies conducted in Idaho and Montana have indicated that the use of vaccines have produced a significant degree of immunity. The Committee on Diseases of Sheep and Goats recognizes the need for an effective OVA vaccine in the United States and, therefore, recommends that State and Federal regulatory bodies support the production of a vaccine in this country.

RAM EPIDIDYMITIS

Ram Epididymitis (REO) is presently causing considerable concern and loss to the sheep industry of the United States; and due to the widespread commerce in sheep, particularly of breeding sheep, it is expected that this disease will continue to spread and losses will increase.

The California project has shown that vaccination is a very useful tool and can provide effective control of the disease in an infected flock. Last year your committee reviewed evidence and concluded that the commercial vaccine was a good product, and outlined recommended vaccination procedures. More study is needed to determine the natural spread of the disease, to evaluate the present methods of diagnosis and develop improved methods of diagnosis. Workers of the
Animal Health Division at the National Disease Laboratory (NADL) have developed an improved media for culturing the organism in semen.

The Committee on Diseases of Sheep and Goats recommends that State and Federal regulatory bodies give continued support to the work being conducted in California, Idaho, and at NADL project; urge and support the initiation of similar projects in other States; give continued support of studies to develop improved diagnostic methods; and strongly urge all States to determine the incidence of the disease and vigorously control the movement of infected and exposed sheep. Since several organisms in addition to REO are known to cause epididymitis and/or orchitis, the committee also strongly urges that when clinical evidence of epididymitis is found that considerably more attention should be given to identifying the specific causes of the infection.

SCRAPIE OUTBREAKS DURING FISCAL YEAR 1968

Scrapie was reported in ten flocks in five States during Fiscal Year 1968, the same number of outbreaks as occurred during the previous year. The ten outbreaks involved eight Suffolk flocks and two in sheep of the Montadale breed. These are the first reported cases of scrapie in the Montadale breed, a breed developed in Missouri by crossing Cheviot rams on Columbia ewes. The number of flocks under surveillance has dropped to 270, the lowest since the beginning of the Scrapie Eradication Program in 1952.

The ten outbreaks occurred, one each, in Franklin County, Kansas; Champaign, De Kalb, Hancock, Tazewell, and Washington Counties, Illinois; Cecil County, Maryland; Ulster County, New York; and Cooke and Tarrant Counties, Texas.

One of the outbreaks was reported by a veterinary practitioner; three were reported by the flock owners; one was found during a scabies inspection; and five were found on routine surveillance inspections.

All bloodline sheep were either slaughtered or taken to Mission, Texas, for use in the Scrapie Field Trial. With the exception of the infected flock in Maryland and a source flock in New York where the nonbloodline exposed sheep are being held under quarantine for 24 months, all exposed sheep in infected and source flocks have been slaughtered.

Nonbloodline exposed sheep sold from infected and source flocks in Illinois, Maryland, and New York are being traced and placed under 42 months quarantine.

SCRAPIE FIELD TRIAL – MISSION, TEXAS

The Scrapie Field Trial has been underway since November 1964 (43 months); however, most of the animals taken to Mission for this study have been obtained since March 1965.

On infected premises No. 3 there are some 1,322 animals from ten States. The bloodline sheep brought into this flock are of the Cheviot, Hampshire, Montadale, and Suffolk breeds and were purchased from flocks involved in 26 outbreaks in Indiana, Illinois, Maryland, Missouri, New York, Texas, and Virginia. These sheep represent 20 bloodlines in which scrapie has been found in the United States. The nonbloodline animals in the flock are Hampshire, Rambouillet, Suffolk, and Targhee sheep breeds; and Angora, Nubian, and Toggenburg goat breeds. These
animals were purchased from Oklahoma, Montana, Texas, and Wyoming flocks and represent 17 individual bloodlines. In each case, the other of the twin pair or two of triplets are maintained on the farm of origin as controls.

None of the nonbloodline, previously nonexposed, sheep or goats have shown signs of scrapie. However, none of these animals have been subjected to scrapie exposure for longer than 36 months. The first progeny of these animals were born at Mission in the spring of 1966 and have, therefore, only been exposed for about 28 months.

Fifty-one cases of scrapie have been diagnosed on infected premises No. 3 from December 1964 through June 1968. These cases have occurred in the following categories:

<table>
<thead>
<tr>
<th>CATEGORIES</th>
<th>NUMBER OF SCRAPIE-AFFECTED SHEEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Progeny of affected ram and ewe</td>
<td>1</td>
</tr>
<tr>
<td>B. Progeny of affected ewes</td>
<td>11</td>
</tr>
<tr>
<td>C. Progeny of affected ram</td>
<td>3</td>
</tr>
<tr>
<td>D. Full-siblings to affected sheep</td>
<td>4</td>
</tr>
<tr>
<td>E. Half-siblings to affected sheep via the sire</td>
<td>14</td>
</tr>
<tr>
<td>F. Descendants of sires of affected sheep</td>
<td>4</td>
</tr>
<tr>
<td>G. Exposed sheep (not known to be related to scrapped sheep through three generations)</td>
<td>1</td>
</tr>
<tr>
<td>H. Exposed mixed breed dairy goat</td>
<td>1</td>
</tr>
<tr>
<td>I. Field suspects held for observation</td>
<td>12</td>
</tr>
</tbody>
</table>

The exposed mixed breed dairy goat, the only known goat to be affected with natural scrapie in the United States, was a 5-year old animal born on a Missouri farm (Missouri Flock No. 8) in the spring of 1963 where she was used as a nurse goat for Suffolk lambs until April 1967. During these four years this goat was exposed to six purebred Suffolk sheep that developed scrapie: a pair of twin Suffolk ewes affected in the Missouri flock in May 1967; a Suffolk ram sold into a second Missouri flock where he was affected in March 1967; a Suffolk ram sold into a Kansas flock affected in August 1967; and two Suffolk ewes taken to Mission, Texas, affected in March 1967.

Missouri Infected Flock No. 8 was slaughtered in April 1967, except for 35 bloodline sheep and three nurse goats taken to Mission, Texas. The affected goat, one of the three above, was observed to show typical scrapie signs 13 months after arriving at Mission in May 1968. The goat was destroyed on May 29, 1968, and histopathological examination of the brain revealed widespread degenerative changes of scrapie throughout the brain stem. This incident and experiments conducted in Scotland and France prove that goats born and reared in a natural scrapie environment can develop the disease by contact.

The Scrapie Field Trial has demonstrated that if bloodline exposed sheep are held under observation, instead of being slaughtered, many of them will develop scrapie. Scrapie losses in these bloodlines often run as high as 10 percent per year and can affect as many as 40 to 60 percent in certain bloodlines over a 4-year period. Therefore, the Scrapie Field Trial has demonstrated that bloodline sheep
and their descendants which have been exposed to scrapie in infected and source flocks continue to develop scrapie at rather high incidence rates and would be a potential source of widespread dissemination of the disease if allowed to remain alive and moved in the normal commerce of this country.

Your committee recommends that this organization continue to oppose passage of legislation which would restrict regulatory officials in their efforts to carry out the cooperative scrapie eradication program.

RESOLUTION

WHEREAS, 9 CFR, Part 71.3 provides that sheep found to have foot rot while in transit or upon arrival at a feed lot, stockyard or marketing center are allowed interstate movement under d(1) to immediate slaughter or d(4) to be quarantined, and

WHEREAS, this places an unfair and unwarranted burden upon owners whose sheep are found to have foot rot on farms or ranches, and

WHEREAS, these sheep should be allowed similar movement,

THEREFORE, BE IT RESOLVED that this Association request the Secretary of Agriculture to amend 9 CFR, Part 72 so as to afford such sheep the privilege of similar movement.
REPORT OF
THE COMMITTEE ON LAWS AND REGULATIONS

JUNG HOTEL
New Orleans, Louisiana
October 7 - 11, 1968

G. B. Rea, Salem, Oregon, Chairman; L. N. Butler, Phoenix, Arizona, D. E. Flagg, Bismark, North Dakota; F. W. Hanson, Hyattsville, Maryland; J. F. Hudelson, Topeka, Kansas; T. A. Ladson, College Park, Maryland; D. L. Smith, Indianapolis, Indiana.

During the past year six major problems have been presented to this committee for consideration. One subject, "The Identification of Livestock in Interstate Trade," had been previously studied. The five new topics are:

1. The need for establishment of certain requirements or standard operating procedures to be used as guide lines for the proper and meaningful inspection of livestock prior to completion of interstate health certificates.

2. The need and authority for the establishment of uniform health requirements for the interstate movement of zoo animals and for improvement in the quarantine facilities provided at foreign points of origin.

3. Indemnification for animals caused to be destroyed because of outbreaks of exotic or foreign animal diseases.

4. Identification of carcasses during slaughter procedures.

5. Recommendations pertinent to the recent circuit court decisions in Florida which relate to that state's program of Brucellosis eradication.

1. Guidelines for Interstate Health Inspections

Realizing that all accredited veterinarians have been properly and completely schooled in the professional techniques necessary for meaningful health inspections and recognizing also that the several states have established certain health requirements which the veterinarian must certify to, there remains only the circumstances under which these inspections are made, which might be questionable or of concern to the officials in the state of destination.

With these facts in mind, the committee recommends that the chief livestock officials of the several states promulgate regulations speaking to circumstances such as sufficient light, whether artificial or natural, amount of confinement deemed necessary under the various conditions pertaining to his state, and the lapse of time between actual inspection and the writing of the certificate.

2. Inspection of Zoo Animals in Interstate Trade and Quarantine Facilities at Points of Origin.

The committee has been advised that the ninety day quarantine required at the point of origin before importation of foreign or exotic animals is many times of questionable value due to private ownership of facilities.

We are further advised that most of the importers of these animals recognize the value of proper quarantine procedure with the attendant checks and tests. The
committee was asked whether or not there might not be some offshore island under United States supervision which could be used for the ninety day quarantine period prior to entry into the Continental limits.

In studying this question the committee found there were many advantages to such a facility over the present method, and in the name of the United States Livestock Sanitary Association asks the ANH of ARS to study the possibility of such a facility, as well as the probable costs. Zoo representatives have suggested that using agencies or organizations would find such facilities valuable enough to warrant underwriting some of the expense involved.

In discussing the problem of interstate movements of zoo animals, the committee, although recognizing the possible need for certain health requirements, did not feel that they had sufficient information to make meaningful recommendations. It is, therefore, the desire of the committee that next year’s members discuss this problem with the individuals involved in their home states and be prepared for recommendations at the 1969 meeting.

3. **Indemnification for Animals Slaughtered Due to the Outbreak of Exotic or Foreign Diseases.**

The financial burden which might accrue when exotic or foreign animal diseases are found in large public stockyards or feedlots would most likely be beyond the ability of most of our states. A resolution from an auxiliary regulatory group, and questions from individuals concerned, prompted committee consideration.

In searching for solutions to the problem, our committee discovered that the Emergency Disease Staff of the Animal Health Division is cognizant of the problem. The present restrictions which limit federal indemnity payments are based on regulations - not statutes. The staff has recommended that these regulations be changed to allow federal payment of 100 percent indemnity under certain specified circumstances. The whole matter is presently under study by the Department’s legal staff. Your committee applauds the initiative that the Emergency Disease Staff took in this matter and heartily supports its action.

4. **Identification of Carcasses During Slaughter Procedures**

The committee recognizes the importance of maintaining owner identification in the slaughter house as necessary to the proper function of the Market Cattle Testing Program. We are advised that the Consumer and Marketing Service has already caused to be printed in the Federal Register regulations which would require all identification devices except brands to remain with the carcass until after final post mortem inspection. Comments have already been received and studied, and the second printing is about to be made. Since these requirements are so important to disease control and surveillance, and since they are not yet actually in being, a resolution in support of this action has been presented to the Resolutions Committee (Res. No. 1).

5. **Recommendations Pertinent to the Recent Circuit Court Decision in Florida Which Relate to that State’s Program of Brucellosis Eradication.**

Concern over the recent Circuit Court decision affecting the maintenance of the Modified Certified Brucellosis Free status and continuing eradication
procedures in the State of Florida have caused other state officials to consider steps necessary to prevent reinfection of their own herds.

After considerable discussion, it was the committees decision to, by means of this report, alert all states of the situation pertaining to Florida so that they may take those steps deemed necessary on a local state basis to provide the needed protection and assurances.

6. Identification of Livestock in Interstate Trade

The committee recognizes the increasing importance of animal identification in the eradication of livestock diseases, and that the continuing reduction of field testing and vaccination activities will tend to reduce the number of animals identified by conventional ear tags. In order to continue and expand surveillance efforts through the identification of animals in marketing channels, the committee recommends:

(a) Efforts be made to copyright or otherwise reserve the oval, white and yellow, MCT backtag, or a similar device, for animal health purposes, and that further work be done in developing a uniform method of identifying animals in commerce.

(b) That a resolution requiring all bovine animals over 2 years old, except steers or spayed heifers, moving interstate for slaughter be identified with a device the use of which is restricted to regulatory officials.

A resolution to this effect has been given to the Resolutions Committee.

This report is respectfully submitted for consideration of the Executive Board.
REPORT OF THE COMMITTEE ON NOMINATIONS, RESOLUTIONS AND INTERNAL AFFAIRS

A total of eleven resolutions will be offered for your consideration. In addition, the Committee has instructed the Secretary to resubmit to the appropriate authorities copies of Resolution No. 6 adopted at Phoenix in 1967. This resolution concerned itself with the control of poultry disease.

**RESOLUTION NO. 1**

WHEREAS, many private distributors of bovine semen may custom freeze and sell semen from bulls without regard to the health status of the bulls;
WHEREAS, semen from some bulls that were rejected by artificial insemination studs because of health examinations can be sold by private distributors;
WHEREAS, some semen handlers keep no fertility records on their bulls, may fail to keep proper records on the identity of bulls, semen and offspring, may sell semen from genetically inferior bulls and also neglect the service aspect to the dairymen - upon which the Artificial Insemination Industry was founded.

BE IT THEREFORE, RESOLVED: that the United States Livestock Sanitary Association work with the American Dairy Science Association, the United States Department of Agriculture and National Association of Artificial Breeders to develop standards designed to regulate semen distribution and thereby protect dairymen and the purchasers of bovine semen.

**RESOLUTION NO. 2**

WHEREAS, the United States Livestock Sanitary Association joins with the American Veterinary Medical Association, American National Cattlemen's Association and the National Livestock Feeders Association in recognizing the vital, continuing need for chemotherapeutic and biological agents to aid in the control of infectious diseases of cattle,

THEREFORE BE IT RESOLVED that the United States Livestock Sanitary Association recommends that a task force committee be appointed by the National Academy of Sciences to study and demonstrate any potential hazard resulting from the use of these agents, thereby assisting the Food and Drug Administration in obtaining reliable scientific information from this task force committee that a hazard does exist prior to limiting the use of these agents which are so vital to the welfare of the cattle industry and the nation.

BE IT FURTHER RESOLVED that a copy of this resolution be mailed to the aforementioned groups and the Hearings Clerk of the United States Department of Health, Education and Welfare.

**RESOLUTION NO. 3**

WHEREAS the need for additional training of employees concerned with Salmonella control is evident;

THEREFORE BE IT RESOLVED that a training program for state and federal
inspection personnel for inedible rendering establishments be developed and implemented by the Animal Health Division of Agricultural Research Service, United States Department of Agriculture, in cooperation with the National Renderers Association.

RESOLUTION NO. 4

WHEREAS, there appears to be a need for additional specific information regarding isolations of salmonella serotypes

THEREFORE BE IT RESOLVED: that the Animal Health Division of Agricultural Research Service, United States Department of Agriculture be requested to publish on a regular periodical basis, a summary of the recovery of Salmonellae by species, by host species or source, and by geographical location of origin as confirmed by the National Animal Disease Laboratory or other approved serotyping laboratories.

RESOLUTION NO. 5

WHEREAS, all states rely on animal identification through slaughter to locate herds infected with Tuberculosis, Brucellosis and other diseases; and

WHEREAS, the meat packing industry experiences a significant loss through condemnation of carcasses and parts due to disease; and,

WHEREAS, the loss of identification of animals through slaughter precludes traceback to herd of origin of animals condemned or found infected with Tuberculosis, Brucellosis, or other diseases; and

WHEREAS, the maintenance of complete identification of slaughtered animals would make possible the accurate traceback of animals, whether in herd of origin or other herds of which the animal may have been a member;

NOW, THEREFORE, BE IT RESOLVED: that the United States Livestock Sanitary Association support the amendment to 9 CFR 310.2 of the meat inspection regulations published for comment in Vol. 33 No. 104 of the Federal Register on Tuesday, May 28, 1968, which will require that identifying devices be retained with carcasses until post mortem examination is completed.

RESOLUTION NO. 6

WHEREAS, the identification of animals through slaughter is an important tool in locating foci of diseases; and

WHEREAS, this method of detection of disease has proven to be efficient and has greatly reduced the need for funds for area testing procedures; and

WHEREAS, the number of properly identified animals moving to slaughter should be increased to improve the efficiency of this screening procedure; and

WHEREAS, the Consumer and Marketing Service has recently published for comment an amendment to 9 C.F.R. 310.2 requiring slaughtering establishments to maintain complete identification through slaughter;

THEREFORE, BE IT RESOLVED that the United States Livestock Sanitary Association recommend to the Animal Health Division that regulations be developed requiring all bovine animals over 2 years of age except steers and spayed heifers that are moving interstate for slaughter, to be identified with a device the use of which is restricted to regulatory officials.
RESOLUTION NO. 7

WHEREAS, there is concentrated and increased activity toward total elimination of certain animal diseases; and
WHEREAS, there is attention directed toward elimination of improper and indiscriminate use of medication, pesticides, insecticides and food additives through a systematic drug review and surveillance program; and
WHEREAS, the Wholesome Meat and Wholesome Poultry Products Act demand increasing utilization of fully competent veterinarians in regulatory technology as well as administrative ability; and
WHEREAS, the ability to properly administer these expanding veterinary public programs are the responsibility of veterinarians in the employ of local, State or Federal agencies; and
WHEREAS, regulatory veterinary administrators cooperate and coordinate with members of the veterinary profession of foreign countries, many of which function at the Cabinet level

THEREFORE BE IT RESOLVED that the American Veterinary Medical Association consider the establishment of a specialty board - American College of Regulatory Veterinary Practice - for those qualified veterinarians whose normal duties relate to the administration, management, direction and control of regulatory programs at the local, state and federal levels and for those veterinarians engaged in teaching, disease control and meat hygiene, and the principles of regulatory programs as well as veterinarians engaged in industrial endeavors and extension efforts.

BE IT FURTHER RESOLVED that the United States Livestock Sanitary Association initiate action to establish an organizing committee for the development of this specialty board.

RESOLUTION NO. 8

WHEREAS, 9 CFT, Part 71.3 provides that sheep found to have foot rot while in transit or upon arrival at a feed lot, stockyard or marketing center are allowed interstate movement under d(1) to immediate slaughter or d(4) to be quarantined, and
WHEREAS, this places an unfair and unwarranted burden upon owners whose sheep are found to have foot rot on farms or ranches, and
WHEREAS, these sheep should be allowed similar movement,

THEREFORE, BE IT RESOLVED that this Association request the Secretary of Agriculture to amend 9 CFR, Part 72 so as to afford such sheep the privilege of similar movement.

RESOLUTION NO. 9

WHEREAS, the livestock industry, the various States and the Federal Government have spent millions of dollars for the eradication of brucellosis which causes annual losses amounting to millions of dollars and hundreds of cases of undulant fever in humans; and
WHEREAS, 99.4 percent of the counties of the United States are moving
ahead to achieve eradication of the disease; and

WHEREAS, the industry in the remaining .6 percent of the counties of the United States is making little or no effort to reduce the high incidence of brucellosis known to exist; and

WHEREAS, the cost to all concerned of achieving eradication of brucellosis in the United States can be materially reduced if adequate controls are provided to prevent the spread of brucellosis; and

WHEREAS, existing Federal Interstate Regulations and those being proposed do not adequately protect the industry in these counties where the industry is eradicating brucellosis; and

WHEREAS, even calves from cows or herds infected with brucellosis may spread brucellosis to herds where the disease is being eliminated,

NOW, THEREFORE, BE IT RESOLVED that the United States Livestock Sanitary Association, assembled in convention at New Orleans, Louisiana this 11th day of October 1968, requests the Secretary of Agriculture to adopt interstate regulations effective January 1, 1969, requiring that all cattle moving interstate originate from Modified Certified Brucellosis Areas, Certified Bovine Brucellosis-Free Areas, or from herds known not to be affected with brucellosis; except (1) cattle moved only for immediate slaughter, or (2) cattle moved to quarantined feed lots which are under the direct supervision and control of the State Livestock Health Official and from which feed lot all animals are moved only for immediate slaughter in accordance with established procedures for handling quarantined animals.

BE IT FURTHER RESOLVED that a copy of this resolution be sent immediately to the Secretary of Agriculture with the request that no revision of Part 78, Title 9, Code of Federal Regulations, be published before this resolution has been considered.

(This resolution was adopted by the Resolutions' Committee with one dissenting vote cast by the member from the State of Florida.)

RESOLUTION NO. 10

WHEREAS, chemicals used as insecticides against ticks, lice, flies, etc. on livestock are registered with the Pesticides Regulation Division, Agricultural Research Service, United States Department of Agriculture, and

WHEREAS, chemicals used against mite infestation of livestock must be registered as drugs, and

WHEREAS, it is much more difficult and costly to obtain clearance for use, thereby inhibiting the development of miticides,

BE IT THEREFORE RESOLVED that compounds used externally against ectoparasites, including all species of mites, be permitted registration as pesticides rather than as drugs.

RESOLUTION NO. 11

WHEREAS the seventy-second annual meeting of the United States Livestock Sanitary Association held in New Orleans, Louisiana, October 6-11, 1968 has proven to be one of the most successful in the long history of the organization; and
WHEREAS the officers and members of the Association recognize that this success is the result of the unstinting efforts of all those who have devoted themselves to that end;

THEREFORE, BE IT RESOLVED that the special thanks and appreciation of the United States Livestock Sanitary Association be extended to

- The Committee on Local Arrangements
- The staff of the Louisiana office of the Agricultural Research Service, Animal Health Division
- The Veterinary Medical Association of New Orleans
- The New Orleans Chamber of Commerce
- The staff of the Livestock Sanitary Board, Louisiana Department of Agriculture
- The Commissioner of Agriculture of the State of Louisiana
- The Ketchum Manufacturing Company
- The National Band and Tag Company

BE IT FURTHER RESOLVED that the Secretary forward a copy of this resolution to each of those named.
STATISTICALLY IMPROVED ANIMAL TESTS FOR VACCINE EFFECTIVENESS

Bruce E. Mackey¹, Gary V. Richardson¹, J. M. Hejl²

Agricultural Research Service
United States Department of Agriculture

One of the functions of the Veterinary Biologics Division of Agricultural Research Service is the regulation of animal vaccines produced for interstate commerce. This service has in part been rendered by the development of standard requirements which the vaccines must meet before they are marketed. These standard requirements often involve animal tests for vaccine effectiveness which are applied on a serial to serial basis by one producer. The producer is also required to send a sample of each serial to Veterinary Biologic’s testing lab at Ames, Iowa where they have the opportunity to verify the producer’s tests. The purpose of this report is to discuss some of the problems involved in the present efforts to improve these tests.

The tests referred to here involve the challenge of two groups of animals with a disease agent. The “vaccinate group” is a group of animals that is vaccinated prior to challenge and the “control group” is a group of unvaccinated animals. The results of the test are measured by the number of animals “surviving” in each group. In this context, “survival” means failure to show symptoms of the disease in question.

In order to begin a study of the properties of the present tests, operating characteristic (O-C) curves were constructed for each test from the standard requirements. In this case, these curves are found by graphing the probability of accepting a vaccine against the entire range of possible vaccine test effectiveness levels. Rules concerning acceptance of a vaccine are specific to each requirement, i.e. the numbers of animals used plus the number required to survive in the vaccinated and control groups. Probability of acceptance is therefore synonymous with the probability of occurrence of these required animal survival rates for a particular vaccine test effectiveness level. Vaccine test effectiveness is defined as the proportion of susceptible animals protected by the vaccine. This test effectiveness refers to lab conditions only unless prior knowledge allows for translation to field conditions.

Exhibit one is constructed for a test using 2 vaccinates and 2 control animals with a possible range of 86-96% disease challenge. This disease challenge would be expected to produce positive disease reaction in 86-96% of the unvaccinated animals. Note that each curve represents a single disease challenge in this range. These curves show that if a 40% test effective vaccine were subjected to this test, it would have an approximate 18% chance of acceptance. For this test, both

¹Biometrical Services, Agricultural Research Center, Beltsville, Maryland
²Veterinary Biologics Division, Hyattsville, Maryland
vaccinates and neither of the controls must survive for acceptance. The probability of this occurrence is .18 if the vaccine is 40% test effective. If one or two controls survive, the test is called a “no test” and is repeated. For the exhibit one test, products must be nearly 100% test effective to obtain a reasonable probability of being accepted. This test would be poor if the maximum attainable test effectiveness were 90% because these upper range vaccines would only be accepted 80% of the time. This test would also be poor if there was a large proportion of products near 60% test effectiveness which we would like to reject because about 35% of these vaccines would pass.

The O-C curve for exhibit 8 shows that vaccine serials above 80% test effective have an excellent chance of passing this test. Recent data on the Blackleg Bacterin show a test effectiveness of near 100% indicating this test is probably too liberal in accepting 80-90% serials. Comparison of exhibits one and eight indicate that if Blackleg Bacterin has a greater test effectiveness than the products of exhibit one, the development of these tests was paradoxical.

Exhibit 9 illustrates the effect of increasing the number of animals on the test. This test is good if one wants to reject products below 70% test effective and accept products above 90% test effective. Increasing the number of animals allows us to distinguish between products in a smaller range of test effectiveness. However, we must then determine whether or not it is economically feasible to eliminate products below for example 70% test effective. If products in this category are very rare, it may well be economically un-sound to try to test for them. Of course the number of animals used in the exhibit 9 test may be impracticle for serial to serial testing, but it illustrates that a balance must be obtained between the benefits of stronger tests and their cost.

One can see that the O-C curves can not be used by themselves to evaluate the tests. Knowledge of the actual test effectiveness of serials being produced must be combined with the curves for each product separately. This will allow us to answer the question; what proportion of serials accepted and rejected meet our standards for test effectiveness?

The major problem which emerges from this study is determining what are the actual effectiveness levels of products being produced. It would be wrong to try to make a general statement in this matter for all products, but there are two schools of thought on the variability of effectiveness levels for the important products.

One idea is that a particular product will always be very close to the same effectiveness; and even if lab tests show variability for the product, it would be uniformly effective in the field. The argument for the latter assumption is that the disease challenge is greater and the product more dilute as tested in the lab. Recommendations in this case would be to obtain data on translation of lab tests to field conditions, and effectiveness testing would simply be to determine if enough units of immunizing agent are present in a particular vaccine serial. An animal test would be used to check on antigenicity, but it would require a relatively small number of animals.

There are equally reliable sources who maintain some products are variable in their effectiveness. They feel that variability in tissue culture materials and methods as well as variability of the disease agent itself can result in variable effectiveness. They also feel that differences shown in the lab tests may be important when
translated to the field. The recommendation in this case is to estimate this variability of test effectiveness levels in the lab and design the animal tests accordingly. If the variability proves to be great, a large number of animals are needed to separate the products of lower test effectiveness.

If either recommendation is followed, the required data will be expensive. Large scale projects would be needed to gain any useful translation data of lab to field results. In order to determine whether or not a product is variable in test effectiveness from serial to serial, a large number of animals is required on each serial. If a particular product does prove to be variable, the number of animals required for a worthwhile effectiveness test may be impractical. As long as neither type of data is available, we can only rely on the inductive reasoning of people who work with a product, and choose the O-C curve and corresponding test they think is best.

APPENDIX:

Exhibit 1  V8, V3 Canine Hepatitis Vaccine (killed)  
            V5 Feline Distemper Vaccine (killed-cat test)  
            V9 Ovine Ecthyma Vaccine  
            V1, V3 Canine Distemper Vaccine (killed)  

Exhibit 2  V5 Feline Distemper Vaccine (killed-mink test)  
            V39 Fowl Pox Vaccine  
            B20 Leptospira Bacterin  

Exhibit 3  V4 Equine Encephalomyelitis Vaccine  

Exhibit 4  V26 Newcastle Disease Vaccines  
            V27 Laryngotracheitis Vaccine  

Exhibit 5  V29 Newcastle Disease Vaccine  

Exhibit 6  B46 Erysipelas Bacterin  

Exhibit 7  V11 Rabies Vaccine, MLV  

Exhibit 8  B23 Blackleg Bacterin and Proposed for Clostridium Novyi Bacterin  

Exhibit 9  Forty-five vaccinates and ten controls.  
            Thirty-eight or more vaccinates and two or less controls must survive for acceptance.  

Note: The vaccine effectiveness labels on the graphs refer to test effectiveness.
Two vaccinates and two controls.

Both vaccinates and neither control must survive for acceptance.
ANIMAL TESTS FOR VACCINE EFFECTIVENESS

V8 Feline Distemper Vaccine (Killed-mink test)

V39 Fowl Pox Vaccine

B20 Leptospira Bacteria

Five vaccinees and five controls.
Four or five vaccinees and zero or one
controls must survive for acceptance.
Ten vaccines and ten controls.

Eight, nine or ten vaccinated and zero, one or two controls must survive for acceptance.
Ten vaccinates and five controls.

Eight, nine or ten vaccinates and zero or one controls must survive for acceptance.
Ten vaccines and ten controls.

Nine or ten vaccinees and zero or one control must survive for acceptance.
ANIMAL TESTS FOR VACCINE EFFECTIVENESS

B46 Erysipelas Bacteria

Four vaccines and four controls.

Three or four vaccines and zero or one
controls must survive for acceptance.
Ten vaccinates and five controls.

Seven or more vaccinates and zero or one controls must survive for acceptance.
B23 Blackleg Bacterin and proposed for Clostridium novyi Bacterin.

Test one: Five vaccinates and ten controls.
Four or five vaccinates and two or less controls must survive for acceptance.

Test two: Ten vaccinates and ten controls.
Seven or more vaccinates and two or less controls must survive for acceptance.

If the vaccine fails test one, test two is applied.
This example shows the effect of increasing the number of animals on the operating characteristic curve of the test.

Forty-five vaccinates and ten controls.
Thirty-eight or more vaccinates and two or less controls must survive for acceptance.
REPORT OF THE VETERINARY BIOLOGICS COMMITTEE OF U.S.L.S.A.


The field of Veterinary Biologics development, production, evaluation and reevaluation is rapidly changing. It is difficult to keep abreast of these changes even when one is involved with them on a day-to-day basis. Your Committee has discussed some of these problems brought about by an expanding technology in microbiology which have indicated a need for more searching evaluation of some of these products. This report will highlight a few of these problems.

CHANGE IN VIRUS-SERUM-TOXIN ACT

The Veterinary Biologics Division and the Agricultural Research Service of the U.S. Department of Agriculture are developing proposed amendments to the 55 year old Virus-Serum-Toxin Act for the purpose of updating, defining and clarifying requirements for production and testing of modern veterinary biologics.

ANTIBIOTICS IN VETERINARY BIOLOGICS

Antibiotics have been used in live virus vaccines as preservatives for several years. Now, with the concern for antibiotic residues in meat, milk, and eggs, the level of antibiotics used in vaccines has been reviewed. The levels of antibiotics used by different manufacturers were not always the same and the levels were also not the same for all products. Therefore, in addition to the residue concern, there is now a concern that antibiotic levels must be limited to those which will not interfere with valid tests for bacterial contaminants. If permitted levels are set too low, the biologics producers are faced with a probable high discard rate of product during processing.

STERILE LIVE VIRUS VACCINES

Live virus vaccines which are subject to many manipulations in preparation and use of living tissue in their cultivation are quite easily subject to minor contamination by environmental bacteria or fungi. Thus very low levels of bacterial contaminants have been permitted in the final product. Now, there is interest expressed in having the Veterinary Biologics Division of the Agricultural Research Service to require all such products to be sterile. Producers of these products agree that this is not impossible but is very impractical (or unnecessarily costly) due to an unwarranted waste of safe and effective vaccines. The unwarranted increase in cost
would have to be borne by the uninformed user of such products. The Veterinary
Biologics Division is contemplating issuance of proposed requirements for sterility
of live virus vaccine, first for those which are injected parenterally. Your comments
will be solicited. Manufacturers of these vaccines hope you will consider the
increased cost potential of this move against the need for it when you make such
comments as we do not feel it is necessary for the safety of these vaccines.

NEW METHODS FOR EVALUATING VETERINARY BIOLOGICS

In the past year, the following new methods of testing licensed biologics were
imposed or proposed for further trial:

Standards Adopted
1. Clostridium novyi bacterin-toxoid.
2. Parainfluenza 3 vaccine.

Proposed
1. Rabies vaccine tissue culture origin live virus.
2. Rabies vaccine tissue culture origin killed virus.
3. Changes in potency tests
   a) Clostridium perfringens Type C toxoid.
   b) Clostridium perfringens Type D toxoid.
   c) Clostridium perfringens Type C anti-toxin.
   d) Clostridium perfringens Type D anti-toxin.

Methods Under Development
Potency test method for Clostridium hemolyticum bacterin.

NEW PRODUCTS LICENSED THIS YEAR

Special Licenses
1. Allergenic Extracts for use in desensitizing small animals to certain
   proteins.
2. Mycoplasma synoviae plate test antigen.

Regular Licenses Issued
1. Streptococcus vaccine Group E (avirulent live culture).
2. Clostridium chauvoei-Septicum Novyi-Sordelli Bacterin.

TESTING RESULTS THIS YEAR

The Veterinary Biologics Division Laboratory at Ames, has tested a substantial
percentage of licensed veterinary biologics with the following results:

<table>
<thead>
<tr>
<th>Type of Test</th>
<th>Percent of Industry Output Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Sterility</td>
<td></td>
</tr>
<tr>
<td>or Mycoplasma</td>
<td></td>
</tr>
<tr>
<td>Contamination</td>
<td>50%</td>
</tr>
<tr>
<td>Potency</td>
<td>25%</td>
</tr>
</tbody>
</table>
Lots rejected  
(All products for any cause)  

22%  

This 22% rejection rate is reported to be reduced from a former 13-14% rejection rate.  

All lots of poultry live virus vaccines have been tested for Mycoplasma contamination and none have been rejected.  

**PROPER USE OF VETERINARY BIOLOGICS**  

Several factors involved in the proper use of bacterins and vaccines were discussed, such as:  

1. Best routes of injection.  
2. Maternal antibody interference.  
3. Relation of age to response to antigens.  
4. Best routes of administration:  
   a) For maximum response.  
   b) To minimize injection residue potential.  

While these questions can be answered for some products, it isn't possible to generalize. Therefore, the Committee strongly recommends that the label and direction sheet instructions for each product be followed carefully since these factors are considered for each product and labeling is prepared to direct its use for safety and effectiveness.
AN ATTENUATED ANAPLASMA MARGINALE VACCINE

by

Miodrag Ristic,* D.V.M., Ph.D., Stevan Sibinovic,** D.V.M., M.S., and C. Joseph Welter,*** Ph.D.

Anaplasmosis is an infectious and transmissible disease of cattle, characterized by progressive anemia associated with the appearance of intraerythrocytic bodies named *Anaplasma marginale.* The disease causes severe economic losses to cattle industry in the United States and throughout tropical and subtropical regions of the world. In the absence of factual knowledge of the nature of *A. marginale* and in view of the close resemblance of certain clinical signs of anaplasmosis to protozoon babesiosis, it was thought that the *Anaplasma* organism was also a blood protozoon parasite.

The breakthrough into the mysterious nature of the marginal *Anaplasma* body started with the study by De Robertis and Epstein which revealed that the organism was not a compact simple entity, the so-called "chromatin body," described by Theiler, but was actually an inclusion body consisting of a number of smaller subunits. More definite understanding of the structure and mode of development of *Anaplasma* came from a series of studies by Ristic, Ristic and Watrach, and Ristic and Kreier, which showed that the subunit of the marginal body, termed the initial body by these investigators, was the true etiologic agent of anaplasmosis and that the ability of the initial body to invade mature erythrocytes and multiply in them by binary fission led to the formation of the marginal inclusion body. The structural and developmental resemblance between the initial *Anaplasma* body and the rickettsia has contributed to the classification of the *Anaplasma* under the order of Rickettsiales.

Immunizing injection is considered the only practically functional and lasting type of immunity in naturally occurring anaplasmosis. The underlying mechanism of this immunity seems to be based on the persistence in the bovine host of *Anaplasma* at low levels of development necessary for maintenance of immulologic processes. These processes, specific or nonspecific and humoral or cellular in nature, all seem to be essential prerequisites for immunity in anaplasmosis. Immunization of cattle using blood containing live *Anaplasma centrale* has been practiced in certain areas of Africa for more than half a century and during the last decade *A. centrale* was introduced in some South American countries and

From the Department of Veterinary Pathology and Hygiene, College of Veterinary Medicine, University of Illinois and the Department of Research, Diamond Laboratories, Inc., Des Moines, Iowa.

*Professor of Veterinary Pathology and Hygiene and of Veterinary Research, University of Illinois.

**Research Veterinarian, Bionetics Research Laboratories, Kensington, Maryland.

***Director of Research, Diamond Laboratories, Inc.

This investigation was supported in part by a research grant from Diamond Laboratories, Inc., Des Moines, Iowa.
ANAPLASMA MARGINALE VACCINE

Australia. Recent serologic studies revealed that *A. centrale* and *A. marginale* was produced. On the basis of cross-vaccination and cross-challenge of cattle with the two strains, it was demonstrated that the protection offered by the method is partial and clinically insufficient. In addition, it was clearly demonstrated that *A. centrale* is too virulent and thus cannot be used without risk as a immunizing agent.

Some eight years ago we started experimental work toward development of an attenuated vaccine for anaplasmosis with the following objectives in mind: (1) The strain should be *A. marginale* (2) the attenuated organism should be sufficiently immunogenic to confer complete protection against challenge with the virulent strain, (3) it should be attenuated to a degree that it can be safely inoculated into susceptible adult cattle, (4) the attenuated organism should not revert to virulence when transmitted by means of ticks or subinoculation of blood from vaccinated to susceptible cattle, and (4) finally, we felt that it would be mandatory that this vaccine be produced in nonbovine hosts because of the hazards of transmitting other bovine infections in the process of vaccination.

This report describes the development of an attenuated *A. marginale* vaccine which meets the above objectives.

**MATERIALS AND METHODS**

The *A. marginale* isolate which was used in these studies was originally secured by Dr. A. A. Sanders* as a pool sample from naturally infected cattle in various sections of Florida.

All animals employed for the attenuation of *A. marginale* were splenectomized at least 2 months before use in the experiment. They consisted of 4 to 9 month old Holstein calves, 1 to 3 year old ewes, and 1 to 2 year old white-tailed deer (*Dama virginiana*). Venous blood samples collected directly in sterile tubes or bottles containing heparin, the final concentration of which was approximately 25 units per ml., were used throughout the study.

The basic methodology used for the attenuation of *A. marginale* has been: (1) Acceleration of the rate of dissociation of a virulent *A. marginale* by exposure to X-ray irradiation (2) selection of an avirulent *A. marginale* variant by serial passages of irradiated organisms in nonbovine ruminants including deer and sheep. Ten milliliter of blood with 70% of its erythrocytes parasitized obtained from an experimentally infected calf was first exposed to 60,000 roentgens of X-ray irradiation for 45 minutes. The blood was then inoculated *via* the jugular vein into a deer. Five hundred milliliters of blood from this deer was transfused into another deer 12 days later. Approximately 500 ml of blood was then transferred from this deer into an ewe 15 days later. Thereafter, serial blood passages of quantities varying between 300 and 700 ml. were made in 58 ewes in intervals between 14 and 30 days (Fig. 1).

Blood quantities varying between 5 and 50 ml. were obtained from passages 51

*Department of Veterinary Science, University of Florida, Gainesville, Florida.*
to 58 and inoculated subcutaneously into corresponding numbers of splenectomized susceptible calves in order to establish the virulence of this organism for cattle. It was concluded from these experiments that the organism had lost its virulence for cattle as evidenced by the survival of infected splenectomized calves and, moreover, absence of clinical signs of the disease in these animals. The attenuated *A. marginale* strain was subjected to additional 77 consecutive sheep passages for a total of 135 passages.

Whole blood, containing 15 to 30% infected erythrocytes was harvested from the donor sheep in 500 ml. bottles containing ACD solution.* A final concentration of 10% glycerol was added to the blood and the mixture was suspended in 5 ml. vials and frozen at -65°C. Frozen vaccine was thawed by dipping the vials in lukewarm water for 2 to 4 minutes. The vial was then manually agitated and the content administered intramuscularly in the hind upper leg.

**RESULTS**

**A. Study of the safety of the vaccine**

1. **Inoculation of pregnant cattle**

Three Holstein, two Angus, and six Hereford cows which were four to eight months pregnant, each were inoculated subcutaneously with 10 ml.

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*Baxter Laboratories, Inc., Morton Grove, Illinois*
of vaccine. All of these animals delivered healthy calves. The calves were observed from a period of two to six months. Antibodies were detected in the serum of these calves by the Capillary Tube Agglutination (CA) test,** for at least the first two months of life.

In another experiment, 20 Holstein *Anaplasma*-free dairy cows in the sixth to eighth month of pregnancy were each inoculated intramuscularly with 5 ml of the attenuated *A. marginale* vaccine. The average peak parasitemia was 8.7% and occurred on an average of 39 days following vaccination. The average initial packed cell volume (PCV) of 35% decreased to an average low of 19% approximately 44 days after vaccination (Table 1). A prompt reticulocyte response averaging 3.5% was noted in all animals approximately 42 to 49 days following vaccination. None of the 20 vaccinated cows showed signs of anaplasmosis. Nineteen cows delivered healthy calves. One cow (426) delivered a dead calf 45 days following vaccination. Clinical and bacteriologic examination of the cow revealed bacterial metritis and septicemia.

<table>
<thead>
<tr>
<th>NO. Animals</th>
<th>Vaccine Dose and Route of Inoculation</th>
<th>AVERAGE VALUES</th>
<th>NO. Calves Delivered</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Pregnant Dairy Cattle</td>
<td>5 ml i.m.</td>
<td>PCV Initial</td>
<td>35%</td>
<td>Peak Parasitemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lowest</td>
<td>19%</td>
<td>(44 days)</td>
</tr>
</tbody>
</table>

*Calving occurred before, during and after the period of parasitemia.

**Bacterial metritis and septicemia.

Table 1: Safety study of attenuated *A. marginale* vaccine. Hematologic responses in 20 pregnant dairy cattle inoculated with the vaccine. None of the animals showed clinical signs of anaplasmosis.

2. *Attempts of reversion to virulence by blood subinoculation.*

Three trials of this type were conducted employing susceptible cattle, at least two years old. In the first trial, a milliliter of blood obtained from vaccinated animals at the peak of parasitemia was subinoculated through a series of 10 consecutive passages. In the second trial, 5 ml quantities of blood collected from an animal approximately 3 months after vaccination were carried through similar series of six passages. With the exception of occasionally transient slight temperature increase, no other signs of anaplasmosis were observed.

In the third trial 12 pregnant and Anaplasma-free Holstein cows were used to make 4 additional blood passages from the 20 vaccinated pregnant cattle described above. One milliliter of fresh citrated blood, collected from the donor animal at the time of maximal parasitemia and inoculated subcutaneously into recipient animal, was used for all back passages. No signs of anaplasmosis were noted in any of the 12 cows. These cows delivered live and healthy calves. The information pertaining to the average initial and lowest PCV in 5 cows used in the first passage, 3 cows used in the second passage and 2 cows used in each fourth and fifth passage is shown in figure 2.

<table>
<thead>
<tr>
<th>Passage</th>
<th>Initial PCV</th>
<th>Lowest PCV</th>
<th>Average PCV</th>
<th>Average PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>464</td>
<td>470</td>
<td>451</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>+ PCV 14%</td>
<td>+ PP 15%</td>
<td>+ PCV 12%</td>
<td>+ PP 3%</td>
</tr>
<tr>
<td>2nd</td>
<td>455</td>
<td>456</td>
<td>459</td>
<td>460</td>
</tr>
<tr>
<td></td>
<td>+ PCV 23%</td>
<td>+ PP 1%</td>
<td>+ PCV 16%</td>
<td>+ PP 7%</td>
</tr>
<tr>
<td>3rd</td>
<td>303</td>
<td>304</td>
<td>301</td>
<td>473</td>
</tr>
<tr>
<td></td>
<td>+ PCV 24%</td>
<td>+ PP 5%</td>
<td>+ PCV 28%</td>
<td>+ PP 6%</td>
</tr>
<tr>
<td>4th</td>
<td>396</td>
<td>395</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ PCV 33%</td>
<td>+ PP 6%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PCV = Packed cell volume of the donor animal at the time passage was made. No clinical signs of anaplasmosis were observed in any of the animals.

Figure 2: Back passage of attenuated *A. marginale* in pregnant dairy cattle. No clinical signs of anaplasmosis were observed when 1 ml. quantities of parasitized blood originally obtained from vaccinated animals was passed four times consecutively in 12 pregnant dairy cattle.

3. **Inoculation of massive doses of the vaccine**

Eight susceptible Holstein cows, five to six years of age, were each inoculated intravenously, subcutaneously, or intramuscularly with quantities ranging from 25 to 150 ml. of the vaccine. All of the eight cows which received vaccine developed erythrocytic inclusions and showed hematocrit values which dropped from the original value to a maximum of 14% yet none of the animals developed any clinical signs of anaplasmosis. A similar Holstein cow which received 5 ml. of the virulent *A. marginale* developed fever, anorexia, weight loss, severe anemia, and subsequently died.

4. **Inoculation of cattle infected with Leptospira Hardjo**
Three yearling Herefords (one of which was pregnant) artificially infected with *Leptospira hardjo* were each inoculated intramuscularly with 20 ml. of the vaccine. All animals responded serologically in the CA test approximately 5 weeks later. Deviations in hematocrit readings after vaccination were very minimal, from 2 to 6%. Percent infected erythrocytes in vaccinated animals ranged from 0.1% to a maximum of 2%. There were no deviations in hematocrit levels or signs of anaplasmosis in any of the animals after challenge with 5 ml. of carrier blood.

5. *Inoculation of lactating dairy cattle*

Use of the vaccine in 18 lactating Holstein dairy cattle, 6 of which were pregnant, gave the following results. In the first experiment, 10 of 18 vaccinated animals developed clinical signs of anaplasmosis and were treated. The average packed cell volume in these animals ranged from an initial 33% down to 17%. All vaccinated animals, however, recovered completely within four weeks after onset of the post-vaccine reaction. In the second experiment involving 4 lactating Holstein dairy cows, none of the animals developed clinical signs of anaplasmosis as a result of vaccination. A sharp drop in average PCV, from 32% to 15% was noted. The PCV of the four animals returned to prevaccination values within 2 weeks without treatment.

6. *Tick transmission studies*

Adult *Dermacentor andersoni* ticks, obtained from the National Institute of Health's Rocky Mountain Laboratory in Hamilton, Montana, and known to be pathogen free, were used to study the natural transmission of the vaccine strain. The infection was not transmitted with the vaccine strain by the tick feeding technique or by inoculating susceptible cattle with homogenates of various tick tissues. These findings were further substantiated by the results obtained from the examination of various tick tissues by means of the fluorescent antibody method and electron microscopy. On the other hand, *Anaplasma* was found in the Malpighian tubules, salivary glands, and ovaries of ticks fed on calves inoculated with the virulent strain of the organism and the infection was transmitted to cattle with these ticks and their tissues.

In another experiment, mechanical transmission of the vaccine strain was demonstrated in one of two contact calves which were held in isolation with two vaccinated tick-infested calves.

**B. Studies of Protection Conferred by the Vaccine.**

1. **Laboratory experiments**

A total of 238 adult (2 to 14 years old) cattle of mixed breeds were used in various experiments to ascertain the degree of protection, if any, conferred by the vaccine.

In a typical experiment, 15 Hereford cattle from 4 to 14 years old were used (Fig. 3). Their susceptibility to infection with *A. marginale* was determined by examining their blood serum by means of the capillary tube agglutination (CA) and complement fixation (CF) test. In addition, blood samples from each animal were stained by the Giemsa and acridine
Eleven of the animals were inoculated subcutaneously with the vaccine; six received 10 cc. and five received 5 cc. The remaining four animals were kept as controls. Five to 6 weeks later, all vaccinated animals became positive to the CA and CF tests and their erythrocytes contained between 3 and 7 percent of Anaplasma inclusions. Hematocrit values dropped from prevaccination values, to between 2 and 12%, the average reduction being 6%. No clinical signs of anaplasmosis or other abnormalities were observed in any of the animals. At 72 days, both principals and controls were inoculated subcutaneously with 10 ml. of blood from a virulent A. marginale carrier. All four animals in the control group succumbed to clinical anaplasmosis five weeks after challenge. Three of the animals died and one slowly recovered four months after challenge.
However, no clinical or hematological abnormalities were observed in any of the vaccinated animals.

From the remainder of this series of experiments, it was learned that the success or failure of the vaccination could best be measured by the persistence or disappearance of the CA titer following vaccination. The persistence of the CA and CF reactions indicated the persistence of immunity, while the disappearance of the CA and CF reactions indicated that the animal was no longer resistant to *Anaplasma* infection. It was further observed that immunity conferred by a single vaccination with the attenuated *A. marginale* strain stimulated persistent protection against infection for at least three years. Our studies covered only a three year period, and hence duration of protection may last even longer. Animals as young as two months and as old as 14 years appeared to be equally responsive to inoculation with the vaccine.

2. *Challenge of immunity with foreign isolants of A. marginale*

It was of further interest to ascertain the resistance conferred by the vaccine by challenging vaccinated cattle with *A. marginale* from different geographic regions. These experiments included several trials in certain Latin American countries. A typical experiment of this kind was conducted by the scientists of the National Center of Animal Pathology in Lima, Peru. In this experiment, eighteen Brown Swiss cattle, 2 to 6 years old and free of *Anaplasma*, were divided into 3 groups. Group 1 was composed of 6 animals; each were inoculated with 5 ml. of the vaccine.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>2-YEAR BROWN SWISS CATTLE</th>
<th>VACCINE DOSE</th>
<th>POST VACCINATION AVERAGE</th>
<th>POST CHALLENGE AVERAGE</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Packed cell Volume</td>
<td>Temperature°C</td>
<td>Maximum Parasitemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Lowest</td>
<td>Initial</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>5 ml</td>
<td>44</td>
<td>28</td>
<td>38.5</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>10 ml</td>
<td>48</td>
<td>28</td>
<td>38.5</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>non vaccinated</td>
<td>43</td>
<td>39</td>
<td>38.3</td>
</tr>
</tbody>
</table>

High initial PCV is due to the fact the experiment was started on the day the animals were introduced from the High-Lands of Sierra (11,000 feet) to the Lima Area (Sea Level). In addition, the animals were transferred without water and feed for two days.

**Table 2: Study of attenuated *A. marginale* vaccine in Peru by the National Center of Animal Pathology in Lima.** Clinical and hematologic data on 11 vaccinated and 5 control cows, each of which were challenged with 5 ml. of blood of an *Anaplasma* carrier from Tingo Maria jungle area, are given.
The 7 animals of group 2 were each inoculated with 10 ml. of the same vaccine. The 5 animals (group 3) served as non-vaccinated controls. Approximately 2 months later all 18 animals were each challenged with 5 ml of blood from a carrier of virulent isolant of *A. marginale* originally secured from a cow which died of anaplasmosis in the Tingo Maria jungle area of eastern Peru. All inoculations were made intramuscularly. The five control animals developed typical signs of anaplasmosis and died within 45 days after challenge. The vaccinated animals had no hematological or clinical abnormalities following the challenge. (Table 2)

Figure 4: Electron micrographs of ultrathin sections and photomicrographs of fluorescent antibody staining of erythrocytes infected with *A. marginale* (A, a) and *P. caudatum* (B, b), respectively, A-29, 000 X; a-1, 200 X; B-34, 000 X, and b-1100 X. Electron micrograph (B) was obtained through the courtesy of Drs. C. F. Simpson, J. M. Kling, F. C. Neal and the Journal of Cell Biology.
3. Challenge of immunity with isolants containing *A. marginale* and *Paranaplasma caudatum*.

Mixed infections with *A. marginale* and *P. caudatum* are known to occur in cattle in various regions of the United States and in certain foreign countries. *Paranaplasma caudatum* is known to share serologically some but not all antigens with *A. marginale*. In addition, *P. caudatum* possesses certain structural features not found in *A. marginale* (Fig. 4) It was deemed important to observe the response of vaccinated cattle to a challenge with blood containing *A. marginale* and *P. caudatum*. Figure 5a illustrates a typical response noted in one of the three vaccinated 4-year old Hereford cows following challenge with the Oregon isolant* of *Anaplasma*, approximately 3 months after vaccination. The strain was known to contain both *A. marginale* and *P. caudatum*.

Approximately 27 days after challenge the animal had the lowest PCV (22%) which constituted an 18% drop from the initial 40%. The recovery of PCV, however, was rapid. Within approximately 2 weeks the PCV returned to pre-challenge values. The parasitemia was maximal (11%) at approximately the 26th day following challenge. In this animal, *P. caudatum* organisms in lysed erythrocytes seen by phase-contrast microscopy and marginal bodies seen in Giemsa-strained blood films appeared at

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*Figure 5: A three year Hereford cow inoculated with attenuated *A. marginale* vaccine and challenged with blood containing both *A. marginale* and *P. caudatum* approximately three months after vaccination. Notice that *P. caudatum* predominately developed following the challenge. The animal did not show any clinical signs of anaplasmosis (a).

Hematologic response of a cow similar to that above, which was challenged with a pure strain of *A. marginale*. No anemia or parasitemia were observed in this animal (b).*

*Received through the courtesy of Dr. O. H. Muth, Department of Veterinary Medicine, Oregon State College, Corvallis.
the same level, and disappeared at the same time. The finding indicated
that P. caudatum predominately developed following the challenge. The
animal, however, did not show any clinical signs of anaplasmosis. This is in
contrast to the absence of parasitemia and anemia in vaccinated animals
challenged with pure strain of A. marginale. Figure 5b)
4. Field experiments

These experiments were designed to determine the stability of the
attenuated vaccine strain under field conditions and the extent of the
protection conferred by the vaccine to natural challenge. More than 300
animals were vaccinated in various trials. Forty two-year old Hereford
cattle in Mexico City (the Vera Cruz experiment) underwent the most
drastic exposure to environmental stress and field challenge.\textsuperscript{22} Twenty of
these animals were vaccinated and 20 were not before they were shipped
to the Vera Cruz jungle. The animals were not dipped or sprayed to
protect them from ticks until near the end of the study. They were left in
the jungle for six months, during the spring, summer and fall of 1967. One
of the authors visited the area. The climate there is tropical, with
temperatures averaging 95° to 105° F and humidity ranging between 80
and 100%. Many varieties of ticks infest this area which is known to have
extremely high prevalences of babesiosis and anaplasmosis. The experi-
tment was carried out under the direct supervision of the officials of the
Mexican government. No adverse effects attributable to vaccination were
observed either during the long transport to or period in the jungle. At the
end of the 6 months in the jungle, 7 of the vaccinated and 13 of control
animals had died. Unfortunately, the cause of death of none (vaccinated or
nonvaccinated) was determined. However, it was known that in addition
to anaplasmosis, deaths of cattle could be attributed to a variety of factors
ranging from snake bite, massive invasion by ticks or other arthropods,
heat and humidity, babesiosis, etc.

Similar experiments were carried out in Venezuela,\textsuperscript{23} Colombia,\textsuperscript{24}
and Peru\textsuperscript{25} and the results of these studies are most encouraging. In the
latter country,\textsuperscript{26} 20 vaccinated and 5 nonvaccinated 2 to 5 year old cattle
were introduced into the tick infested area of eastern Peru from the high
Sierras. Within a month after introduction, 3 of the control animals died
of anaplasmosis with the remaining 2 animals being saved through
application of systemic tetracycline therapy. At 9 months after intro-
duction, the last time observation was made, vaccinated animals were free
of any evidence of anaplasmosis or any other disease. Several other field
experiments involving small groups of animals have produced similar
results.

Comments and Conclusions

The first attenuated A. marginale vaccine developed by selecting an avirulent
variant of the organism in an atypical host has been described in this report. With
the exception of a few lactating Holstein dairy cattle, some of which had mild signs
of anaplasmosis following vaccination, all cattle studied, numbering more than 900
in laboratory and field experiments, showed no signs of anaplasmosis. Experimental animals ranged in age from 3 months to 14 years and among these were also pregnant cattle and splenectomized calves. The attenuated Anaplasma organism did not revert to virulence when passed consecutively 10 times in mature cattle and 5 times in pregnant cows. Dermacentor andersoni ticks did not biologically transmit infection with the attenuated Anaplasma and there was no serologic or electron microscopic evidence that the organism had grown in the tick. On the other hand, infection was transmitted by the same type of ticks when these were fed on calves inoculated with a virulent strain of A. marginale and the organism was detected in the Malpighian tubules, salivary glands and ovaries of these ticks. Administration of the vaccine into cattle experimentally infected with Leptospira hardjo did not produce any adverse reaction.

All vaccinated animals developed solid and durable immunity as revealed by their ability to resist the massive laboratory and field challenge doses with virulent A. marginale to which they were exposed 8 weeks to 3 years following administration of the vaccine. Upon challenge, PCV of all vaccinated animals generally continued to maintain prevaccination levels and maximal parasitemia seldom exceeded 3 percent. When vaccinated animals were challenged with blood containing both A. marginale and P. caudatum, a transitory decrease of PCV followed by appearance of the latter parasite in the peripheral blood were noted. In general, the animals did not develop clinical signs of acute anaplasmosis and improvement of PCV was rapid.

The serum from vaccinated animals reacted in the CA and CF tests approximately 4 to 6 weeks following administration of the vaccine. Persistence of the agent and thus resistance to clinical reinfection was directly related to persistence of the CA and CF persistence of these reactions depends on endurance of latent or carrier form of anaplasmosis. Thus it could be concluded that resistance in anaplasmosis depends on persistence of a latent infection in a given animal. It is the latter syndrome that is initiated and maintained in animals inoculated with attenuated A. marginale vaccine.

Losses due to anaplasmosis in the United States are greatest in endemic areas. In these areas, high percentages of cattle are already infected and the remaining "clean" cattle are under continuous danger of the disease. Authorities who have been close to the anaplasmosis problem in these areas agree that isolation of carrier animals from herds provides for increase in death losses due to the disease. Application of the attenuated vaccine to cattle in these endemic regions would afford United States' cattlemen the long sought and patiently awaited protection against anaplasmosis. The mechanism by which protection is conferred by the attenuated vaccine is based on and has been substantiated by scientifically accepted facts. It is truly the only type of protection known to safeguard cattle from severe exposure to Anaplasma organisms as it frequently occurs in regions where the disease has been traditionally established due to the existing arthropod vector and, possibly, wild animal reservoirs.

Outside the United States, in tropical and subtropical areas of the world, anaplasmosis is still one of the most severe hemotropic diseases and, as such, has greatly hampered the development of livestock industries. The United States is
providing substantial economic assistance to many of the underdeveloped countries in these areas. Introduction and implementation of the attenuated vaccine in these areas would augment the growth of their livestock industry by enabling the domestic and improved cattle to graze safely on the newly developed pasture lands.

The authors express appreciation for their cooperation in certain experiments reported in this paper to Drs. A. G. Castillo, San Marcos University, Lima, Peru; H. Esparaza, National Center for Animal Disease Research, Palo Alto, Mexico, D. F.; K. L. Kuttler, Texas A and M, College Station, Texas; Cesar Lora, Center of Animal Pathology, Lima Peru; Carlos Leon, Center of Veterinary Investigation, Maracay, Venezuela; M. E. Mansfield, University of Illinois, Urbana, Illinois; N. A. Popovic, Walter Reed Army Institute of Research, Washington, D. C., R. L. Taylor, University of Nevada, Reno, Nevada; and W. F. Schroeder, Center of Veterinary Research, Maracay, Venezuela.

REFERENCES

COMPARISON OF CARD AGGLUTINATION WITH COMPLEMENT FIXATION FOR DETECTING BOVINE ANAPLASMOSIS


Complement fixation (CF) and capillary agglutination (CA) are serologic procedures currently available for diagnosing bovine anaplasmosis. These procedures, best performed in the laboratory, do not lend themselves to field use. Results from a rapid card agglutination test were encouraging when used recently on herds of known anaplasmosis status. Because of its ease and rapidity, the card test could be used for diagnosing anaplasmosis under field conditions and, therefore, warrants further study.

Through the cooperation of Federal and State veterinarians in Arkansas and Virginia the efficacy of the card test conducted on farms was compared with that of the CF test conducted in the laboratory. The study involved 2 breeding beef herds of 188 cattle, and 2 dairy herds totaling 265 head. Three herds were selected because they had a history of anaplasmosis and because the owners were cooperative and interested in controlling the disease. The fourth herd was selected because it was known to be anaplasmosis-free.

During the studies, anaplasmacidal compounds were administered to some CF reactors. Administration of treatments constitute one of the procedures in a different study aimed at determining the effectiveness of tests and treatments of reactor animals as a means of eliminating anaplasmosis from a herd. This report concerns (1) the efficacy of the card test in relation to the CF test, and (2) the effects of treatments on the CF and card test reactions in relation to the presence or absence of anaplasmosis in the animals tested.

PROCEDURES

Erythrocytes from cattle in the acute phase of anaplasmosis were subjected to high pressure to remove the parasites. The accumulated organisms, after processing, served as antigen for the card test. For use, the antigen was stained with fast green dye to facilitate reading of agglutination reactions on Brewer diagnostic test cards. Card testing was conducted as the blood samples were taken. Blood was obtained from the tail vein, using micro blood plasma collectors containing an anticoagulant. One-half ml. of blood was sufficient for a test. Immediately after

*From the Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705

**From the Animal Health Division, Agricultural Research Service, U. S. Department of Agriculture, Federal Center Building, Hyattsville, Maryland 20781
TABLE 1
Comparison of Anaplasmosis Complement-Fixation and Card Agglutination Reactions.

<table>
<thead>
<tr>
<th>CF Reactions</th>
<th>Number Tested</th>
<th>Card Positive</th>
<th>Reactions</th>
<th>Percentage of Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. marginale Infected Herds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>79</td>
<td>65</td>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td>Suspect</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>87</td>
</tr>
<tr>
<td>Negative</td>
<td>216</td>
<td>21</td>
<td>195</td>
<td>90</td>
</tr>
<tr>
<td><strong>A. marginale Non-Infected Herd</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>150</td>
<td>2</td>
<td>148</td>
<td>99</td>
</tr>
</tbody>
</table>

collection, the collectors containing the blood were centrifuged, in a small 12-volt centrifuge* that plugged into the cigarette lighter outlet of an automobile. Five one-hundredths of a ml. of the plasma was mixed with one drop (approximately 0.015 ml.) of antigen and the mixture was rotated mechanically (approximately 100 rotations per minute) for 4 minutes with a card test rotator.* The reactions were read immediately, the results recorded and later compared with results of CF tests subsequently conducted in the laboratory.

An Arkansas herd (Herd 1) had 4 deaths attributable to anaplasmosis, in the fall of 1967. Our first tests, CF and card, were conducted in January, 1968. The herd at that time, consisting of 82 head, was segregated into non-reactors and reactors, on the basis of CF test results. The reactors were then fed a diet containing Aureomycin** for 30 days. Both tests were again conducted in June, 1968. At that time the herd consisted of 63 animals, because 19 CF-negative cattle had been sold during the spring. Forty ml. blood samples from all treated cattle were collected for calf inoculations. The blood was collected in tubes containing 5 ml., sterile 20 percent sodium citrate solution. Immediately after collection, the samples were placed in a styrofoam box containing ice and shipped air express to the laboratory. Within 24 hours after collection each sample was injected intravenously into a splenectomized calf with 2 exceptions. One calf received a pooled sample from 2 CF and card-negative cows. Another calf received pooled blood from 3 CF suspect cattle (2/ reaction, 1:5 dilution). Blood from each of 5 CF reactor cattle, 4 of which were card-negative, was inoculated into individual splenectomized calves. (See Table 2)

A Virginia herd (Herd 2) consisted of 104 animals, 55 of which were beef cows. Two herd tests, (CF and card) were conducted in March and July and are considered in this report. On the basis of the July test results, 15 animals were

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*Wynson Westcott and Dunning, Inc., Baltimore, Md. 21201

**Aureomycin (fed in the form of Auromycin 50) American Cyanamide Co., Princeton, N.J.
TABLE 2

*Anaplasma marginale*; Comparison of Complement-Fixation (CF) and Card Agglutination Reactions with Results of Calf Inoculation Trials

<table>
<thead>
<tr>
<th>Donor number</th>
<th>CF reaction</th>
<th>Card reaction</th>
<th>Results of calf inoculations for <em>A. marginale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Herd 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1:40</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>1:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 (pooled)</td>
<td>2+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7 (pooled)</td>
<td>2+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8 (pooled)</td>
<td>1+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9 (pooled)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 (pooled)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Herd 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (pooled)</td>
<td>1:40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 (pooled)</td>
<td>1:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3 (pooled)</td>
<td>2+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 (pooled)</td>
<td>2+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7 (pooled)</td>
<td>Trace</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8 (pooled)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Untreated cattle, Herd 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1:5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>1:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>2+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>2+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*3.5 months after treatment with Aureomycin 50
**3.5 months after treatment with a ethoxyethylglyoxal dithiosemicarbazone

selected for calf inoculation studies. Eleven of these received Gloxalzone* after the March test because they were reactors. Blood was collected from these cattle in the same manner as that collected in Arkansas, brought to the laboratory, refrigerated overnight and then inoculated into susceptible calves. Two calves received pooled blood samples as indicated in Table 2.

Splenectomized cattle used in all the inoculation trials were 4-12 months old; blood samples taken for CF testing and microscopic examination, were negative for anaplasmosis. After inoculation, blood samples were taken 3 times a week from

*a ethoxyethylglyoxal dithiosemicarbazone (injected intravenously) Burroughs Wellcome & Co. Tuckahoe, New York.*
each calf for 60 days; hematocrit determinations were made and Giemsa-stained blood films were examined. Rectal temperatures were recorded daily, and at 30 and 60 days, CF tests were performed. At 60 days, the susceptibility of the calves that had not shown evidence of anaplasmosis was verified by inoculating them with 5 ml of blood from a steer with acute anaplasmosis.

Another Virginia herd (Herd 3) an infected dairy herd, consisted of Holstein, Jersey and Brown Swiss cattle comprising 115 head in all. The herd was tested in March of 1968, and the results are included in this report. No inoculations of blood from CF reactor animals were made nor were therapeutic treatments for anaplasmosis administered.

The fourth herd studied, an anaplasmosis-free herd at the Agriculture Research Center at Beltsville, Maryland, consisted of 150 cattle. No indications of anaplasmosis had ever been observed in this herd nor had previous CF tests disclosed any reactors to the test. Card and CF tests were conducted on the entire herd.

RESULTS

An overall comparison of the serologic findings in the 3 infected herds and the anaplasmosis-free herd is given in Table 1. These data are the first test results obtained in each herd prior to any treatment and during the winter when possible transmission of the disease by vectors would be minimal. Eighty-two percent of the CF positive samples were also card-positive, whereas 87 percent of CF reactions that were classified as suspect were positive to the card test. Of 216 samples negative to the CF test obtained from infected herds, 195 (90 percent) were also negative to the card test. Moreover, 87 cattle (29 percent) in the 3 infected herds were reactors or suspects to the CF test whereas 93 cattle (31 percent) were positive to the card test. When all samples were considered, the CF and card tests agreed 89 percent.

Results of the CF and card tests conducted on the anaplasmosis-free herd closely agreed (99 percent). Two of 150 samples negative to the CF test reacted to the card test. Both samples gave low level reactions. Possibly some blood group factor was responsible for these reactions or a cross reaction occurred with some unknown agent.

The CF test results obtained in January, indicated that 15 of 82 cattle (18 percent) in herd 1 were infected with A. marginale. These 15 cattle also reacted to the card test. When this herd was tested 3.5 months later, after the CF reactors had been separated and treated, 5 remained reactors and 3 remained suspicious to the CF test. Of these 8, only 1 was positive to the card test. Blood from this cow produced anaplasmosis when inoculated into a susceptible calf (see Table 2). Fifteen days after the calf was given the infected blood, a 2 percent parasitemia was noted in Giemsa-stained blood films. At 22 days, the calf was suffering from acute anaplasmosis and was euthanatized. At this time, 33 percent of the erythrocytes contained A. marginale; the hematocrit reading was 11 percent.

Another calf inoculated with blood from a CF reactor cow in this herd became infected; the cow’s CF titer was 1:5 but the card test was negative. As with the previous calf, 15 days after receiving the infected blood, 1 percent of the calf’s erythrocytes contained A. marginale; 22 days after inoculation, the calf died of acute anaplasmosis. At this time, 40 percent of its erythrocytes contained A.
marginale and its hematocrit reading was 9 percent.

Although blood from 6 other cows with reactor or suspect titers to the CF test was inoculated into susceptible calves, none had signs of anaplasmosis. Examinations of Giemsa-stained blood films did not disclose A. marginale nor did any of the calves have anemia, CF titers, or temperature increases during the 60 days post inoculation. Likewise, calves that received pooled blood from the 2 cows that were card and CF reactors to the January tests but negative to both tests in June, remained normal. All the above-mentioned calves that did not succumb to the disease, received 5 ml of blood from a steer with acute anaplasmosis and proved susceptible to the disease.

Herd 2, in March, contained 37 CF reactor cattle, 26 of which were positive to the card test and 6 with suspect CF titers, 5 of which were card-positive. Based on the CF test, the herd at this time contained 35 percent CF reactor cattle. When this herd was tested 3.5 months later in July, there were 26 reactors and of the 11 CF reactors that had been separated and treated after the March test, 5 remained CF reactors and 4 had suspect titers to the CF test. None of the 11 treated reactors were positive to the card test. Blood from these 11 cattle, inoculated into susceptible calves failed to produce A. marginale infection. The results of 8 of the above blood inoculations in calves observed for 60 days are listed (Table 2). Results from the other 3 inoculations are not shown because the calves that received the inoculum died of eperythrozoonosis before any evidence of anaplasmosis occurred. Blood from 4 other cattle that had not been treated but had reactor titers to the CF test did produce anaplasmosis when inoculated into calves. Three of these cattle were positive to the card test, the fourth was not (Table 2).

Herd 3, a dairy herd, had 27 reactors and 2 suspects out of 115 head indicating a possible infection rate of 25 percent. The CF and card tests closely agreed in this herd. Of the 29 reactor and suspect cattle to the CF test, 26 or 90 percent were positive to the card test.

**DISCUSSION**

The technical skill needed to perform the card test and the limited supplies required in its application directly contrast to those required for CF testing. Excluding the roles of antigen and antibody, the CF test required close attention to pH, ionic strength, and complement titration as well as heat inactivation and incubation under precisely controlled laboratory conditions. However, the card test requires only that the operator add 1 drop of antigen to 0.05 ml of plasma and mix the 2 for 4 minutes before reading the results. Moreover, the test can be run at the farm or ranch, thus making it a valuable survey tool.

In this study, the card test titers receded sooner than did CF titers in samples from cattle treated with Aureomycin or Gloxazone. Because of the earlier elimination of card test titers after treatment, the test could be useful in determining the effect of drug treatment on Anaplasma-infected cattle. The persistence of CF titers after drug treatment has previously been recognized, and a study conducted by Jatkan showed that the CA test became negative 90 days following treatment whereas the CF test remained positive.

It was also observed that the card agglutination test failed to react in two
instances with plasma from infected cattle. Possibly in a disease situation such as anaplasmosis, the antibody level in an infected animal could at times recede to a level below the sensitivity of the card test. Serological tests vary in their degree of sensitivity, and in this study, the CF test has greater sensitivity in detecting lower levels of antibodies.

The data strongly indicate that the card agglutination test has definite potential as a useful diagnostic aid for detecting anaplasmosis. We should now seriously consider this test for large-scale field trials, preferably in herds where calf inoculations could substantiate its specificity and sensitivity.

SUMMARY

Field evaluation studies of a rapid card agglutination test for bovine anaplasmosis indicated that the test could be successfully conducted in an automobile at the farm. Results from the card test in the field agreed closely with those from the complement-fixation test in the laboratory. In 3 infected herds of 303 cattle, results from the 2 tests agreed 86 percent. On samples from 150 cattle known to be anaplasmosis-free, results agreed 99 percent.

Comparative serologic studies made on samples from anaplasmosis-infected cattle successfully treated with Aureomycin or a-ethoxyethylglyoxal dithiosemicarbazone showed that card test reactions became negative sooner than did complement-fixation reactions. The results of inoculations of blood from 18 treated and 4 untreated cattle into susceptible calves proved that the test accurately classified the anaplasmosis status of 20 (87 percent) of such cattle tested.

The authors appreciate the cooperation and assistance of Dr. G. S. Warner and Mr. G. J. McMillion of Hynson, Westcott and Dunning, Baltimore, Md.; Dr. A. J. Roth of the Virginia Department of Agriculture and Commerce, Richmond, Va.; Dr. Paul Becton, Veterinarian in Charge, Animal Health Division and Dr. David Ibsen, Extension Veterinarian, Little Rock, Ark. We also acknowledge the technical assistance of Mr. J. C. Caudill and Mr. R. L. Sealock, Beltsville Parasitological Laboratory.

REFERENCES

REPORT OF THE ANAPLASMOSIS COMMITTEE, 1968

Dr. J. W. Safford, Chairman

Members: Dr. W. E. Brock; Dr. W. Burgdorfer; Dr. J. F. Christensen; Dr. T. E. Edds; Dr. L. E. Foote; Dr. N. L. Garlick; Dr. R. J. Hidalgo; Dr. D. W. Marble; Dr. J. L. O’Harra; Mr. J. O. Pearce; Dr. C. C. Pearson; Dr. R. I. Port; Dr. T. O. Roby; Dr. M. Ristic; Dr. J. C. Trace; Dr. C. J. Welter; Mr. L. Williams; Mr. J. D. Manke.

The Fifth National Anaplasmosis Conference was held in February, 1968, at Stillwater, Oklahoma. The 39 papers presented are soon to appear in the Proceedings of the Conference, now in press. All persons interested in recent developments relating to anaplasmosis are urged to review those Proceedings. Inquiries may be directed to Dr. L. E. Foote, Louisiana State University, Baton Rouge, Louisiana, 70803.

The Committee discussed research trials using experimental living anaplasmosis organisms. A preliminary report of such investigations underway at Gainesville, Florida, under the direction of Drs. F. C. Neal and G. T. Edds, is presented as follows:

"Highly satisfactory resistance could be produced in mature Florida cattle by a single 5.0 ml. injection of an attenuated Anaplasma marginale vaccine. Five lots of vaccine have been tested in 64 cattle and vaccination reactions were inapparent except in one lactating dairy cow and one young dairy bull. The average vaccine parasitemia was 4.8 percent and the average drop in hematocrit was 6.4. The percent of cattle protected by a single 5.0 ml. injection of the first three lots of vaccine tested was unsatisfactory. However, modification in the last two lots of vaccine has apparently increased resistance to 100 percent of Florida cattle. Further evaluation of this aspect of the problem is needed.

Two groups of five serologically positive cattle were challenged 63 and 90 days, respectively, after vaccination. No clinical symptoms of anaplasmosis or drop of hematocrit could be detected in the vaccinates after challenge with doses that caused severe clinical signs or death in controls. Challenges six months and one year after vaccinations are planned with other groups.

Two mature vaccinated cows were each challenged, 90 days following vaccination, with 1.0 ml. of infective blood containing seventy percent infected erythrocytes. Thirty-nine days later, 10 ml. of blood from each of these animals was sub-inoculated into two susceptible mature cows. The latter cows died at 35 and 39 days, respectively.

Other studies on the safety of this product are in progress."

It was announced by Dr. Paul D. DeLay, Director of the ADP Division, ARS, USDA, that a project has been funded to investigate reservoirs and vectors of anaplasmosis. The work is underway at the University of California at Davis.
The objective is to determine whether or not deer and ticks are significant and persistent sources of *Anaplasma* infection in the absence of cattle.

A number of articles on anaplasmosis have appeared in the literature during the past year. A bibliography of these reports is appended. One of the articles (Christensen, J. F., and McNeal, D. W. Am. J. Vet. Res. 28 (1967): 599-600) reported natural infections of *Anaplasma* marginale in deer in the foothills of the Sierra Nevada Mountains in California, an area in which bovine anaplasmosis is known to be enzootic. The deer, crosses between Columbian black-tailed and mule deer, were shown to be infected by inoculating an intact calf subcutaneously with pooled deer blood.

A progress report on the new national anaplasmosis reporting system was given by Dr. B. C. McCallon of the ANH Division, ARS, USDA. The system has not been in operation long enough to warrant a statistical summary as yet. The greatest value in the system may be to spot changing trends in the various States. It is not expected to provide reliable information on the absolute incidence of the disease. Statistically valid surveys to determine the true incidence of anaplasmosis are being contemplated by the USDA; these are encouraged by the Committee.

The Committee recommends that the USLSA request the USDA to update and republish the *ARS Special Report on Anaplasmosis* which was issued several years ago. The primary purpose of the revision would be to provide the cattle industry with a working knowledge of the disease and the tools available to combat it. The revision would be most useful if it became available next spring, prior to the 1969 anaplasmosis season.

Dr. J. F. Frank, of Canada, reported the first known outbreak of anaplasmosis in Canadian cattle. It occurred in Southern Manitoba during this past August. There was a history of cattle having been imported from the State of Kansas in previous years. Anaplasmosis was known to have been a problem on the Kansas ranch of origin. Several possibilities exist concerning the mode of transmission within the Canadian herds. The tick, *Dermacentor variabilis*, is prevalent in the area, mosquitoes were unusually numerous, and tabanid flies were also present. However, common vaccine needles, and possibly other man-precipitating transmissions, were used.

There are nine ranches in the affected area, with 1,600 cattle. A complement-fixation testing program was instituted to identify all infected animals. Reactors were found on two premises, with most of them in one herd. All reactors were removed for slaughter. The owners received indemnity payments from the Canadian Government for all animals slaughtered. Because these were predominately feeder cattle, the losses did not greatly exceed the salvage value. Chemotherapy was not tried because it was decided it would be best to quickly eradicate the disease, if possible. Additional herd tests are anticipated during the coming winter.

Canada is now considering the adoption of testing requirements for cattle entering the country from endemic areas.
ANAPLASMOSIS PUBLICATIONS - 1968

DNA HOMOLOGY RELATIONSHIPS WITHIN THE GENUS BRUCELLA

Norman B. McCullough, Ph.D., M.D.
Department of Microbiology and Public Health and
Department of Medicine
Michigan State University
East Lansing, Michigan 48823

Deoxyribonucleic acid (DNA), the genetic material of the cell, consists of a double helical structure composed of complementary strands of polynucleotides. The nucleotides in each strand are joined by phosphate linkages between the sugar residues, and the strands are held together by hydrogen bonding between complementary purine and pyrimidine bases (guanine-cytosine; adenine-thymine). When heated, or subjected to other treatment which disrupts hydrogen bonds, single stranded DNA results. Under appropriate conditions, the complementary strands of this single-stranded DNA will reassociate. The degree of recombination varies with the complexity of the DNA; that derived from relatively simple genomes, such as those of viruses and bacteria, reassociates to a high degree. Since reassociation occurs between complementary polynucleotide sequences, similar interactions will also occur with the separated strands of heterologous DNA's sharing similar polynucleotide sequences. This, determination of the degree of interaction between DNA's provides a measurement of genetic relatedness among microorganisms. In practice, one measures the degree of interaction between immobilized, single-stranded DNA and radiolabeled, single-stranded DNA fragments. The terms binding, duplexing, reannealing, homologizing, hybridization, and double strand formation have been used to describe these reactions.

The detection and measurement of DNA interactions have been facilitated by the DNA-agar procedure\(^1\), wherein high molecular weight, single-stranded DNA is immobilized in agar, and later by the Denhardt modification\(^2\) of the membrane filter technique\(^3\). In the latter, the high molecular weight, single-stranded DNA is immobilized on nitrocellulose membranes. In both instances, the degree of binding of radiolabeled, single-stranded DNA fragments to the immobilized, high molecular weight DNA is measured by determining the amount of the radiolabel which is bound to the immobilized DNA. The introduction of competition reactions\(^4,5\), which measure the interference of homologous or heterologous nonlabeled DNA fragments in the binding of radiolabeled DNA fragments to homologous, immobilized DNA, has reduced the number of experimental variables and improved the reliability of these determinations.

In the last few years DNA homology studies have been done on a variety of microorganisms and have served to confirm, clarify, and extend our knowledge of microbial relationships. Thus, they have become an invaluable aid in taxonomy.

The purpose of this discussion is to present the results of DNA homology studies on members of the genus \textit{Brucella}, and, in particular, to display the
relationships of the organisms of contagious canine abortion \((B. \text{canis})^{6,7}\) and of ram epididymitis \((B. \text{ovis})^{8}\) to the classical members of the genus. The methods of preparation, purification, and standardization of high molecular weight, single-stranded DNA, and of \(^{32}\text{P}\)-labeled DNA fragments, as well as the details of performing the homology experiments have been described previously in detail\(^9,10\) and will not be repeated here.

In our initial studies\(^9\) we employed the DNA-ager procedure\(^1\). Radiolabeled, single-stranded DNA fragments of Type I of each of the three classical species of \(\text{Brucella} (B. \text{abortus}, B. \text{melitensis}, \text{and } B. \text{suis})\) were reacted with their homologous DNA's in the presence and absence of competing DNA. A series of reciprocal competition reactions was done by adding, in separate experiments, graded amounts of nonlabeled single-stranded DNA fragments from each of the three species to each of the homologous systems. For each experiment the percentage of the radiolabeled DNA bound to the homologous, immobilized DNA was determined. These data generated a series of competition curves. In every instance the heterologous nonlabeled DNA fragments competed equally as well as the nonlabeled homologous fragments in reducing the binding of the radiolabeled fragments to their homologous DNA's. These reciprocal competition experiments failed to distinguish between the DNA's of the three classical species. Within the limits of the sensitivity of the experimental procedure, these findings indicate equal and reciprocal polynucleotide sequence similarity among the three species. Results of experiments using other competitors added to any one of the homologous systems should then apply equally to all three systems. \(B. \text{neotomae}\)^{11} DNA, added as a competitor to one of these systems, competed as effectively as did DNA from any of the classical species, confirming the placement of this recently described organism in the genus \(\text{Brucella}\). However, the DNA of the ram epididymitis organism did not compete as effectively in the two systems tested as DNA of any of the classical species, there being a small but detectable difference. As controls on the specificity of the reactions, DNA of \(\text{Escherichia coli}\) and a mammalian DNA were also employed; both failed to alter the binding of radiolabeled \(\text{Brucella}\) DNA fragments to homologous embedded DNA. Likewise, the DNA of \(\text{Francisella tularensis}\) failed to compete in these systems, indicating a lack of polynucleotide homology with \(\text{Brucella}\).

In later studies\(^{10}\), we investigated the relationship of the organism of contagious canine abortion to the genus \(\text{Brucella}\) and further explored the relationship of the ram epididymitis organism \((B. \text{ovis})\). The Denhardt modification\(^2\) of the membrane filter technique\(^3\) was employed in these studies. Reciprocal competition experiments were done using DNA from \(B. \text{suis}, B. \text{ovis}\), and the canine abortion organism. Nonlabeled, single-stranded DNA fragments of the canine abortion organism competed equally as well in the \(B. \text{suis}\) homologous system as did such fragments of \(B. \text{suis}\) DNA, and vice versa. Within the limits of the sensitivity of the experimental procedure these data indicate equal and reciprocal polynucleotide sequence similarity between the DNA of the canine abortion organism and that of \(B. \text{suis}\), and by implication from the previous data, between the DNA of the canine abortion organism and that of \(B. \text{abortus}, B. \text{melitensis}, \text{and } B. \text{neotomae}\). Once again, the DNA of \(B. \text{ovis}\), although preponderantly similar, was detectably different. In both the canine abortion
organism and B. suis homologous systems the DNA of B. ovis did not compete as effectively as that of the other two organisms. However, in the B. ovis homologous system, the DNA of both the canine abortion organism and B. suis competed equally as well as that of B. ovis. These data suggested that B. ovis DNA differs from that of other members of the genus by lacking some of the polynucleotide sequences shared by the other members. That is, it could be a deletion mutant occurring in nature. To test this hypothesis we attempted to obtain a fraction of B. suis DNA enriched in polynucleotide sequences not present in B. ovis. This selection was accomplished by reacting B. suis DNA fragments of high specific radioactivity with fixed B. suis DNA in the presence of a relatively large amount of unlabeled B. ovis DNA fragments. Theoretically, that fraction of radiolabeled B. suis DNA which became fixed to the immobilized B. suis DNA on the filter should be enriched for the unique sequences. The radiolabeled B. suis fragments which became fixed to the filter were recovered and resubjected to the enrichment procedure. They were again recovered and used in competition experiments. Enrichment did indeed occur. These selected, radiolabeled fragments bound to either B. suis or B. ovis fixed DNA about the same as unselected fragments in the absence of competitors. However, B. ovis competitor did not reduce the binding to B. suis fixed DNA as effectively as it reduced the binding of unselected fragments. No enrichment was demonstrated when similar experiments were done with B. ovis or B. suis competitors in the B. ovis reassociation system. It was effectively demonstrated that B. suis DNA contains polynucleotide sequences not present in B. ovis. The reverse was not demonstrated. These data support the thesis that B. ovis is a deletion mutant of one of the classical species of the genus Brucella.

An effort was made to define further the nature of the unique fraction of B. suis DNA not present in B. ovis. Fractional thermal elution from hydroxylapatite columns has been shown to be effective in fractionating DNA fragments on the basis of their guanine plus cytosine (G plus C) content. When the temperature is raised in increments and fractions are collected, those eluted at the lowest temperatures will be relatively enriched in their adenine plus thymine content, and those at higher temperatures in G plus C content. Such a fractionation was carried out with radiolabeled B. suis DNA fragments. The 32P-labeled DNA in the fractions collected was processed and used in competition experiments. In the B. suis homologous system, B. ovis DNA competed less effectively with all of these preparations than did the homologous B. suis fragments. This finding suggests that the polynucleotide sequences present in B. suis, but not in B. ovis, are probably present throughout the genome. If this is true, then it is unlikely that B. ovis arose as the result of a single deletion, but rather by multiple small DNA deletions. One would not then expect B. ovis to reacquire the portion of its genome which is lacking by a sudden one step process.

To further define the DNA homology relationship of the members of the genus Brucella to other groups of bacteria, we included in these studies DNA's of Serratia marcescens, Alcaligenes faecalis, and Bordetella bronchiseptica. All of these DNA's failed to compete in the B. suis homologous system, and hence lack homology with the members of the genus Brucella. In the earlier study, lack of homology was established for the DNA's of E. coli and F. tularensis. The named organisms were selected because the G plus C content of their DNA's was close to that of the
members of the genus *Brucella*, or in two instances (*F. tularensis* and *B. bronchiseptica*) because in the past they were considered members of the genus.

It is apparent that the recognized members of the genus *Brucella* have very extensive DNA polynucleotide sequence similarities. The DNA of the organisms of ram epididymitis, and of contagious canine abortion share in this extensive homology. On the basis of the data discussed, together with the characterizations obtained by the more classical methods, we have recommended that the ram epididymitis organism, *B. ovis*, and the canine abortion organism, *B. canis*, be recognized as new species in the genus *Brucella*.

**LITERATURE CITED**

A status report, to be complete, must include both accomplishments and shortcomings or weaknesses. We are pleased to report that during the past year the accomplishments of the program far outweigh the weaknesses or shortcomings. The advances made in the eradication of brucellosis have been substantial, and we can be proud of them. However, if the goal—a Certified Brucellosis-Free Nation in 1975—is to be reached, improvements must be made in our rate of progress toward that goal. Seven years is a relatively short period of time in which to completely eliminate the disease in those areas where little preliminary work has been done. It becomes increasingly urgent that all counties be placed under an area program immediately, which will lead to a modified certified status not later than 1971.

During the past year, satisfactory progress has been made in the modified certified States as measured by counties and States achieving brucellosis-free status. Three States—Michigan, New York, and Maryland—qualified during the last fiscal year as Certified Brucellosis-Free. New Jersey has since qualified making a total of 14 States, plus the Virgin Islands, which are currently Certified Brucellosis-Free Areas.

Likewise, during fiscal year 1968, three States—Alabama, Colorado, and Wyoming—achieved modified certified status. Currently, there are 28 modified certified States. Combining the certified free and modified certified States, makes a total of 42. Eight States—Florida, Mississippi, Louisiana, Texas, Oklahoma, Nebraska, South Dakota, and Hawaii—have not yet reached this status. It is expected that at least five of these eight States will become modified certified before the next meeting of this Association. With the exception of Florida and Texas, area work is in progress in all counties. In Texas, only 13 counties remain in which area work is not underway. Five counties in Florida have not started area work; only 18 in the entire Nation. (Figure 1)

1Chief Staff Veterinarian, Brucellosis Eradication, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
2Senior Staff Veterinarian, Cattle Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
During fiscal year 1968, a total of 258 counties became Certified Brucellosis-Free; 41 more than achieved this status during fiscal year 1967. As of September 15, 1968, there were 1,159 certified-free counties which represents 36 percent of all counties in the United States.

Progress, as measured by the number of counties which qualified as modified certified, was substantial during the year with 90 counties qualifying. At the end of fiscal year 1968, 1,837 counties were modified certified areas. The 2,957 modified certified and certified free counties at the end of the year represented over 94 percent of the total U. S. Counties. Only 0.6 remain to begin area work. (Figure 1)

Although the number of herds found infected in certified free States has increased over the last year, the number of free States has also increased. However, over one-half of these infected herds were found in the three States qualifying during the year prior to the date they qualified as free States. These herds had been freed of the infection prior to the States qualifying. During 1967, in the 10 States that were at that time certified free, 82 infected herds were found. In those same 10 States during 1968, only 75 infected herds were located.

The number of infected herds found in modified certified areas remained approximately the same with the same number of States involved as last year. Similarly, there was little change in the number of herds found infected in the
noncertified States. It must be noted, however, that at the close of the fiscal year there were only 8 States in this category compared to 11 the previous year. This actual increase in infected herds found reflects the high level of activity in most of the remaining noncertified States. (Figure 3)

**BRUCELLOSIS INFECTED HERDS FOUND**

In Noncertified, Modified Certified and Certified - Free States

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**STATES WHERE INFECTED HERDS FOUND**

<table>
<thead>
<tr>
<th>States Where Infected Herds Found</th>
<th>Certified-Free</th>
<th>Modified Certified</th>
<th>Noncertified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961</td>
<td>6,548</td>
<td>1,324</td>
<td>36,000</td>
</tr>
<tr>
<td>1962</td>
<td>5,000</td>
<td>1,324</td>
<td>36,000</td>
</tr>
<tr>
<td>1963</td>
<td>4,943</td>
<td>1,423</td>
<td>35,000</td>
</tr>
<tr>
<td>1964</td>
<td>5,000</td>
<td>1,423</td>
<td>35,000</td>
</tr>
<tr>
<td>1965</td>
<td>8,287</td>
<td>1,100</td>
<td>34,000</td>
</tr>
<tr>
<td>1966</td>
<td>14,813</td>
<td>1,423</td>
<td>34,000</td>
</tr>
<tr>
<td>1967</td>
<td>14,264</td>
<td>1,423</td>
<td>34,000</td>
</tr>
<tr>
<td>1968</td>
<td>157</td>
<td>1,423</td>
<td>34,000</td>
</tr>
</tbody>
</table>

**NUMBER STATES**

- Non-certified
- Modified Certified
- Certified-Free

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Non-certified</th>
<th>Modified Certified</th>
<th>Certified-Free</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961</td>
<td>24</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>1962</td>
<td>21</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>1963</td>
<td>18</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>1964</td>
<td>14</td>
<td>32</td>
<td>4</td>
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<tr>
<td>1965</td>
<td>13</td>
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<td>1966</td>
<td>12</td>
<td>29</td>
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</tr>
<tr>
<td>1967</td>
<td>11</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>1968</td>
<td>8</td>
<td>29</td>
<td>13</td>
</tr>
</tbody>
</table>
It is also of interest to note that the percentage of Certified Brucellosis-Free counties in which no reactors were found remained at 55, the same as in 1967; although the number of these counties increased from 471 to 615. Likewise, the percentages of counties with singleton and multiple reactors remained approximately the same. Four certified free States had two or less infected herds, while Alaska, Rhode Island and the Virgin Islands revealed none. (Figure 4)

The progress toward eradication is further demonstrated by the fact that 14 States had 10 or less infected herds and 19 States had 20 or less.

Although our record is good in most of the certified free States, as measured by the number of infected herds found, even greater progress can be accomplished by making full use of the tools at our disposal. In these States, every possible source of infection must be searched out and eliminated. In reaching our goal in the shortest possible time, we cannot afford the carelessness of unused epidemiological procedures. (Figure 4)

![BRUCELLOSIS-FREE COUNTIES Diagram](image)

**TOTAL FREE COUNTIES 1,120**

As of June 30, 1968  
Fiscal Year 1968

U. S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL RESEARCH SERVICE

It is of interest that there was no change from last year in the percentage of herds that revealed reactors on only a single test in the brucellosis-free counties. This percentage remained constant at 71 with 63 percent of the herds revealing only a singleton reactor on one test and 8 percent, two reactors on one test. These herds had a history of being free of brucellosis and subsequent to the test on which reactors were disclosed, they have remained free. (Figure 5)
STATUS OF ERADICATION PROGRAM

REACTOR HERDS FOUND
In Certified Brucellosis-Free Counties

71% of Reactor Herds Disclosed on ONLY 1 TEST
Fiscal Year 1968

U S DEPARTMENT OF AGRICULTURE

BLOOD TESTING: CATTLE

FISCAL YEAR
There was little change in the total number of tests made and reactors found compared with last year. Total blood tests remained at approximately 12 million with 149,000 reactors being found. The relative relationship of market cattle tests to total tests remained at approximately 40 percent. The adoption of the yellow and white flip tag by over 500 markets was responsible for a 53 percent increase in the number of backtags applied. Almost 4,000 infected herds with over 21,000 reactors were found as a result of testing MCT reactor herds of origin.

Considerable improvement was made in reducing the percentage of MCT reactors that could not be traced to herds of origin. In fiscal year 1967, 12.5 percent could not be traced; while in 1968, this percentage was reduced to 5.4 percent. In spite of this improvement, there were still over 2,000 reactors that could not be traced to their source. It is important that better use be made of this vital and economical means for locating diseases. (Figure 6)

The number of ring tests conducted continued to decline which reflects the decreasing numbers of dairy herds in the U.S. However, the estimated number of cattle represented by such tests increased due to herds becoming larger. The percentage of herds showing suspicious ring tests was again reduced from 0.6 percent in 1967 to 0.4 percent this past year, reflecting the continued reduction of infection in dairy herds. As should be expected, the decrease in BRT suspicious herds is greatest in the certified States. (Figure 7)
A total of 7,057 suspicious ring tests were made resulting in 6,257 followup herd tests which located 1,388 infected herds with 3,781 reactors. Since the success of the eradication program depends greatly on the effectiveness of our surveillance programs, it is important that they be reviewed frequently. For adequate coverage of the dairy population, the BRT should be conducted at least 3 times a year, and any herds suspicious should be blood tested promptly. (Figure 7)

**Brucellosis Eradication**

**Calf Vaccinations**

Percent Change, Fiscal Years 1967-68

Almost one-half million fewer calves were vaccinated this past year than in 1967—the fourth consecutive year in which a decrease has occurred. Vaccine has been of considerable value in lowering the incidence of brucellosis in those areas where there was previously a high degree of infection. Such areas have now been reduced in number and size to the point that widespread continued use of vaccine cannot be justified.

Unfortunately, increases in use of vaccine did occur in many of the States which are now modified certified or certified free. On the other hand, decreases occurred in some areas not yet certified which still have relatively high infection rates. Compared with 1967, vaccination increased in 14 States, including 5 certified free and 6 modified certified. In all other States, decreases occurred, including 5 noncertified States. (Figure 8)

The use of Strain 19 in certified States must be drastically reduced. Continued use of vaccination will only prolong the time that it takes to achieve eradication.
The minimal advantages of Strain 19 vaccination in certified areas are far outweighed by the problems it causes in diagnosis and by the failure of the occasional heifer to overcome the infection induced by Strain 19 vaccination. Although these are not common and as yet there is no evidence that brucellosis resulting from Strain 19 has spread to other cattle, it does cause concern.

The continued use of Strain 19 is justified and encouraged in those few areas not yet certified in which the incidence of brucellosis is still relatively high. Even in those areas where effective eradication programs are rapidly being carried out, it should be recognized that the need for vaccination will decrease rapidly.

The Animal Health Division, in cooperation with Clemson University, is continuing to study the effects of Brucella abortus 45/20 vaccine. As reported in previous years, there is inadequate information available in the reports from Europe and the earlier trial in cooperation with Clemson to accurately judge the effectiveness of the vaccine. The current study is designed to determine what effect the vaccine has on serological tests and what protection it provides against infection after challenge by a measured exposure dose of virulent Brucella abortus.

The preliminary results indicate that although the product is supposedly non-agglutinogenic, some animals do develop titers which may persist as long as four months when the standard plate or tube test is used and as long as 48 weeks on some of the supplemental tests. The cows are to receive their first annual booster vaccination in early November as prescribed by the producers of the vaccine.

**Blood Testing: Swine**

**Herds-Lots Tested**

<table>
<thead>
<tr>
<th>Year</th>
<th>Herds-Lots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>14,919</td>
</tr>
<tr>
<td>1966</td>
<td>59,328</td>
</tr>
<tr>
<td>1967</td>
<td>61,372</td>
</tr>
<tr>
<td>1968</td>
<td>75,760</td>
</tr>
</tbody>
</table>

**Infected Herds-Lots**

<table>
<thead>
<tr>
<th>Year</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>9.5%</td>
</tr>
<tr>
<td>1966</td>
<td>3.1%</td>
</tr>
<tr>
<td>1967</td>
<td>2.4%</td>
</tr>
<tr>
<td>1968</td>
<td>1.8%</td>
</tr>
</tbody>
</table>
The vaccine used in the study is produced by two European biological producers; it is administered as each recommends in their directions. In the case of one vaccine, permanent swellings have developed at the site of injection in most of the cows. The swellings vary in size from a golf ball to larger than a grapefruit.

Challenge of the animals is scheduled for December 1969. A full report of the results should be available for the 1970 meeting of this organization.

There were 75,760 lots of swine tested during fiscal year 1968, an increase of 19 percent over the previous year. The incidence of brucellosis continues to decline with 1.8 percent of the lots disclosing reactors this past year. The number of validated counties increased to 154, while the number of validated herds remains at approximately 2,300. Nevada, Utah, Vermont, and the Virgin Islands continued as Validated Brucellosis-Free Areas. (Figure 9)

BRUCELLOSIS ERADICATION GOALS
Status of County Certification

A year-long pilot project to study the problems associated with swine identification, collection of blood samples, use of the card test, and traceback of reactors will be completed this month. Through the 42nd week, approximately 3,900 lots with 17,000 slaughter swine have been tested. Preliminary observations appear to support the slap tattoo method of swine identification. The use of heart clot samples also appears satisfactory when the card test is utilized. Final reports of this project will be given at a later date.

The individual States have been strongly encouraged to continue development of market swine testing programs to the extent that funds and manpower permit. To date, 9 States, Puerto Rico, and the Virgin Islands are continuing their efforts in
market swine testing. Additional States are planning to begin swine testing programs during fiscal year 1969.

The problem herd program has proven highly effective in those States where it is being utilized. It has proven that the procedures available are fully adequate to eradicate brucellosis. This program is, however, effective only with the full cooperation of the herd owners.

Currently, 6 epidemiologists are available on a regional basis. In most States, there are veterinarians with special training in brucellosis epidemiology who are doing an excellent job of solving the few remaining problems that stand in the way of eradication.

In summary, a review of program goals and achievements are desirable. A compilation of State goals for this past year projected that 1,126 counties would be certified free. This goal was missed by only 6 counties. Likewise, the projected goal for modified certified counties was missed by 7. However, more counties were conducting area work than had been established in the goals. The number of counties not yet conducting area testing was reduced from a projected 80 to 29—a real gain!

The projections indicate that next year at least 1,485 counties will be certified free and all remaining counties will be modified certified with the exception of 87 counties in which area testing will be underway.

These goals are attainable. They must be reached on schedule. Brucellosis can be eradicated from the Nation by 1975. (Figure 10)
REPORT OF THE COMMITTEE ON BRUCELLOSIS
United States Livestock Sanitary Association
Seventy-Second Annual Meeting
October 7-11, 1968

H. G. Wixom, Sacramento, California, Chairman; J. W. Ralph Bishop, Tipton, Indiana; G. E. Burch, Delmar, New York; George B. Estes, Richmond, Virginia; Dean E. Flagg, Bismarck, North Dakota; A. E. Janawicz, Montpelier, Vermont; W. D. Knox, Fort Atkinson, Wisconsin; Robert I. Laramore, Gillette, Wyoming; C. A. Manthei, Ames, Iowa; R. J. McLenaghan, Ottawa, Ontario, Canada; S. H. McNutt, Madison, Wisconsin; J. O. Pearce, Jr., Okeechobee, Florida; E. A. Schilf, Hyattsville, Maryland; Jean V. Smith, Hartford Connecticut; William C. Tobin, Denver, Colorado; A. O. Wilson, Hysham, Montana; Fred Phillips, Keating, Oregon; Joe B. Finley, Jr., Encinal, Texas

Your Brucellosis Committee met in open session on Monday to give opportunity for livestock and regulatory officials and other interested parties to make their recommendations pertaining to the national brucellosis eradication program, as well as explain problems encountered in the program. This open session was well attended. Keen interest still exists in the national brucellosis eradication program and there are still problems to overcome. In spite of certain obstacles encountered during the past year your committee reaffirms that the national goal of eradication by 1975 is still attainable if the knowledge concerning the disease and the proven procedures are applied to this national effort.

There are currently 14 States and the Virgin Islands that are Certified Brucellosis-Free areas as compared to 10 States last year. In addition there are 28 States that are Modified Certified Brucellosis areas. Now there are 94 percent of the counties of the United States that are either Modified Certified or Certified-Free areas as compared with 92 percent one year ago. Approximately 37 percent of these counties have achieved the higher rating as compared to 28.4 percent this time last year. Testing is being conducted in all but 18 counties of the nation. This is a gain of 88 counties. Thus brucellosis eradication has been extended to 99.4 percent of the counties of the United States.

INTERSTATE MOVEMENT OF CATTLE:

The committee, after serious consideration, expresses indignation and concern that steps have not been taken by the United States Department of Agriculture to provide the livestock industry with protection from brucellosis infected and exposed cattle to which they are entitled. We find that 99.4 percent of the counties are moving ahead to achieve eradication, yet the industry in these counties is still exposed to the probability of reinfection from the remaining .6 of 1 percent of the areas. The industry in these remaining areas is making little or no effort to reduce the high incidence of brucellosis known to exist. The committee reaffirms its
recommendation that effective January 1, 1969, all cattle moving interstate originate from Modified Certified Brucellosis Areas, Certified Bovine Brucellosis-Free Areas, or from herds known not to be affected with brucellosis. All cattle from quarantined herds or herds of unknown brucellosis status may move only for immediate slaughter or to quarantined feed lots.

The committee further recommends that the following definition of a quarantined feed lot be adopted and incorporated as a part of the Uniform Methods and Rules and the Federal Interstate Regulations. Definition: A quarantined feed lot shall be a confined area under the direct supervision and control of the State Livestock Health Official who shall establish procedures for accounting of all animals entering or leaving such quarantined feed lot. The quarantined feed lot shall be maintained for finish feeding of animals in dry lot with no provision for pasturing or grazing. All animals leaving such feed lot must move only for immediate slaughter in accordance with established procedures for handling quarantined animals.

The committee recommends that a copy of this recommendation pertaining to interstate movement be forwarded immediately to the Secretary of Agriculture requesting that no revision of Part 78, Title 9, Code of Federal Regulations, be published before this recommendation has been considered.

**AGE OF VACCINATION:**

As eradication of brucellosis is approached, the problems of persistent titers due to vaccination become more evident. Calves vaccinated at the upper ages are more apt to show persistent titers. In harmony with the national advancement in the program more emphasis needs to be given to vaccinating at the lower age levels. The committee recommends that the age of vaccination be established at 3 to 8 months (90 to 239 days) of age for the dairy breeds and 3 to 10 months (90 to 299 days) for the beef breeds. Cattle owners are urged to arrange to have their animals vaccinated as close as possible to the minimum age.

**TESTING OF OFFICIAL VACCINATES:**

Reconsideration was given to when official vaccinates should be tested to assure the continued progress of the program. The committee reaffirms their recommendation as published in the 1967 Uniform Methods and Rules and urges its incorporation in the Uniform Methods and Rules and the Federal Interstate Regulations. This recommendation is:

Beginning January 1, 1970, officially vaccinated heifers of the beef breeds be tested at 24 months of age, and those of the dairy breeds at 20 months of age.

**UNIFORM METHODS AND RULES:**

As the free areas have increased, a problem has been noted in marketing brucellosis reactors. The committee recommends that the Uniform Methods and Rules be amended to permit the sale of reactors through markets in Certified-Free areas in accordance with established procedures for State-Federal approved markets.
In the certification of areas the committee recommends that Brucellosis Ring Tests be conducted not less than three times per year. Qualifying tests should be conducted at approximately equal intervals. Only two states remain which are conducting the Brucellosis Ring Test on a semi-annual basis.

**MODIFIED CERTIFIED BRUCELLOSIS AREAS:**

To assure that proper precautions are provided to the various states accepting cattle from the Modified Certified Brucellosis Areas without further test, the committee recommends the Uniform Methods and Rules and the Federal Interstate Regulations be amended to provide that continuous brucellosis programs are conducted to locate and eliminate infected cattle within any area which is declared Modified Certified.

**EXEMPTIONS FOR CALVES:**

The committee finds that the exemptions for the movement of calves are not consistent in the Federal Interstate Regulations and the Uniform Methods and Rules. We recommend that the exemption be the same - that is - 6 months of age.

**ANIMAL IDENTIFICATION:**

The committee again urges the adoption of regulations by the Federal Government requiring identification of animals moved interstate. Such regulations were discussed and will be published in the Federal Register in the near future.

**SWINE BRUCELLOSIS:**

Some progress has been made during the year in expanding the market swine testing program. However, the progress of establishing Validated Brucellosis-Free States has not progressed as rapidly as hoped. The Committee recommends an alternate method of Validating States and encourages the States to implement the recommendations.

Our recommendations are:

1. Develop and implement a market swine testing program.
2. Support the swine identification program as an integral part of programs for eradicating swine diseases.
3. The various States, in cooperation with the United States Department of Agriculture, that have conducted market swine testing programs and intensified swine brucellosis eradication programs are to be commended. We recommend that the States implement and enforce regulations to prohibit the interstate movement of breeding swine without official test unless they originate in Validated Brucellosis-Free herds or areas.

Provisions should be made for Validating States as swine brucellosis free when:

1. all herds selling breeding stock are tested and declared Validated, and
2. sows, boars, and stags are tested at slaughter, and
3. when reactors are found, the herd of origin is Validated or sent to
slaughter.
States complying with these provisions for a one year period should be declared Validated.

RESEARCH:

In reviewing the brucellosis eradication program the livestock industry requested that additional research be undertaken to develop information that would expedite eradication of brucellosis. Therefore, we urgently recommend that the United States Department of Agriculture, State Agencies and private industry, expand research to develop improved immunizing products and diagnostic procedures immediately; Furthermore, that adequate funds be provided to implement this research.

I wish to thank the members of this committee for their interest and hard work. They spent long hours in studying the problems of the brucellosis program. It has been a real pleasure for me to work with them.

H. G. Wixom, Chairman
PREVENTION OF INTRAMAMMARY INFECTION IN DAIRY CATTLE DURING LACTATION: ROLE OF TEAT SKIN DISINFECTION AND MILKING UNIT SANITATION

John S. McDonald, D.V.M., Ph.D.*
Ames, Iowa 50010

Methods of milking time hygiene have been shown\textsuperscript{10,11,12} to reduce the incidence of new \textit{Intramammary} (IM) infection (invasion and growth of microorganisms within the mammary gland). Chemical sanitation following machine milking\textsuperscript{1,3} decreased the number of colony-forming units (DFU) of bacteria within the \textit{milking unit} or \textit{Cluster} (the 4 teat cups, claw, and long milk tube). Application of heat to the cluster has been shown to be more effective in decreasing bacterial numbers than chemical methods of sanitation.\textsuperscript{3} Dipping of teats in a disinfectant after each milking decreased the CFU on the teat skin.\textsuperscript{16,17}

The relative effectiveness of each of the above procedures in \textit{prevention} (a zero incidence) of new IM infection has not been reported. It has been recommended that all contaminated items used in the milking process should be sanitized in order to prevent the spread and growth of microorganisms that can cause new IM infection\textsuperscript{4}; however, these procedures do not prevent spread of bacteria from cow to cow during milking.\textsuperscript{12,13}

This paper describes two studies on a method of milking time hygiene. In the first study, the method of milking time hygiene was utilized for prevention of new IM infections. In the second study, experimental challenge and evaluation of the method of milking time hygiene was carried out.

In the study on prevention of IM infection, 20 cows, maintained in a closed herd, were utilized for one lactation. The incidence of new IM infection in the same herd during control periods both before and following the study are presented (Figure 1). Methods for examination of milk samples and inflations, and criteria of infection have been described previously.\textsuperscript{5} Secretions from all quarters were bacteriologically negative at the start of the lactation. When a quarter became infected, treatment was administered to eradicate the infecting microorganisms. The cows were machine milked with the aid of a vacuum system maintained at 35.6 cm Hg. A cyclic vacuum fluctuation of 10 to 14 cm Hg existed during peak milk flow.\textsuperscript{6} Irregular vacuum fluctuation did not occur.\textsuperscript{15}

The method of milking time hygiene consisted of four procedures. The lower surface of the udder and all of the teats were washed with disinfectant-free warm (43 C) running tap water.\textsuperscript{7} Material on the udder and teat skin surfaces was removed manually during washing. Following washing, the skin on the udder and teats was dried with individual disposable paper towels. The cluster was sanitized with circulating hot (93 C) water before each cow was milked. Within 30 minutes

after removal of the teat cups, all teats were dipped in a disinfectant solution (10,000 PPM chlorhexidine* containing 6 oz. glycerine/gallon).**

The results of this study showed that heat sanitation of the cluster was lethal to all bacteria that commonly cause IM infection, but small numbers of gram-positive sporeforming rods could be recovered from the inside of the teat cups. It was estimated that dipping teats in the disinfectant solution lowered the number of bacterial CFU on the teat skin by 99%. Figure 1 shows the monthly incidence of IM infection before, during, and following the experimental period. The monthly incidence of new IM infection during the experimental period was 0.57%.

![Incidence of New Quarter Intramammary Infection Before, During, and After First Milking Time Hygiene Study.](image)

The study showed that it is possible to prevent most IM infections in the dairy cow but did not demonstrate whether all procedures were necessary or which procedure was most effective.

The cost of these milking time hygienic procedures was estimated at 7.5 cents/cow/day.

In order to evaluate the hygienic procedures used in the first study, an

*Nolvasan, Fort Dodge Labs, Fort Dodge, Iowa.

**This teat dip was used throughout the studies reported in this paper.
experiment was designed which utilized 12 lactating cows. For contamination of the teats and cluster, a 4-hour 1% serum broth culture of *Staphylococcus aureus* (Slanetz 10 strain) was diluted in fresh whole milk so that each milliliter of contaminating fluid contained 2 million CFU. The experiment consisted of 5 periods of 3 weeks each, with sufficient time between consecutive periods to insure that all quarters were bacteriologically negative before the start of the next period. When a new IM infection occurred, it was treated to eradicate the microorganisms as in the first study. During all periods, boiling water was circulated through the cluster twice a day only just before milking time. The disinfectant teat dip was bactericidal to *S. aureus* on the teat skin within 5 minutes.

During period 1, only the cluster was contaminated before each cow was milked. Within 30 minutes after milking, all teats were dipped in the disinfectant solution. No new IM infection resulted. In period 2, both the cluster and all teats were contaminated before each cow was milked and all teats were dipped after milking as in period 1. Results were analogous to those obtained in period 1. During period 3, a 4-minute overmilking period was carried out. The cluster and teats were contaminated only prior to the overmilking period. After overmilking, all teats were dipped as in period 1. Two new IM infections occurred.

In period 4, the cluster was sanitized before each cow was milked and all teats were contaminated after milking. Thirty minutes before the next regular milking, all teats were dipped in the disinfectant solution. Four new IM infections occurred during the period. During period 5, the cluster was contaminated before each cow was milked. All teats were contaminated both before and after milking and no disinfectant solution was used. Seventeen new IM infections resulted.

The weekly incidence of new IM infection was as follows: period 1, 0.96%; period 2, 0%; period 3, 1.11%; period 4, 2.22%; and period 5, 9.47%. No IM infection occurred when either the cluster or teats or both were contaminated before milking and all teats were dipped in the disinfectant solution after milking. However, two IM infections resulted when the cluster and teats were contaminated only prior to overmilking. When bacteria were placed on the teats after milking and allowed to remain throughout the time between milkings, the incidence of new IM infection was high.

The cost of the disinfectant solution for teat dipping was 1/2 cent/cow/day.

These 2 experiments demonstrate, as others have suggested,9,13,14 that the most important hygienic procedure in prevention of new IM infection is the dipping of teats in an effective disinfectant solution after every milking. This one procedure virtually eliminated the bacteria on the teat skin. This result indicates that the bacteria present on the teat skin between milkings are the most important source of new IM infection.

There are rigid requirements for an acceptable and effective teat dip2 for use in prevention of new IM infection. These include the following: 1. Bactericidal against all bacteria capable of causing IM infection. 2. Bactericidal in the presence of milk. 3. Residual bactericidal effect on the surface of the teat skin. 4. Non-irritating upon prolonged usage. 5. Minimal interference with tissue repair. 6. Residues do not appear in the milk. Few disinfectant teat dipping materials meet all these requirements.

In summary, milking time hygiene, mainly application of an effective
disinfectant teat dip after each milking, appears to be the best approach in prevention of new IM infection. Initiation of this procedure for the prevention of IM infection in field herds encounters 2 basic problems: 1. Many quarters are already infected and, 2. some quarters will continue to become infected, especially during the dry period. To be effective on a herd basis, this procedure must be combined with an effective treatment program both during lactation and the dry period.8,11

We need field herd application of this research on prevention of IM infection. The basic program includes: 1. Dipping all teats in an acceptable and effective disinfectant after every milking and 2. circulation of boiling water through the cluster prior to every milking period and after milking 50 to 100 cows. This program is simple and practical, and should be acceptable in the field. Treatment of IM infection and correction of faults in the milking machine and milking technique should aid in early mastitis abatement.

BIBLIOGRAPHY

REPORT OF THE USLSA MASTITIS COMMITTEE, 1968


The USLSA Mastitis Committee met October 7, 1968. Ten members and interested guests attended.

As a follow-up to last year's committee report, a survey form was circulated to the 50 states for the purpose of determining current interest and facilities for the eradication of mastitis caused by Streptococcus agalactiae. Analysis of the data received is incomplete at the present time. However, preliminary evaluation indicates that five states are presently conducting some degree of a S. agalactiae eradication program, while 12 additional states expressed a positive interest. The USLSA Mastitis Committee recognizes that for such an eradication program to be successful, a coordinated nation-wide effort is essential.

In an attempt to achieve coordination, the committee proposes to meet coincident with the 1969 National Mastitis Council meeting in Chicago, and to invite representatives of the Council and the American Veterinary Medical Association mastitis committee to participate in its deliberations.

In a further attempt to achieve national coordination, the committee approved unanimously the following recommendation: "The mastitis committee of USLSA in its 1967 report urged Federal and State regulatory officials responsible for animal disease control to institute appropriate measures to control and eradicate Streptococcus agalactiae infection. In support of that proposal the mastitis committee further recommended that the United States Department of Agriculture invite representatives of the USLSA, American Veterinary Medical Association and the National Mastitis Council to meet for the specific purpose of devising an acceptable and effective nation-wide S. agalactiae eradication program."

The committee reviewed the long-range objectives delineated at the 1967 meeting, and agreed that they are appropriate and are perhaps more urgent now. These objectives are as follows:

1. Create among dairymen, veterinarians, regulatory officials and extension workers a more general awareness of the importance of mastitis as an infectious disease of dairy cattle.
2. Encourage Federal, State and other regulatory agencies to develop and improve mastitis control programs.
3. Encourage expanding activities in mastitis research.
4. Encourage the establishment of laboratory facilities for the diagnosis of mastitis.
5. Encourage and stimulate funding for diagnostic facilities, mastitis research and mastitis control programs.

6. Encourage veterinary practitioners to assume a more active role in mastitis control.

The committee recommends that the USLSA maintain continuing membership in the National Mastitis Council.

Respectfully submitted,
Donald S. Postle, Acting Chairman
THE MOVEMENT OF FEEDER CATTLE.
MANAGEMENT FACTORS CONTRIBUTING TO THE RESPIRATORY DISEASE COMPLEX:
PART V AUCTION BARN SALES

Richard F. Bristol D.V.M., M.S.
Professor Department of Veterinary Clinical Science
Iowa State University, Ames, Iowa

During the fall of 1967, three auction barns in Northern, Central and Southern Iowa were observed from September 1, 1967 to January 1, 1968, a period of 15 weeks.

These barns were observed for methods of handling animals, source of livestock, clientele and general method of operation.

This report concerns itself primarily with the source of animals for auction barns and the clientele purchasing animals from these barns.

During the time of observation, a total of 124,068 animals were sold by these barns. This averaged 41,356 animals per barn and 2,750 animals per barn per week. The animals were consigned in 6,432 lots. A 19.2 animals per lot average.

Source of Animals

Cattle consigned by ranchers and farmers directly to the barns numbered 810 lots and 11,745 cattle. This represented 12.6 percent of the lots and 9.5 percent of the animals. Known dealers of cattle consigned 5,622 lots of 112,323 cattle. This represented 87.4 percent of the lots consigned and 90.5 percent of the cattle. Those cattle consigned listing joint ownership of animals (grouped) numbered 636 lots of 23,199 animals or 9.8 percent of the lots and 18.69 percent of the animals. Lots of one animal numbered 1,581 or 24.5 percent of the lots and 1.2 percent of the animals.

Sales of Animals

Sales to the farmer feeder of animals amounted to 4,323 lots and 103,548 animals representing 67.2 percent of the lots and 83.46 percent of the animals. Bid ins and repurchases by dealers numbered 2109 lots and 20,520 animals representing 32.8 percent of the lots and 16.5 percent of the animals.

Discussion

The observations made upon these sales barns are not meant to represent all of this type of means of exchange. The type, variety and volume size of sales barns is too varied to be represented here.

In general, there are three basic types of auction barns in Iowa. The first is a local variety auction of low volume and a varied consignment consisting of a variety of species and certain other merchantable goods. The clientele is usually local. The second is the specialty barn in areas where cow calf programs and/or dairy programs are the mode of farming. The consignments consist of cull and slaughter cows, replacement heifers and calves. These barns will also sell other species during one
day a week and during periods of low volume consignments will sell all species on a single day. The third type of auction barns acts as a meeting place for buyer and seller in areas where cattle feeding is the type of agriculture engaged in. These barns deal primarily in replacement animals for the feedlot and are usually high volume barns particularly so during the fall of the year when traditional restocking of feedlots begins. The clientele of these barns is primarily dealers in cattle, feedlot operators, order buyers of various types and abattoir representatives. The high volume and reputation auction barns attract clientele from many surrounding states in addition to Iowa.

The source of animals for this type of barn appears to be the cattle dealer rather than the farmer. It usually is a large operator with sufficient capitalization to purchase large numbers of cattle from varied points, assemble these animals and ship to the auction barn. These operators will usually furnish some animals for 2 to 3 barns. Shipping unsold or bid in animals from one barn to the other during the selling period.

It is quite rare to find auction barn owners and operators listed as owners of animals sold in that barn; although, it has been established that they do indeed buy and sell animals through “front men”. This same devious practice is used for “bid in cattle” or those for which too low a price is bid for profitable sale. A single dealer may have as many as 5 persons “fronting” for him in the sale ring and clientele area. These “bid ins” may amount to as much as 60 percent of the total animals in the sale, and average 32.5 percent of the lots sold and 16.5 percent of the cattle.

Grouping of small lots is a common practice of dealers and sales barn operators. These grouped cattle usually consist of repurchased cattle from previous sales, animals rejected by purchasers, ill animals that have been treated and recovered.

Single lots of animals seem to be the greatest risk in auction barn purchases. These animals are rejects, ill and generally of poor type. Upon permission of the owner of one sales barn, all single sales were examined for illness. Of a total of 486 single sales in this barn, 305 were found to have a serious defect. The tentative diagnosis rendered in these instances were: infectious bovine rhino tracheitis, chronic bronchial pneumonia, bovine virus diarrhea, infectious keratitis, papilloma, lymphosarcoma, ergot poisoning, chronic pleuritis, fracture of the femur, radius, third phalanx, separation of the scapula, malignant edema, infectious pododermatitis, vegetative pododermatitis, dilation of the esophagus, internal and external parasitisms, laryngeal edema, juvenile pregnancy, calf diphtheria and numerous abrasions, contusions and lacerations. These animals represent a total of 24.5 percent of the lots and 1.2 percent of the cattle sold.

Attempts were made to trace animals sold from sales barns to the final destination. This proved to be an impossible task, less than .5% of the animals could be consistantly traced. Records are not properly kept with attempts to trace seller and buyer highly confusing.

Identification of state of origin of animals is lost after the first sale and there remains no way to identify animals from other states and countries. We were able to identify on an inconsistent basis animals from 14 different states and one foreign country.

Conclusions
The method of handling, obtaining and selling animals in auction barns would seem to contribute to the respiratory disease complex in cattle; stale, stressed and diseased animals both perpetuate and perpetrate the disease complex. The problem of tracing animals remains the greatest puzzle in this method of marketing and identification of animals seems to be all but impossible.

There is a general laxity in enforcing existing federal and state regulations in identification of animals moved from one state to another. Inspection of animals is perfunctory.

Recommendations
1. More conscientious enforcement of state and federal regulations in identification of lots and animals shipped interstate.
2. Identification of animals regardless of their economic role on a national basis.
3. Stringent enforcement of animal disease regulations in regard to the selling of animals with or exposed to infectious disease conditions.

SALES

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CONSIGNMENTS

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AVERAGE

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REPORT OF THE COMMITTEE
ON
INFECTIOUS DISEASES OF CATTLE


A discussion was held concerning the reasons why the 1967 report was not accepted by the USLSA Executive Committee, yet was printed in the USLSA proceedings. The Committee then proceeded to rewrite the 1967 report by more clearly explaining the specific intent of the Committee in its deliberations.

The Committee requests that USLSA recommends to the Secretary of Agriculture that he appoint a national “task force” group to study and recommend to the USLSA a national program of “guidelines” for animal identification. It is suggested that initially, this program should start with the cattle industry. This “task force” should include representation from the total animal industry; animal producers, livestock organizations, marketing organizations, breed associations, livestock disease associations and animal health related interests.

On the subject of “preconditioning”, the Committee defined the term to mean a total program of preparing cattle to withstand the stress of movement and to prevent the spread of diseases peculiar to the movement of these animals. The Committee recognizes the work of the National Preconditioning Coordinating Committee and supports the continued need for research and development of a “preconditioning” program that would involve individual animal identification, herd identification, promote good management practices, disease prevention and control practices which are so essential to the economic well being of the total animal industry.

The Committee discussed and approved the following resolution as presented by the American Dairy Science Association:

WHEREAS, many private distributors of bovine semen may custom freeze and sell semen from bulls without regard to the health status of the bulls;

WHEREAS, semen from some bulls that were rejected by Artificial Insemination studs because of health examinations can be sold by private distributors;

WHEREAS, some semen handlers keep no fertility records on their bulls, may fail to keep proper records on the identity of bulls, semen and offspring, may sell semen from genetically inferior bulls and also neglect the service aspect to the dairymen - upon which the Artificial Insemination Industry was founded,

BE IT THEREFORE RESOLVED THAT: the Health and Breeding Committees
of American Dairy Science Association be commissioned to work with the United States Department of Agriculture and National Association of Animal Breeders to develop standards designed to regulate semen distribution and thereby protect dairymen and the purchasers of bovine semen.

From previous years' recommendations, the Committee again urged further consideration and appropriate action be instigated on the following items:

a. State Livestock Sanitary Officials should be made aware of the potential for disease transmission through the use of artificial insemination of livestock.

b. State Livestock Sanitary Officials should be made more aware of the existence of the United States Livestock Sanitary Association's recommended regulation and uniform certificate for movement of bovine semen.


This Committee urges the United States Livestock Sanitary Association to recognize the continued efforts of the Study Committee on Leukosis in cattle and encourages full dissemination of their deliberations.

The Committee on Infectious Diseases of Cattle pays tribute to the sponsors of the First Symposium on Bovine Respiratory Diseases held in Athens, Georgia, in June, 1966. We encourage full utilization of the information obtained from the proceedings of the Symposium. Also, we recommend that a second symposium on this subject be held by the sponsoring organizations at the earliest possible time.

The following resolution from the Inter-mountain Veterinary Medical Association adopted in June, 1967, on the subject of diseases of cattle, was considered and approved by the Committee:

WHEREAS: It is recommended by the Animal Health Committee that the Inter-mountain Veterinary Medical Association take note of the serious problems confronting the livestock industry and the veterinary profession in regard to anaplasmosis, calf scours, ram epididymitis and equine infectious anemia,

AND FURTHER, BE IT RESOLVED: That the Inter-mountain Veterinary Medical Association requests the appropriate committees of the United States Livestock Sanitary Association to continue or institute studies leading to the preparation of guidelines for handling these diseases.

Members of the United States Livestock Sanitary Association are reminded of the availability from the Animal Health Division of the United States Department of Agriculture of the 1966 supplement to an earlier publication on the subject of neonatal diseases of cattle.

The Committee recommends the institution of long range disease prevention, control, and disease eradication procedures to be developed for breeding herds of cattle in the United States. This involves re-evaluation of vaccines, testing procedures, and the reporting of morbidity and mortality of the diseases affecting cattle. The Committee recommends that the American Veterinary Medical Association, State Veterinary Medical Associations and state regulatory officials cooperate in the implementation of a morbidity and mortality reporting system. The information to be compiled would be reported back to the veterinary profession in each state and be made available to the United States Department of Agriculture and other interested persons or organizations.
The Committee recommends uniformity in testing by all diagnostic laboratories, particularly those diseases of cattle; namely viral diseases, vibriosis and leptospirosis.

Dr. John B. Herrick, Chairman
Committee on Infectious Diseases of Cattle
AN UNUSUAL INCIDENCE OF CYSTICERCOSIS

George A. Martin, DVM,
Acting Assistant to the Deputy Administrator,
Consumer Protection, USDA, Washington, D. C.

It is indeed a pleasure for me to have the opportunity to visit with you today. The United States Livestock Sanitary Association has been a leader for years in the field of disease control and problems confronting that phase of agriculture that supplies this nation with a meat supply unequal to any in the world. We, in the Consumer and Marketing Service of the United States Department of Agriculture, are honored to have the opportunity to work with such a fine organization.

The Consumer and Marketing Service administers a number of programs such as Food Stamp, Commodity Distribution, the National School Lunch Program, Meat Grading, Meat and Poultry Inspection, and many other similar programs having to do with the welfare of the consumer. Recently, the meat and poultry inspection services were combined under the Deputy Administrator for Consumer Protection. I represent the Slaughter Inspection Division of this Service.

For many years, we in the Slaughter Inspection Division, have been concerned with the problem of *cysticercus bovis* and its effect on the American meat supply. Cysticercosis is a very costly disease to producers and packers. Animals found extensively infected are condemned. This amounted to $28,000 in 1967. Those carcasses with slight infection are trimmed and refrigerated to assure that the meat is safe for human consumption. This amounts to approximately ten cents a pound or about $70 per animal. Last year losses from this cause totaled almost $300,000 in federally inspected plants alone. It is estimated that during 1967, $740,000 was spent in inspecting for cysticercosis in plants operating under Federal inspection. This does not include cost in state or municipal inspected plants.

*Slide 1* - This slide shows the total number of cases of cysticercosis reported in packing plants operating under Federal meat inspection from 1959 through 1967. As you can see, we had 13,375 cases reported in cattle during 1959 and 14,407 cases in 1967. These figures indicate that we have not made much progress toward reducing the number of cases of cysticercosis in the past nine years.

*Slide 2* - Further studies indicate that most cases of cysticercosis reported in the United States during 1967 were located in California, Texas, Colorado and Arizona. In fact, 10,455 of the 14,407 cases were in California. So our major problem area is the Southwestern and Western part of the United States. Evidence points to the likelihood that this is caused by migrant laborers contaminating areas and material with which cattle come in contact. Recently, we had a very unusual outbreak of this disease in two feedlots located in the Texas panhandle.

On March 15, 1968, a “lot” of 63 cattle were slaughtered in Oklahoma City, Oklahoma. Of this “lot”, 42 animals were infected with *cysticercus bovis*. This is certainly an alarmingly high incidence. Fortunately, the Consumer and Marketing Service meat inspector in this plant was able to obtain information that enabled us to trace the origin of these animals to a feedlot near Gruver, Texas. This information was passed on to the Animal Health Division of USDA's Agricultural
Research Service, to local and state health officials, and to U.S. Public Health
officials at the Communicable Disease Center in Atlanta, Georgia.

Immediately these officials launched a complete epidemiological investigation of
this outbreak. The owner of this feedlot promised to cooperate and to ship cattle
only to slaughter plants having inspection. He also agreed to notify inspectors at
destination before shipping animals from the infected premise. During the ensuing
weeks, cattle from this premise showed up in seven states from California to Florida
and many times under names not readily associated with this feedlot. There was no
notification to anyone regarding the movement of these animals. We kept Animal
Health and Public Health officials informed of these shipments of diseased cattle.
After it was obvious that a voluntary cooperative program could not be worked
out, Animal Health officials placed a quarantine on the premise where these
diseased animals originated.

Under the quarantine, all remaining cattle moved to packing plants having
adequate inspection. The average infection rate for cattle slaughtered in federally
inspected plants during 1967 was 0.05%. Some lots of cattle from this quarantined
herd had almost 100% infection. This feedlot had a capacity of 8,000 head and an
animal production of 20,000 head.

Another "lot" of cattle found to have a relatively high degree of infection was
traced to a feedlot near Hereford, Texas. This feeding operation was capable of
feeding 15,000 animals at one time. It was also placed under quarantine.

Information obtained by the Public Health epidemiologist investigating this
outbreak of cysticercosis, revealed that there were employees working in these
feedlots who were passing *Taenia* eggs in their stools. One of these men had the job
of cleaning the water troughs. Some employees quit their jobs rather than be
examined for tapeworms. Those found to be positive were treated to rid them of
tapeworms. There was some evidence of contamination of ensilage in trench silos
by human feces.

As a result of this outbreak of cysticercosis, a national task force was formed to
attack this problem. Represented on this task force are officials from Animal
Health Division in USDA, the U.S. Public Health Service, State and local health
agencies, industry, and the Consumer and Marketing Service of USDA. A plan of
eradication was worked out. This involves a team effort by all represented on this
task force.

A new reporting form was developed by Animal Health people that will
encourage better reporting and will supply much needed information for such a
program to be a success.

Consumer and Marketing Service meat inspection personnel will initiate all
action by reporting all cysts found during slaughtering operations. They will also
collect specimens for Animal Health personnel to use in their epidemiological
study. Animal Health will investigate all reported cases and attempt to locate the
source of infection. Where indicated, Public Health personnel and, in some cases,
local officials will be called in to assist in eradicating this problem.

Representatives of industry will actively participate in an educational program to
school employees in knowledge relating to the life cycles of the tapeworm and the
importance of suitable latrines that are properly used.

This *team* approach to the age-old problem of cysticercosis should significantly
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<td>1964</td>
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<td>Cattle</td>
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<td>4,540,430</td>
<td>13</td>
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<td>11</td>
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<td>1965</td>
<td>.05</td>
<td>Cattle</td>
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### TABLE II

**INCIDENCE OF CYSTICERCOSIS IN U.S. - 1967**

<table>
<thead>
<tr>
<th></th>
<th>Number of Cases</th>
<th>With Restriction (5011)</th>
<th>Refrigeration (5111)</th>
<th>Cooking (5021)</th>
<th>Condemned (5031)</th>
<th>Total No. of Cases</th>
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<tr>
<td>United States</td>
<td>14,407</td>
<td>10,100</td>
<td>4,102</td>
<td>112</td>
<td>93</td>
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<tr>
<td>California</td>
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<td>7,084</td>
<td>3,323</td>
<td>10</td>
<td>38</td>
<td>10,455</td>
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<td>777</td>
<td>487</td>
<td>275</td>
<td>11</td>
<td>4</td>
<td>777</td>
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<tr>
<td>Colorado</td>
<td>334</td>
<td>258</td>
<td>70</td>
<td>6</td>
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<td>334</td>
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<tr>
<td>Arizona</td>
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<td>130</td>
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<td>Calif., Ariz.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Texas &amp; Colo.</td>
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<td>3,798</td>
<td>27</td>
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<td>Remainder U.S.</td>
<td></td>
<td>2,103</td>
<td>304</td>
<td>885</td>
<td>46</td>
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Recently, Secretary Freeman announced a merger of the poultry and meat inspection programs into one food inspection component in C&MS. The eight regional food inspection offices will be in full operation by the end of 1968, replacing the 12 existing field units – seven in meat and five in poultry. Under the merger, each regional office will be responsible for all field operations of the combined Federal meat and poultry inspection programs.

The regional offices will be located as follows: Northeast, Philadelphia, Pa.; Mid-Atlantic, Raleigh, N.C.; Southeast, Atlanta, Ga.; North Central, Chicago, Ill.; Northern, St. Paul, Minn.; Central, Kansas City, Mo.; Southwest, Dallas, Tex.; and Western, San Francisco, Calif.

During recent years, the poultry and meat industries throughout the world have taken on greater economic significance than ever before. The production of poultry and meat and international commerce of these products are on the increase. Governments of other countries are encouraging and promoting the growth of poultry and meat production, processing, and exporting of these products. Exports of United States meat and poultry products have been made to virtually every country in all continents of the world. Many countries are writing us for information concerning getting their meat and poultry inspection programs approved by the United States.

Until the poultry and meat inspection acts were amended, they covered only plants which ship poultry and meat products in interstate or foreign commerce. These federally inspected plants processed about 87 percent of the total poultry and meat produced in the United States. About 13 percent (1.6 billion pounds) of the poultry sold off farms is not prepared for distribution in “commerce” as defined in the Act, and under present law, is not subject to Federal inspection. Since only four States have active mandatory poultry inspection programs, the majority of these poultry products receive no inspection. These poultry and meat products are permitted to be intermingled in the retailing process (intrastate sales) with federally inspected products for sale to the public.

The poultry and meat inspection laws, as amended by the Wholesome Meat Act and the Wholesome Poultry Products Act, go a long way toward providing consumers the protection they need and expect. The object of these laws is to assure consumers that the poultry and meat products they buy are wholesome, unadulterated, honestly packaged and properly labeled.

Those plants which are already under the Federal inspection programs will not be basically affected by the amended laws. The Wholesome Poultry Products Act of 1968 was closely patterned after the Wholesome Meat Act of 1967. Let’s look at their basic provisions.

*Both of the Acts provide the necessary tools to establish with each State a new nationally-uniform meat and poultry inspection program. The laws provide for broader consumer protection authority within the traditional interstate concept. They provide for full intrastate protection through authority for Federal assistance to the State for the purpose of improving State meat and poultry inspection.
DR. R. K. SOMERS

programs to an acceptable level. USDA can assist States through furnishing advisory assistance in planning, developing and implementing State programs; by providing technical, laboratory, and training assistance; and finally by contributing up to 50 percent of the State program costs.

Aid to the State is contingent on the State law meeting certain minimal requirements; for example, imposing mandatory ante- and post-mortem inspection, sanitary and reinspection requirements on some classes of intrastate plants and cooperating in the development of a documented improvement plan for each State program.

On meat inspection, we feel we are reaching our objective of a truly cooperative relationship in implementing the new meat inspection law. We have cooperative agreements with 27 States under the Wholesome Meat Act. Several others are pending. Since passage of the Wholesome Meat Act, 18 States have enacted or amended their meat inspection laws to bring their inspection program in line with the requirements of the Wholesome Meat Act. Reaching the same objective in a cooperative poultry inspection program should move along quickly and smoothly since the procedures have already been developed.

*Another of the basic features for both laws provides for the Secretary of Agriculture to appoint an advisory committee consisting of State Officials to assist in implementing Federal-State cooperation provisions of the Acts.

A 17-member National Advisory Committee on Meat Inspection was promptly appointed after passage of the Wholesome Meat Act and is active. It has been meeting monthly and is a "working" committee. Many recommendations have been made, reviewed and adopted. This same committee's responsibilities will be extended to include implementation of the Wholesome Poultry Products Act. The States not represented directly on the Advisory Committee also have an opportunity to assist in formulating policy. As appropriate policies and regulations are generated, they are being forwarded to the poultry and meat inspection officials in each State for review and comment prior to changes being made in regulations and procedures. Reports of committee meetings are also sent to all States.

*The Acts give USDA broader consumer protection authority in the areas of enforcement, especially in the control of illicit operations. They allow us to check on any members of those allied industries that have been suspected of diverting inedible products, intended for nonhuman use, back into human food channels. These authorities impose a requirement for registration, recordkeeping, and inventory review on such firms as brokers, wholesalers, renderers, animal and pet food producers, as well as those who also handle, transport and slaughter, in commerce, dead, dying, diseased and disabled poultry and livestock. Federal assistance to States will be provided for an effective State program in this area for intrastate operations.

Title II of the Wholesome Meat Act and Section 11 of the Wholesome Poultry Products Act are entirely new areas of coverage for those industries which are related to the production of animals and poultry, movement and processing of poultry and animals and their products, and the storage and distribution of these products. These regulations will facilitate carrying out the provisions of the Acts which are designed to keep unwholesome, adulterated, and unfit meat and poultry products out of the food supply.
*Under the laws, we now have detention and seizure authority which enables us to control meat and poultry food products that are moving illegally or which have become adulterated or misbranded outside of official premises.

*The new laws authorize the Secretary of Agriculture to provide Federal inspection at any meat or poultry plant immediately, even if it sells only to customers within a State, if the plant poses a hazard to human health and the State fails to take appropriate corrective action to remove the hazard.

State officials and members of the Advisory Committee deserve commendation for their many efforts this year to strengthen State inspection programs and implement the Wholesome Meat Act. Their cooperation with USDA's Consumer and Marketing Service has been outstanding and has demonstrated that the cooperative State-Federal approach to this area of consumer protection is indeed practical.

As a means of developing and strengthening State meat inspection organizations, the Talmadge-Aiken Act has been used in several States very effectively. Under this Act, plants applying for Federal inspection can be staffed with trained and licensed State inspectors who perform Federal inspection under the Federal regulations and procedures.

Products prepared in plants under the Talmadge-Aiken program are federally inspected and can move in interstate and foreign commerce. Federal supervision of these plants is provided in much the same manner as at regular federally inspected plants staffed with Federal inspectors. The cost of providing inspection is shared equally by the State and Federal Government in the same manner that the cost is shared with those States which have signed a cooperative agreement with USDA under the Wholesome Meat Act.

We do not anticipate that plants coming under Federal inspection will be staffed under the Talmadge-Aiken procedure except in those instances where such arrangements appear needed to maintain and strengthen the State inspection organization. It must be understood that federally inspected plants which are staffed now with Federal inspectors will not be changed to staffing under the Talmadge-Aiken procedure.

Under the Wholesome Meat Act, a number of amendments to the Federal meat inspection regulations are needed. These proposed regulations have been drafted, reviewed by the Advisory Committee, and are now being processed by our General Counsel's office. They should be ready for publication as proposed amendments shortly.

*Continuing our discussion of the basic provisions of the new laws, it might be well to touch on a special feature of the Wholesome Poultry Products Act. It has to do with a clarification in the authority under which Federal inspectors condemn diseased poultry. When poultry is condemned because of disease, the reason for it must be supported by scientific fact, information, or criteria. And the condemnations must be achieved through uniform inspection standards and uniform application. It is clear from the legislative history of the new laws that it is not the intent of Congress for diseased meat and poultry to be passed for human food.

*The legislative history of the new laws clearly indicates the intent of Congress that both Federal and State inspection programs are to be financed by appropriated
funds and not through registration or other fees which are collected from the plants receiving inspection service.

*The laws authorize more control over misbranded products and *may* require additional labeling on certain nonconsumer packaged poultry products when they leave the official establishment. This could be done by regulations if determined necessary by the Secretary of Agriculture. As an example, wing tags might be required on ice-packaged poultry carcasses.

*There are modifications of certain producer and processor "exemptions" from Federal poultry inspection. The exemptions will vary, depending on the volume of poultry products produced or processed. All exempted products must be sold intrastate. Producers are exempt from the Act for 250 turkeys or 1,000 chickens of their own raising - in a given calendar year - provided they do not buy and sell poultry other than that produced on their own farms and that none of the product moves in commerce. A producer can sell more than 250 live turkeys which he raised on his farm, but he cannot process more than 250 head without inspection. There will be certain poultry inspection requirements waived for the annual volume between 250 and 5,000 turkeys (1,000 to 20,000 chickens). The inspection requirements on sanitation, records, healthy birds, unadulterated products, and labeling will be provided for by regulations for these operators.

*The laws give the Secretary of Agriculture authority to withdraw or refuse inspection service to a plant if the applicant or recipient is found to be unfit to engage in any business requiring inspection under the Acts.

*The laws require the Secretary of Agriculture to submit annually to the House of Representatives Committee on Agriculture and the Senate Committee on Agriculture and Forestry, a detailed written report on the administration of the Acts.

*The laws provide for punishment of anyone who interferes with an inspector while performing his official duties. To carry out an efficient inspection program, inspectors must not have their safety placed in jeopardy.

There are other minor changes in the laws as amended, but I believe we have covered the ones considered basic. There are a few other points I would like to make relative to our inspection programs.

Maintaining uniformity throughout the Federal inspection programs is a matter that has received special attention for some time. Because of the highly perishable nature of the products involved, it has been necessary to delegate authority for making decisions to all inspectors. Measures taken to achieve uniformity begin with orientation and training of new employees in the policies and procedures of the program. Close supervision is also provided because of the important decisions which inspectors make throughout their tour of duty. The inspection program organization that has been adopted will provide the Slaughter and Processed Food Inspection Divisions with operating staffs who have the responsibility for reviewing the inspection program nationally to assure that it is being applied uniformly.

Comments that I have received from time to time indicate that these measures have not been entirely effective in achieving the standard of uniformity which is needed. It is apparent that there is not enough communication between establishment management and inspection supervisors concerning instances where the inspector's decision is thought to be incorrect. Some managers have indicated
that they have failed to communicate because an appeal might offend the inspector or the inspector might tighten up inspection if his decisions were questioned.

If there is any incident of retaliation by an inspector, I can assure you that the program supervisors will deal with it in a forthright manner. I consider that all inspectors have a responsibility to explain to plant management the reasons for any decision which they make. Similarly, plant management has the responsibility for accepting inspector's decisions which are in accordance with regulations and procedures.

We have increased our chemistry and bacteriology support in assuring compliance with the regulations of poultry and meat food products. We are studying specific products to determine how processing sanitation can be improved, and we are intensifying the surveillance of these products for potential adulterants. We plan to do much more in updating this phase of our program. Steps are being taken to replace obsolete laboratories, automate analytical procedures, and tighten our criteria for excellence.

Preventive sanitation will be an important part of the new nationally-uniform inspection programs. Since meat and poultry plants are in essence, large kitchens, they must be clean--inside and out--and must provide a suitable environment for preparing food. Regular sanitation checks and reports are essential. Operators must take responsibility for clean-up before operations are permitted.

Our objective is to eliminate Salmonella and other pathogens, as well as food-spoilage organisms, from the food supply. Pilot plants are now operating on this basis. This will take a great amount of research and improvement in food processing and handling techniques. We expect that bacterial standards for meat and poultry products will become a reality.

It is quite probable that in connection with developing some standards, for product composition, public hearings will be held for the purpose of making sure that all interested persons including consumers will have the opportunity to participate. We intend to guard against any procedures which would interfere with the prompt marketing of new products. More complete standards which are developed on the basis of full information and reflecting the needs of both producers and consumers should be of considerable value to the meat and poultry industries.

There is another significant development in the consumer protection program which I would like to comment on. This is in the area of training. Four regional training centers have been established--at St. Paul, Omaha, Fort Worth, and Los Angeles. Another is planned in the Southeast. All new meat and poultry inspection personnel go through these training centers before being assigned to inspection duties. The initial formal training of meat and poultry inspectors takes four to six weeks, while for veterinarians it takes twelve weeks. The training orients the new employee on inspection policies and instructs him in uniform and effective inspection techniques and procedures for beginning inspection duties.

We are training a large number of State inspection personnel, as well as Federal personnel, at these training centers. Training in processing inspection is also being provided. We expect in the near future to begin processing training at one university. We consider this a very significant development since it will give us full access to the scientific capabilities which are available. If this program is successful,
it will probably be extended to other universities and should provide the means for substantial upgrading of scientific and technical capability of our nonveterinary inspectors.

As a result of opening these additional training centers, we are closing our Chicago Meat Hygiene Training Center, which has for a number of years made significant contributions in the training of meat inspection personnel.

In closing, let me comment on the subject of communications. The need for better communications within our inspection organization, within the industry, between industry and State and Federal Governments and with consumers is demonstrated every day. We have been going through a period when communications between the consumer protection program and State governments have received much attention. There are many things that can be done to improve communications, and we are going to do as much as we possibly can in this area. We certainly would welcome any suggestions which you can offer.

We are planning a single, regular publication which will go to State and Federal Inspection officials, industry and all other interested persons. This publication would contain all of the official issuances by the consumer protection programs. Only in case of a real emergency would an issuance be made outside of this publication. Progress is being made on this project and we hope to begin the publication by January 1969.

The coming year will bring many changes — some anticipated and some not. We in consumer protection shall do our very best to handle all problems and changes in a practical manner. We shall expect cooperation from the states and we pledge cooperation by the consumer protection program, in achieving of our mutual objective of assuring a clean, safe, wholesome, and truthfully labeled supply of meat and poultry for all Americans.

REPORT OF COMMITTEE ON MEAT,
POULTRY AND MILK HYGIENE

72nd ANNUAL MEETING

United States Livestock Sanitary Association
New Orleans, Louisiana
October 6 to 10, 1968


Your committee reviewed its report of the 71st Annual Meeting of the United States Livestock Sanitary Association, Phoenix, Arizona, October 1967. A continuing study of all major topics covered in that report has been conducted during the past year.

A subcommittee on the Disease of Importance to Meat Inspection is being established in the committee.

Active liaison with the presently established United States Livestock Sanitary Association’s committees concerned with mastitis, public health, and salmonellosis is being maintained.

Each of the thirteen subcommittees met the day prior to that designated for the full committee meeting.

The reports of each of the Subcommittees were reviewed by the full committee and the following was approved:

I. Proposed Position Statement on the Wholesome Meat Inspection Act and Wholesome Poultry Products Inspection Act


2. When these laws are implemented, the consuming public will be better assured of wholesome meat and poultry products nationwide than has heretofore been possible.

3. These new laws will significantly improve protection of the health and welfare of the consumer, enhance consumer acceptance and assist in
the marketing of these products.

4. This Association urges all states to accept their share of responsibility under the Acts. These include the passage of necessary legislation and adequate funding to provide for state inspection programs which meet the requirements of the Acts.


The rapid implementation of the provisions of the Wholesome Meat Act is dependent on the states cooperating with the United States Department of Agriculture. Since its passage, USDA has entered into cooperative agreements with 27 states and 4 agreements are pending (at this writing).

USDA has established 24 Federal-State cooperative officer positions to assist the states in developing their meat and poultry inspection programs. Additionally 307 state and 538 federal inspection employees have been trained in USDA training centers.

A joint Federal-State survey has been conducted in intrastate plants in all states.

A 17 member National Advisory Committee was appointed by the United States Secretary of Agriculture. This committee is composed of appropriate state officials who will consult and advise the Secretary in implementing the Wholesome Meat Act. The committee has met at regular intervals since their appointment. They have made over sixty recommendations of which fifty-two have been accepted and are being implemented. The National Advisory Committee is assisting with the development of new Federal regulations which are to be published soon in the Federal Register.

The recently passed Wholesome Poultry Products Inspection Act is in the initial stages of implementation. This Act is almost identical in authority and scope to the Wholesome Meat Inspection Act. Significant exceptions are two inspection exemption provisions.

Your committee recommends the continued close cooperation between the appropriate state agencies and the United States Department of Agriculture in implementing these new consumer protection laws.

III. Proposed Curriculum for Food Hygienists

The committee reiterates its recommendations of 1967 (1) "that the prerequisites for employment of non-veterinary food inspection technicians be reviewed and steps be taken to strengthen such prerequisites to assure employment of better qualified technicians to assist in the food inspection areas."

(2) "That the appropriate agencies of the Federal Government be requested to furnish assistance - financial, administrative, and instructional aid - to the Colleges of Veterinary Medicine in the development of their instructional programs in meat and poultry hygiene."

(3) "That such assistance also be offered to states in the development of their inspection systems, with strong emphasis and active support in the area of training of their supervisory and inspectional personnel - professional and subprofessional."
The successful completion of a two year college course in food hygiene should be established as a prerequisite for all meat and poultry inspection, non-veterinary supervisors and program reviewers. This training is essential to inspection personnel who must evaluate increasingly complex production methods in regards to consumer protection. It is also essential in carrying out more sophisticated inspection techniques.

The curriculum for training these food hygienists should include: 1) Basic mathematics, 2) general microbiology and food microbiology, 3) general chemistry and food chemistry, 4) English (oral and written communications), 5) zoology (anatomy), 6) food plant sanitation, 7) food preservation and deterioration, and 8) basic statistics.

Longer range plans should call for all non-veterinary meat and poultry inspection personnel to have completed this two year course prior to employment. With the increased competence and ability of non-veterinary inspection personnel, the veterinary food hygienists will be able to assume a more professional role in inspection programs.

The Committee further recommends that the curriculum for the food hygienists be developed by a committee appointed by the Council on Education of the American Veterinary Medical Association.

IV. Post Doctoral Training of Veterinarians in Food Hygiene

Your committee recommends that the College of Veterinary Medicine provide intensified and in-depth training in food hygiene and inspection techniques and procedures at the Master's and Ph D levels for those veterinarians who will be required to assume positions of responsibility in administration and supervision of food hygiene and in teaching. Veterinary colleges are the logical educational institutions to assume leadership in providing such graduate training to develop the necessary expertise for veterinarians engaged in this area of veterinary medicine - essential to the health and welfare of man and the control, prevention and eradication of diseases of animals. It is further recommended that those veterinarians qualified by such training be encouraged to seek Diplomate status in an appropriate specialty board.

V. Utilization of non-Veterinary Personnel in Ante Mortem and Post Mortem Inspection

Faced with the responsibility for recommending ways of covering the inspection in thousands of meat plants, the National Advisory Committee on Meat Inspections has recommended the following changes in the assignment of inspectors: "In slaughtering plants that lay inspectors be allowed to hold questionable animals for final disposition by a veterinarian, that animals that the lay inspectors determines unquestionably unfit for human food be condemned by him with a right of appeal by the owner of the animals."

The shortage of veterinarians has been mentioned as the reason for assigning to lay inspectors responsibility for making certain condemnations, including carcasses. Your Committee does not accept this extension of non-professional responsibility as being in the public interest. Veterinarians have the necessary
professional background to make correct judgment on the health of animals. This background is essential to assure that all meat is wholesome.

Veterinary ante mortem and post mortem inspection is required by most countries to which our meat may be exported. Consumer confidence in the United States must not be shaken. Our foreign markets must not be jeopardized.

Meat packers and animal owners have a right to expect that all dispositions are made correctly. They have learned that frequent appeals do not make for harmonious relations. In the case of animals bought on a grade and subject to inspection, the owner would in most cases be unable to protect his own interest.

Veterinarians for these programs have been reported to be in short supply. Much more can be done to involve veterinarians in these programs on a part time and full time basis. Salaries must be raised to the point where they are competitive. With proper documentation and support, increases can be obtained at the Federal level both in grade and by higher steps in grade. The Department of Agriculture should fully support such moves since they are the major employer of veterinarians.

In order to assure well qualified veterinarians sufficient to provide coverage of the State and Federal programs, we recommend that every effort be made to employ the best qualified veterinarians available to provide necessary post-graduate training and to assign them under circumstances that will fulfill the policies set out in the Wholesome Meat Inspection Act of 1967.

VI. Ante Mortem and Post Mortem Technique Films Available

The Consumer protection program of the U.S.D.A.'s Consumer and Marketing Service has prepared training films depicting each step in ante mortem and post mortem inspection of cattle, swine, sheep and calves. These films are in color and with a complete narration. They average about 15 minutes in length.

The purpose of the films is to aid in training federal and state employees in the mechanics of inspection and to standardize inspection work in this country and abroad.

Each regional office of the Federal Meat and Poultry Inspection Program has several sets of films available for lending to state personnel. Sets are also on hand in each meat inspection training station. Five complete sets are used by C & M S inspection personnel assigned to foreign program review to demonstrate American inspection methods.

States or others desiring sets of films for permanent use may purchase them at a nominal price from the Motion Picture Service, U.S.D.A., Washington, D.C.

VII. Code for Processing Frozen and Dried Egg Products

In line with the recommendations in the report of this Committee at the 71st Annual Meeting relative to development of a code for processing frozen and dried egg products and the possible inclusion of immature fowl ova, the Public Health Service has completed a draft of a code and presented the same to interested public health agencies for evaluation and comment.

VIII. Techniques of Poultry Inspection
Your committee recognizes the need and recommends that attention be directed to possible changes in inspection techniques as an aid in further assuring safety and wholesomeness of food products. Until such time that salmonellae can be eliminated from poultry in the field or controlled to the extent that all poultry presented for slaughter is free from these organisms, more specific control precautions should be taken at slaughter and eviscerating plants. Since these organisms cannot be detected by organoleptic examination, each bird should be handled as if it were known to be affected. These precautions are needed to more positively prevent meat from becoming contaminated. Even a low concentration of these organisms could be considered a public health hazard.

Inspection personnel can be instrumental in encouraging and stimulating industry to initiate high standards of quality control. Orienting and educating plant workers in basic hygienic practices are of paramount importance to these programs.

The following recommendations are submitted for consideration to reduce contamination and other microorganisms in poultry slaughter and eviscerating plants:

1. Establish and require a minimum feed withdrawal time prior to slaughter sufficient enough to at least render the birds' crops empty. Care must be taken not to over stress the birds. It is important that they have access to drinking water as long as possible or practical.

2. Possible slaughter procedures that will allow bleeding and slaughter without opening the outer neck skin and exposing neck meat. A possible approach could be orally.

3. Eliminate the community bath-type scald tank. A possible alternative could be a combination of steam and water-spray in cabinets. These may be used in stages prior to each picking unit, thus eliminating the present need for water flushing at these points.

4. Design open cuts and evisceration procedures that will allow complete evisceration without exposure of the alimentary canal.

5. Improve flow of product for evisceration.

Note: There is no need to maintain identity of the carcass with its viscera at any point past the post-mortem stations. Since the most obvious source of salmonellae is in the digestive tract of the poultry, there should be merit in separating the viscera from the carcass as soon as possible and harvesting the edible visceral organs from a conveyor running in a direction away from the carcass line. The carcasses would be conveyed passed work stations necessary for rendering the carcass ready-to-cook (lung, trachea, oil gland removal, etc.). This ......

6. Chilling procedures should be developed to replace community bath-type chilling. A spray system of refrigerated water in refrigerated compartments may be an answer.

7. All by-products (offal, feathers, condemned carcasses and parts, etc.) not processed for human consumption should be steam sterilized, pasteurized, or otherwise rendered free of salmonellae and other possible disease producing organisms. This should be required either on the premises or the product should be allowed to be shipped under control to approved rendering plants.
IX. *Residues in Milk, Meat and Poultry Products*

Toxic and biological residues in milk, meat and poultry products continues to be a threat to human health. A resolution on this subject has been prepared by and submitted by your committee to the Committee on Resolutions.

X. *Establishing a Specialty Board in Administrative Veterinary Medicine (Temporarily Designated - “American College of Regulatory Veterinary Practice”)*

It is recommended that a specialty board in Administrative Veterinary Medicine be established. It is further recommended that this Committee take the responsibility for initiating and developing an organizing committee in establishing this Board.

This specialty would include, but not be limited to, those veterinarians whose area of specialization is in the areas of food inspection, animal disease control, drug, biologics and feed additives evaluation control. Also, it would include those veterinarians involved in teaching veterinary preventive medicine and meat hygiene and the industrial and extension service veterinarians.

A resolution recommending the establishment of this Specialty Board has been prepared and submitted by your Committee to the Resolutions Committee for appropriate action.

XI. *Recommendations on Food Hygiene at Symposium on Education in Public Health and Preventive Medicine*

The Pan American Health Organization sponsored a Symposium on Education in Public Health and Preventive Medicine March 18 to 22, 1968, at the College of Veterinary Medicine, University of Minnesota. Your committee recognizes the need and supports the nine recommendations made by the Committee on Food Hygiene of that Symposium. A copy of their recommendations is included as an addendum to this Report.

XII. *International Symposium on Meat and Poultry Hygiene*

The committee supports the Conference of Public Health Veterinarians in their effort to interest the Pan American Health Organization in sponsoring an International Symposium on Meat and Poultry Hygiene. Your Committee further supports the suggestions of the Conference to hold this symposium in conjunction with the international meeting of food hygienists previously planned to be held in Germany. It is agreed that the conference President accept the offer of Dr. Morris Shiffman to host the Symposium at the School of Public Health, University of North Carolina, Chapel Hill, North Carolina, or in Washington, D. C.

XIII. *Symposium of World Association of Veterinary Food Hygienists in Yugoslavia*

The Vth Symposium of the World Association of Veterinary Food Hygienists will be held in Opatija, Yugoslavia, September 22-27, 1969. The proposed themes were published in the Journal of the American Veterinary Medical Association, August 15, 1968, and in the May-June 1968 issue of the Federal Veterinarians.
Your Committee strongly recommend that veterinarians from the United States and other Countries of the Western Hemisphere offer papers for this Symposium reflecting the advances that have been made in meat and poultry hygiene. Active participation in such world meetings is essential if we are to develop and hold a position of leadership in this field. Educators, Research Workers and Federal and State Food Inspection Officials should especially be represented.

XIV. Microbiological Standards for Meat Products at Retail Level

Your Committee recommends that the Public Health Service and the United States Department of Agriculture cooperate with states, counties and municipalities in developing microbiological standards for meat products offered for sale at retail. It is emphasized that adequate research, facilities and qualified personnel must be available before such programs can function. Universities should apply for research grants to conduct these studies.

XV. Public Information

Your committee has prepared appropriate releases for use in farm, meat industry and veterinary periodicals and journals, as requested by the President of the United States Livestock Sanitary Association.

Your Committee will continue to disseminate information on meat, milk and poultry hygiene to all interested publics to include — but not limit to — the following:

1. Veterinary Colleges
2. Colleges of Agriculture
3. American Veterinary Medical Association
   a. Council on Education
   b. Council on Public Health and Regulatory Veterinary Medicine
4. State Veterinary Medical Associations
5. Meat and Poultry Industry Organizations
6. Livestock Organizations
7. Farm Organizations
8. Consumer Organizations
9. Agricultural and Home Demonstration Services
10. State and County Fairs
11. Animal Health Division, United States Department of Agriculture

Wm. E. Jennings, Chairman
Committee on Meat, Poultry and Milk Hygiene
The current era is one of great change both in legal aspects of inspection and in food production, transportation and processing technology to meet consumer demand and to protect his health and welfare. Rapid movement of livestock people and goods increases hazards from exotic diseases of both man and animals. The Wholesome Meat Act of 1967 and the pending Wholesome Poultry Products Inspection Act and the Act pertaining to the inspection of fish and marine products place a greatly increased demand on inspection both in numbers of Veterinarians and subprofessional food inspection technicians needed, and the competency of the individuals involved.

It has long been recognized that one of the major responsibilities of the Veterinary Profession is to assure the wholesomeness of human food products of animal origin. To meet the requirements of this increasingly complex situation, it is essential that the Veterinary profession assume leadership in establishing, expanding and improving both the scope and quality of food hygiene in general and hygiene of foods of animal origin in particular.

Therefore, the following recommendations are made:

1. That the Association of American State Boards of Examiners in Veterinary Medicine and the National Board of Veterinary Examiners reevaluate coverage of food hygiene in their examinations. That questions related to food hygiene emphasize scientific knowledge of principles or concepts rather than the ability to perform techniques of food inspection. The Association of Teachers of Veterinary Public Health and Preventive Medicine be requested to provide the questions. Currently, there is a great lack of uniformity between states in the scope of coverage of food hygiene in the examinations. Further, in both the States and National Board Examinations emphasis is frequently placed on minutia of techniques of inspection rather than on the knowledge of principles and concepts.

2. That curriculum of the veterinary colleges include fish hygiene in its food hygiene program. The role of fish as a protein source is increasing tremendously in
part because fish farming is becoming popular. Fish raising, harvesting, and diseases are subjects which are receiving close scrutiny by marine biologists and wildlife scientists. The wholesomeness of fish and marine products should be the concern of the Veterinary Food Hygienists.

3. That the Veterinary profession assume its role of leadership in the proper use of chemicals and other substances which may affect the wholesomeness of the foods of animal origin and human and animal ecological factors. Drugs, biologics, herbicides, insecticides, rodenticides, antimicrobials, hormones, feed additives, growth stimulants, tranquilizers and other chemicals and substances are being used in ever increasing frequency for diverse purposes and acceptable tolerances are being constantly revised. Many new compounds are being developed and used. The full impact on man, animals, ecology of both human and animal diseases and the environment has not been accurately and fully assessed. Residues in foods of animal origin and problems associated therewith are logically Veterinary Public Health.

4. That colleges of Veterinary Medicine provide intensified and in-depth training in food hygiene and inspection techniques and procedures at the masters and Ph. D. levels for those Veterinarians who will be required to assume positions of responsibility in teaching, administration and supervision of food hygiene and inspection. Veterinary colleges are logical educational institutions to assume leadership in providing such graduate training to develop the necessary expertise for Veterinarians engaged in this area of Veterinary Medicine - essential to the health and welfare of man and the control, prevention and eradication of diseases of animals. That those Veterinarians qualified by experience or training (post graduate) be encouraged to seek Diplomate Status in American Board of Veterinary Public Health.

5. That continuing education courses in food hygiene be made available to Veterinarians engaged in food hygiene. These courses are necessary to properly inform of new developments concerning diseases, toxicology, emergency disasters and other topics. This training will provide expertise to safeguard the health of man and animals.

6. That course offerings in all aspects of veterinary food hygiene in the colleges of Veterinary Medicine be made more uniform, be strengthened, improved in quality, modernized, and be made more scientifically-oriented with less emphasis on mechanical techniques. Current and pending legislation requires uniformity of competence by graduates of all colleges of Veterinary Medicine throughout the U. S. Since the shortage of Veterinarians will require that they be used in an appropriately more professional capacity, the training must of necessity provide the Veterinarian with a higher level of professional competence in the area of food hygiene.

7. That microbiological examination be applied in food plant sanitation and in monitoring of processing conditions. This may eventually lead to the establishment of ranges of microbial content as one of the criteria for judgment of wholesomeness of foods. Organoleptic examination is no longer sufficient due to the complexity of food processing. Further, effectiveness of sanitizers in food processing plants can be evaluated only by microbiological examination.

8. That techniques of inspection be subjected to a continuing evaluation and re-evaluation to insure most effective utilization of personnel and application of
new techniques and concepts in control systems. Techniques of inspection as currently applied are essentially the same as when veterinary inspection was first instituted. However, profound changes in disease patterns of man and animals and food processing techniques have taken place. Under certain conditions meat and poultry inspection could be based on a continuing survey of the health conditions in the production unit as a supplement to antemortem and postmortem inspection in the slaughtering plant. This would not only protect the consumer but would also contribute to the prevention of diseases in food, animals and birds. It should also be pointed out that principles of statistical quality control have not yet been generally applied in the inspection of foods of animal origin.

9. That the job specifications for Meat and Poultry Inspector be changed to require certification of completion of an approved two year course in food inspection or its equivalent. That the curriculum for the subprofessional technician be developed by a committee appointed by the Council on Education of the American Veterinary Medical Association, for a two year food hygiene course. That the curriculum for such two year course be under the supervision of Veterinary Food Hygienist. That uniformity in such two year courses be assured thru curriculum content. The more sophisticated techniques of inspection require a better trained, more capable inspector. If Veterinary Food Hygienists are to assume a more professional role in the inspection program, the Meat Inspector must have increased competence and ability. That American Board Veterinary Public Health establish standards for certification and curriculum for the 2 year curriculum.

Wm. E. Jennings
Chairman
Fiscal year 1968 marks the first full year of operation of the Cooperative State-Federal Salmonella Program. This program is conducted according to the procedures outlined in the 1966 and 1967 Report of the Salmonellosis Committee. Current activities are limited to the program for the voluntary control of Salmonella in animal proteins (rendered animal byproducts and marine products) used in livestock and poultry feeds. The primary objective of this activity is to reduce the level of Salmonella contamination in these two critical ingredients of animal feeds.

The number of States participating in this program increased from 26 at the beginning of the fiscal year to 48 at the close of the fiscal year. The participating States are shown in Figure 1. Since July 1, 1968, Puerto Rico has entered the program, and State and Federal officials have reached an agreement for conduct of the program in South Dakota. In December of 1967 the program was extended to include fish meal plants on the same voluntary basis it was being offered to the rendering industry.

The following is a summary of plant inspections and sample collections during fiscal year 1968. There are approximately 924 plants in the United States, Puerto Rico and the Virgin Islands producing animal protein for use in livestock and poultry feeds. Of these 874 are located in the States that entered the program prior to July 1, 1968. Table 1 gives the distribution of the rendering and fishery plants in each of the administrative regions of the Animal Health Division. Approximately 95 percent of the plants in the United States are now covered by the program. Figure 2 gives the distribution of plants by category of rendering operation.

At the end of fiscal year 1968, at least one inspection had been made in 718 of the 874 plants located in the participating States (Table 2). Table 3 gives the number of plants inspected by round and the number and percent of the plants inspected that had all negative samples on each round. There is a decreasing percent
of the plants remaining negative after each subsequent inspection. The difference in number of plants inspected on each round is due to the difference in dates when the States entered the program. The first inspection in some States and the fourth inspection in others were both made during the last quarter of the fiscal year. At the end of the fiscal year, 353 plants had received three inspections. This is the number called for by the program for plant evaluation. Of these 353 plants, 96 or 27 percent had all negative samples on each inspection.

During the inspections indicated in Table 3, 14,512 samples were collected, of which 2,278 or 15.7 percent were positive. A total of 7,075 samples were collected in the plants that had one or more positive samples, of which 2,278 or 32.2 percent were positive. This later figure is approximately the same as the proportion of samples found to be positive during the feed survey. This is understandable when it is considered the product of one renderer may have been included in samples collected at two or more feed mills.

Table 4 shows the distribution of the 353 plants by category of rendering operation and sample results after three inspections. None of the plants in the poultry slaughter renderer category were negative after three inspections, while 9 of the 16 or 56 percent of the marine product plants were still negative. The percent of plants negative in the independent and slaughter renderer category were essentially the same. Table 5 gives the distribution of the 243 plants that have

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**TABLE 1 - STATES AND ESTABLISHMENTS PARTICIPATING BY REGION AS OF JULY 1, 1968**

<table>
<thead>
<tr>
<th>REGION</th>
<th>STATES NUMBER</th>
<th>ESTABLISHMENTS NUMBER</th>
<th>TOTAL NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RENDERING</td>
<td>FISHERY</td>
</tr>
<tr>
<td>NORTHEASTERN</td>
<td>13</td>
<td>151</td>
<td>7</td>
</tr>
<tr>
<td>NORTH CENTRAL</td>
<td>10</td>
<td>237</td>
<td>2</td>
</tr>
<tr>
<td>SOUTHEASTERN</td>
<td>12</td>
<td>186</td>
<td>67</td>
</tr>
<tr>
<td>WESTERN</td>
<td>13</td>
<td>206</td>
<td>20</td>
</tr>
<tr>
<td>TOTAL</td>
<td>48</td>
<td>780</td>
<td>96</td>
</tr>
</tbody>
</table>
STATE-FEDERAL SALMONELLA PROGRAM

48 States Participating—July 1, 1968

Figure 1

DISTRIBUTION OF 874 PARTICIPATING PLANTS UNDER THE SALMONELLA CONTROL PROGRAM

INDEPENDENT (364) 42%

LIVESTOCK SLAUGHTER (343) 39%

MARINE (96) 11%

POULTRY SLAUGHTER 4% (39)

BLENDER 4% (32)

July 1, 1968

Figure 2
### TABLE 2 - PARTICIPATING PLANTS THAT RECEIVED ONE OR MORE INSPECTIONS DURING FISCAL YEAR 1968

<table>
<thead>
<tr>
<th>RENDERING CATEGORY</th>
<th>PLANTS PARTICIPATING</th>
<th>PLANTS INSPECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>NUMBER</td>
</tr>
<tr>
<td>INDEPENDENT</td>
<td>364</td>
<td>314</td>
</tr>
<tr>
<td>LIVESTOCK SLAUGHTER</td>
<td>343</td>
<td>278</td>
</tr>
<tr>
<td>POULTRY SLAUGHTER</td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td>BLENDER</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>MARINE PRODUCTS</td>
<td>96</td>
<td>70</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>874</strong></td>
<td><strong>718</strong></td>
</tr>
</tbody>
</table>

### TABLE 3 - ANIMAL AND MARINE PLANTS INSPECTED AND FOUND CONSISTENTLY NEGATIVE DURING FISCAL YEAR 1968

<table>
<thead>
<tr>
<th>INSPECTION ROUND</th>
<th>PLANTS INSPECTED</th>
<th>PLANTS CONSISTENTLY NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>NUMBER</td>
</tr>
<tr>
<td>FIRST</td>
<td>718</td>
<td>423</td>
</tr>
<tr>
<td>SECOND</td>
<td>499</td>
<td>248</td>
</tr>
<tr>
<td>THIRD</td>
<td>353</td>
<td>96</td>
</tr>
<tr>
<td>FOURTH</td>
<td>243</td>
<td>54</td>
</tr>
</tbody>
</table>
Table 4 - Animal and Marine Plants Negative After Three Inspections, Fiscal Year 1968

<table>
<thead>
<tr>
<th>Rendering Category</th>
<th>Plants Inspected</th>
<th>Plants Negative</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent</td>
<td>188</td>
<td>52</td>
<td>27.66</td>
</tr>
<tr>
<td>Livestock Slaughter</td>
<td>126</td>
<td>33</td>
<td>26.19</td>
</tr>
<tr>
<td>Poultry Slaughter</td>
<td>11</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Blender</td>
<td>12</td>
<td>2</td>
<td>16.67</td>
</tr>
<tr>
<td>Marine Products</td>
<td>16</td>
<td>9</td>
<td>56.25</td>
</tr>
<tr>
<td>Total</td>
<td>353</td>
<td>96</td>
<td>27.20</td>
</tr>
</tbody>
</table>

received four or more inspections according to result on each inspection. Of these 243 plants, 16.87 percent had a positive sample on each inspection, while 22.22 percent had all negative samples on each of these inspections.

The serotypes of the Salmonella isolated from samples collected in program plants during the past fiscal year are listed in Table 6 and Table 7. There were 72 serotypes represented among the 2,175 Salmonella isolates serotyped for program purposes at Animal Health Division or cooperating laboratories. The serotypes are listed in descending order of frequency in Table 7 and in alphabetical order in Table 7. The five most frequent serotypes were S. montivideo (12.5%), S. eimsbüttel (11.45%), S. senftenberg (8.69%), S. infantis (5.15%), and S. oranienburg (5.06%).

Much of the activity during the past year is not reflected in the program statistics. This includes such things as (1) obtaining legal authority or clarification of State authority for conduct of the program, (2) developing laboratory capability to support the program, (3) orientation and training of personnel, (4) establishing working relationship with the rendering and fishery industry, and (5) liaison with other State and Federal agencies with responsibilities in this area.

The following statistics will give you some idea as to how we stand in relation to the criteria for evaluating the effectiveness of this program. The data is based upon the plants inspected during the fiscal year.
TABLE 5 - CLASSIFICATION OF 243 PLANTS INSPECTED FOUR OR MORE TIMES DURING FISCAL YEAR 1968

<table>
<thead>
<tr>
<th>CLASSIFICATION OF INSPECTION ( i )</th>
<th>NUMBER OF PLANTS BY CLASSIFICATION</th>
<th>PERCENT OF TOTAL PLANTS INSPECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1^{st} )</td>
<td>( 2^{nd} )</td>
<td>( 3^{rd} )</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
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<tr>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TOTAL 243 100.00

1/ + (POSITIVE) - (NEGATIVE)
2/ ROUND OF INSPECTION
### TABLE 6--SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1968 (Listed in Descending Order of Frequency)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number of total isolates</th>
<th>Percent of total isolates</th>
<th>Serotype</th>
<th>Number of total isolates</th>
<th>Percent of total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>montlivideo</td>
<td>262</td>
<td>12.05</td>
<td>Salmonella</td>
<td>canoga</td>
<td>8</td>
</tr>
<tr>
<td>simbuettel</td>
<td>249</td>
<td>11.45</td>
<td>takseny</td>
<td>8</td>
<td>0.37</td>
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<tr>
<td>senftenberg</td>
<td>189</td>
<td>8.69</td>
<td>enteritidis</td>
<td>7</td>
<td>0.32</td>
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<tr>
<td>infantis</td>
<td>112</td>
<td>5.15</td>
<td>blockley</td>
<td>6</td>
<td>0.28</td>
</tr>
<tr>
<td>oranienburg</td>
<td>110</td>
<td>5.06</td>
<td>albany</td>
<td>5</td>
<td>0.23</td>
</tr>
<tr>
<td>anatum</td>
<td>101</td>
<td>4.64</td>
<td>Untypable-Group F</td>
<td>5</td>
<td>0.23</td>
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<tr>
<td>derby</td>
<td>78</td>
<td>3.59</td>
<td>braenderup</td>
<td>4</td>
<td>0.18</td>
</tr>
<tr>
<td>binza</td>
<td>71</td>
<td>3.26</td>
<td>newport</td>
<td>4</td>
<td>0.18</td>
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<tr>
<td>schwarsengrund</td>
<td>59</td>
<td>2.71</td>
<td>grampense</td>
<td>4</td>
<td>0.18</td>
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<td>baretly</td>
<td>55</td>
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<td>madeia</td>
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<td>tennessee</td>
<td>54</td>
<td>2.48</td>
<td>halmetad</td>
<td>4</td>
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<tr>
<td>worthington</td>
<td>49</td>
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<td>Untypable-Group E</td>
<td>3</td>
<td>0.14</td>
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<tr>
<td>cerro</td>
<td>48</td>
<td>2.21</td>
<td>simbury</td>
<td>3</td>
<td>0.14</td>
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<tr>
<td>bredeney</td>
<td>47</td>
<td>2.16</td>
<td>corvallis</td>
<td>3</td>
<td>0.14</td>
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<tr>
<td>kentucky</td>
<td>46</td>
<td>2.11</td>
<td>chesapeake</td>
<td>2</td>
<td>0.09</td>
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<tr>
<td>thomasville</td>
<td>42</td>
<td>1.93</td>
<td>harhan</td>
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<td>0.09</td>
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<tr>
<td>typhimurium</td>
<td>39</td>
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<td>alaohua</td>
<td>37</td>
<td>1.70</td>
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<td>0.09</td>
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<tr>
<td>cubana</td>
<td>34</td>
<td>1.56</td>
<td>Untypable-Group C1</td>
<td>1</td>
<td>0.05</td>
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<tr>
<td>heidelberg</td>
<td>33</td>
<td>1.52</td>
<td>Untypable-Group B</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>saint-paul</td>
<td>32</td>
<td>1.47</td>
<td>san diego</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>california</td>
<td>28</td>
<td>1.29</td>
<td>mikawashima</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>neugton</td>
<td>22</td>
<td>1.01</td>
<td>westerstede</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>illinois</td>
<td>22</td>
<td>1.01</td>
<td>Untypable-Group G</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>westhampton</td>
<td>22</td>
<td>1.01</td>
<td>Untypable-Group R</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Untypable-Group Cl</td>
<td>18</td>
<td>0.83</td>
<td>pantana</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Untypable-Group B</td>
<td>18</td>
<td>0.83</td>
<td>san diego</td>
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<td>0.05</td>
</tr>
<tr>
<td>siegburg</td>
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<td>mikawashima</td>
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<td>westerstede</td>
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</tr>
<tr>
<td>reading</td>
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<td>0.69</td>
<td>Untypable-Group O</td>
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<td>0.05</td>
</tr>
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<td>give</td>
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<td>0.64</td>
<td>ruiru</td>
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</tr>
<tr>
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<td>livingstone</td>
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<td></td>
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<tr>
<td>typhimurium var. copenhagen</td>
<td>8</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total isolates 2,175 100.00%
1. Proportion of plants producing a clean product:

<table>
<thead>
<tr>
<th>Plants with all product samples negative</th>
<th>96</th>
<th>27.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants evaluated</td>
<td>353</td>
<td></td>
</tr>
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</table>

2. Ability to maintain status of clean plants:

<table>
<thead>
<tr>
<th>Plants with all product samples negative after four consecutive tests</th>
<th>54</th>
<th>83%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants with all negative samples after three consecutive tests</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

3. Prevalence of Salmonella in finished product samples:

   A. Samples collected in all plants

<table>
<thead>
<tr>
<th>Number samples positive</th>
<th>2,278</th>
<th>15.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples tested</td>
<td>12,234</td>
<td></td>
</tr>
</tbody>
</table>

   B. Samples collected in plants with one or more positive samples:

<table>
<thead>
<tr>
<th>Number samples positive</th>
<th>2,278</th>
<th>32.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples tested</td>
<td>7,075</td>
<td></td>
</tr>
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</table>

After the initiation of this program, there was found to be an overlap in the activities of the Food and Drug Administration (FDA) and those of the Animal Health Division (ANH) and its cooperating State agencies. This duplication resulted from the discharge of statutory responsibilities by FDA, ANH and State agencies in regard to animal disease control and the adulteration of food and feeds.

To avoid this duplication of efforts, ANH and FDA reached an agreement for the coordination of their activities. This agreement permits each agency to meet its responsibilities without a duplication of inspections in individual plants. The essential features of this agreement are:

1. ANH personnel of personnel of the cooperating State agency will conduct routine inspection and product sampling of all byproduct processors including fish meal producers.

2. Establishments found to be producing a contaminated product will be reinspected by ANH or cooperating State personnel. Management of such establishments will be given recommendations of measures to take to eliminate or prevent product contamination.

3. Management will be given a reasonable period of time to take positive steps to eliminate contamination and correct sanitation deficiencies.

4. In those instances where management makes little or no effort to eliminate contamination and clean up (correct sanitation deficiencies), the ANH Veterinarian in Charge will provide the name and address of such establishments to the appropriate FDA authority. However, the ANH Veterinarian in Charge will notify the plant management prior to taking such action. The FDA can be expected to take regulatory action against such establishments as they consider necessary to effect correction.

Seldom, if ever, has all the knowledge desired about a disease agent or control measures been available prior to initiating control or eradication procedures.
Salmonella has been no exception. Our current detection methods are laborious, time consuming, and expensive. There is an ever-present danger to human health in laboratories isolating Salmonella. We have need for a rapid, simple test procedure. A screening test that will identify the negative samples in a shorter period of time will go a long way in meeting the demands of current industry practices. Success in eradication of bovine tuberculosis and brucellosis, for example, can be traced to the development of simple effective diagnostic tools.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number of isolates</th>
<th>Percent of total isolates</th>
<th>Serotype</th>
<th>Number of isolates</th>
<th>Percent of total isolates</th>
</tr>
</thead>
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<tr>
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<td></td>
<td>Salmonella</td>
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<tr>
<td>alachua</td>
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<td></td>
<td></td>
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<tr>
<td><strong>Total Isolates</strong></td>
<td><strong>2,175</strong></td>
<td><strong>100.00%</strong></td>
<td><strong>Salmonella</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the case of Salmonella, the problem is further complicated by the wide number of hosts and the ease with which feed and the environment may become contaminated. Given the right conditions of temperature and moisture, this agent has the ability to maintain itself outside the animal host.

As regulatory officials, we cannot delay action until all the answers or better answers are available. We must act on the basis of current knowledge and technical capability while better answers are being sought.

During the current fiscal year, emphasis should be placed upon completing the evaluation of plants that have not received three inspections and in reducing the number of plants producing a contaminated product.

The recovery of Salmonella from a food or feed item is dependent upon the degree of contamination, the size of the lot sampled, and sampling procedure, and the isolation methods used.

There is some variation between States in the frequency of inspection and the number of samples collected. The adoption of uniform methods and rules at this time will provide guidance for the conduct of the program and uniform data and terminology for evaluating the effectiveness of our control efforts.

This report would be incomplete without acknowledging the cooperation that has been received from the various industry associations. The National Renderers Association played an active role in the initiation of the program in many states. The National Fish Meal and Oil Association, the American Meat Institute, Western States Meat Packers Association, and the Independent Meat Packers Association have all played a part in informing their members of program activities and objectives. The American Feed Manufacturers Association has assisted in cooperative efforts to more accurately define the Salmonella problem as it exists under current commercial feed mill operations.

It is the individual plant owners, however, who have contributed the most to the success of program activities during the past year.
The importance of Salmonellosis as a food borne disease has been recognized for several decades in both man and animals. Recent survey work has established that contaminated feeds constitute a major link in the chain of infection in animals, probably second only to the contaminated environment often found where there is a high concentration of animals in a confined area.

The efforts of the rendering industry and of the animal feed industry to improve sanitary practices to control Salmonellae have been most gratifying. Much, however, remains to be done. A “Uniform Methods and Rules” to be used as a guideline for procedures for the elimination of Salmonellae in animal by-products intended for use in animal feeds, which is delineated later in this report, should give assistance to the animal feed industry. Information gained from the several special studies concerning the feed borne Salmonellae problem has been utilized in preparation of this proposed uniform methods and rules. The work by Dr. William Nape of Springfield, Illinois, presented before this committee establishes some of the problems encountered as to sources of contamination and the subsequent “build up” in animal by-products while in channels of trade.

This, along with the Model Act to regulate inedible rendering establishments adopted by the United States Livestock Sanitary Association in 1967, will offer organized direction for the reduction and control of Salmonellae in animal feeds. A procedure for establishing a “negative” or an “approved” plant is included in the Uniform Methods and Rules.

The concern for the problem of Salmonellae contaminated feeds shown by allied regulatory agencies is given special note. Dr. James L. Goddard, former Commissioner of Food and Drugs, in the Federal Register of March 15, 1967, Title 21, Chapter I, Sub-Chapter A, Part 3, details the concern for the public health due to Salmonellae contaminated food products of animal origin. In fiscal year 1967, 68% of all FDA recalls and seizures due to Salmonella contamination were from livestock and poultry products.

Consumer and Marketing Service defines their official position concerning Salmonellosis in a policy letter of 9-14-67. They state, “Product known to be contaminated (with Salmonellae) will be retained and improvements in sanitation will be required to protect future production. Disposition of retained product will depend upon facts developed in each instance, but will include destruction of Salmonella.”
The Animal Health Division of ARS, USDA, is encouraged to publish on a regular periodical basis, a summary of the recovery of Salmonellae by species, by host species or source, and by geographical location of origin as confirmed by the National Animal Disease Laboratory and by the various cooperating serotyping laboratories. This information is now being accumulated and should be made more readily available to those concerned. An effort should be made to consolidate the reports of the agencies involved in a single document published by the Animal Health Division of U.S.D.A.

The Committee urges all laboratories participating in the isolation and identification of Salmonellae to employ the standard procedures as developed by the American Association of Veterinary Laboratory Diagnosticians and adopted by ANHD, ARS, USDA. (ARS 91-68).

It is suggested that goals for the USAHS committee on Salmonellosis for 1969 be the development of standards for vehicles used for handling and transporting rendered animal by-products; for storage facilities; and for terminal facilities to assure that the products are being handled in such a manner as to prevent re-contamination; and because of the ubiquitous nature of Salmonellae, more emphasis to other areas of involvement should be considered, such as the environmental conditions under which livestock are raised, existing reservoirs of infection, the pathogenesis of the organism, etc.

A training program for inspection personnel for inedible rendering establishments previously proposed by this committee has not been accomplished; therefore, it is urged that the Animal Health Division of the ARS, USDA, D.H.E.W.-F.D.A. and the National Renderers Association cooperate to develop such a training program.

The following details the aforementioned uniform methods and rules for standard procedures for the elimination of Salmonellae in animal by-products intended for use in animal feeds, and adopted by a majority vote of this committee. The committee recognizes the need for a separate set of uniform methods and rules for marine products.

PROPOSED STANDARD PROCEDURES FOR THE ELIMINATION OF SALMONELLA IN ANIMAL BY-PRODUCTS INTENDED FOR USE IN ANIMAL FEEDS

UNIFORM METHODS AND RULES

PART I - DEFINITIONS

A. Animal Product - Bone meal, blood meal, meat meal, meat meal tankage, glands, feather meal, poultry by-product meal, eggs, egg products, hatching; waste, organs, milk and milk derived products, or other parts or products of ruminants, poultry or swine or other animals or fowl, or blended mixtures thereof, to be used in animal feeds.

B. Bone Meal - Ground animal bones.

C. Blood Meal - Dried blood of animals or fowl.
D. *Meat Meal or Meat Meal Tankage* - The rendered and dried carcasses or parts of the carcasses of animals or poultry respectively.

E. *Feather Meal* - Ground hydrolyzed feathers from poultry.

F. *Egg Products* - Processed or unprocessed whole eggs or parts thereof.

G. *Hatching Waste* - Egg shell, membranes, embryos, cull poults, or chicks, and other debris from the hatching of chicken, turkey, or duck eggs, or from any poultry eggs.

H. *Lot* - A lot of animal product that shall be the amount of product for a single shipment or a day's production, whichever is greater.

I. *Official Sample* - A random sample of a finished product, ready for shipment, collected by a State or Federal inspector, in such a manner that it represents the contents of a total lot, which consists of ten (10) sample units with each sample unit of not less than 100 grams each, selected at random from each 1/10 section of the total lot. Each sample unit collected shall be individually packaged and identified.

J. *Official Laboratory* - A State or Federal laboratory or laboratory designated by the cooperating State and Federal animal health official to conduct examinations for Salmonella in rendered products for the purpose of this regulation.

K. *Salmonella Tested* - The laboratory examination of an official sample of a product, collected as outlined in I above, and tested by the procedures and methods recommended by the USAHS and the U.S. Department of Agriculture and F.D.A. for cooperative program activities. (ARS 91-68)

L. *Rendering Establishment* - An establishment that processes animal products as defined in A (this part) for use in animal feeds, whether or not operated in conjunction with a slaughtering or processing plant.

M. *Blending Establishment* - An establishment that grinds, blends, mixes, or further processes animal, marine, or animal and marine protein for the purpose of making such proteins suitable for use in animal feeds.

N. *Quality Control Program* - The sum of methods and procedures employed by an establishment designed to prevent, detect and eliminate Salmonella contamination in its finished product, as outlined in the Recommended Sanitation Guidelines, and includes a test for Salmonella of a representative or composite representative sample of not less than one of the lots produced each week.

O. *Approved Rendering or Blending Establishment* - A rendering or blending establishment that has been given official recognition for consistently producing a negative product and operating in compliance with the Recommended Sanitation Guidelines as described in Part II, paragraph C below.

**PART II - RECOMMENDED PROCEDURES**

A. *Evaluation Phase*

1. **Objectives:**
   a. Determine the presence or absence of Salmonella in the finished product produced by each plant in the State.
b. Determine the plants that are operating in compliance with the Recommended Sanitation Guidelines.

c. Determine the plants that are not operating in compliance with the Recommended Sanitation Guidelines.

2. Classification of Plants:
   a. Plants shall be classified as positive or negative based upon the results of laboratory examination of official samples.
   b. Positive classification - A positive classification shall result from one or more positive sub-samples in an official sample on a single test.
   c. Negative classification - A negative classification shall result from negative tests of all sub-samples on three consecutive tests of official samples. The tests shall be conducted at an interval of not less than 30 days apart.

3. Procedure:
   Conduct three inspections of each plant not less than 30 days apart and the bacteriological examination of an official sample of finished product collected during each inspection.

B. Cleanup Phase

1. Objectives:
   a. Reduce the number of positive plants.
   b. Increase the number of negative plants that remain negative.

2. Procedures:
   a. Positive plants
      (1) Conduct an extensive epidemiological study of each positive plant in an effort to identify the source or sources of contamination.
      (2) Make recommendations to management to eliminate areas of contamination or potential contamination.
      (3) Make recommendations to management for bringing operations into compliance with the Sanitation Guidelines. (ARS 91-47)
      (4) Assist plant in developing a quality control program as related to Salmonella contamination.
   b. Negative plants
      (1) Conduct three inspections at not less than thirty day intervals until the plant has received three consecutive negative tests. Subsequent inspections and tests shall be conducted at not less than 120 day intervals.
      (2) Make recommendations to management to eliminate areas of contamination or potential contamination.
      (3) Make recommendations to management to bring operations into compliance with the Sanitation Guidelines.
      (4) Assist plant in developing a quality control program as related to Salmonella contamination.

C. Approved Phase

1. Objectives:
a. To encourage management to keep trying to produce a product free of Salmonella.

b. To give recognition to plants that demonstrate the capability of consistently producing a Salmonella negative product when sampled and examined by the procedures recommended by the USAHS and the U. S. Department of Agriculture for cooperative program activities.

2. Procedures for qualification:
   A plant may qualify for designation as an approved establishment provided:
   a. It has had three consecutive negative tests of official samples conducted at not less than 30 day intervals.
   b. It is operating in compliance with the Recommended Sanitation Guidelines (ARS 91-47), as verified by a written inspection report by a State or Federal inspector.
   c. It has established a quality control program which meets the approval of the cooperating State and Federal animal health officials.
   d. The management applies for approved status and approval is granted by the cooperating State and Federal animal health officials.

3. Procedure for Maintenance:
   a. A plant may maintain its approved status provided a negative test of an official sample is conducted at an interval of not more than 120 days.
   b. In the event an approved plant has a positive test of an official sample it shall:
      (1) Follow the procedures outlined for positive plants in the Cleanup Phase (Paragraph B-2), this part).
      (2) Obtain three consecutive negative tests of official samples within a six months period. The interval between tests shall be not less than 7 days apart.
   c. It is operating in compliance with the Recommended Sanitation Guidelines (ARS 91-47) as verified by a written inspection report by a State or Federal inspector.
   d. It has established a quality control program which meets the approval of the cooperating State and Federal animal health officials.
RECOVERY OF SALMONELLA FROM MATERIAL IN FEED MILLS

By

William F. Nape, D.V.M.

From the Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, Hyattsville, Maryland 20782. Dr. William F. Nape is Assistant Veterinarian in Charge, Springfield, Illinois.

The writer thanks Lee H. Boyd, American Feed Manufacturers Association, Inc., for coordinating the project with the feed mills; Dr. Gerald G. Jelly, Regional Diagnostic Laboratory, Peoria, Illinois, for isolation and identification of the organisms; and Dr. Claude J. Pfow, Regional Salmonella Epidemiologist for technical assistance.

Smith (1960) demonstrated that Salmonella contaminated feeds did infect swine. In 1953, Galton and her Florida co-workers demonstrated the "build-up" of infection that occurs in marketed swine. Williams (1965) reported that the increased Salmonella excretion in swine may be related to marketing stress, in addition to other risks of infection. Stress could change the farm-infected but non-excreter pig into an excreter. Such increase of Salmonella excretion at slaughter increases the chances of animal-origin food contamination from any breaks in packing plant sanitation.

This survey was designed to define the problem of feed mills manufacturing a Salmonella-free feed.

The Salmonella cycle may be illustrated by Figure 1. A primary cycle involves contaminated feed which infects animals and leads to Salmonella excretion. This may result in a build-up problem when animals infect other animals directly or by contaminating the environment.

The infected animal at slaughter contaminates the animal-origin foods from breaks in packing plant sanitation. These foods then serve as a source of infection for man and his domestic pets. Contaminated offal and dead animals are sterilized when properly heated in rendering plants, but become recontaminated from breaks in rendering plant sanitation.

MATERIALS AND METHODS

FACILITIES—The study was conducted in five feed mills located in the mid-western part of the United States. Figure 2 is a representative feed mill. The material sampled has an arrow pointing to the collection area.

Each mill varied noticeably from the others in their control and removal of ingredient accumulations and dust in the area of sampling; e.g., the Mills A through E had increasing amounts of accumulation and dust inside the conveyor and dust on the bagger. Mill A had the least and Mill E the most of these materials.
Figure No. 1

SALMONELLA CYCLE

PERPETUATED
BY BREAKS IN
SANITATION

Feed mill
Salmonella infected animals
Packing plant

Contaminated feed
Recontaminated animal feed ingredients
Rendering plant
Dead animals
Contaminated offal

Animal origin food

Pen, truck or train
Other animals

Man

Man

Man

Man
FLOW CHART DIAGRAM

The mill flow chart diagram presents in a very simplified form the movement of the many various feed ingredients as they are automatically stored, drawn from storage, weighed and mixed in the process of manufacturing formula feeds.
The characteristics of the conveyor where the accumulations were collected are as follows:

Mill A—Vertical gravity conveyor.
Mill B—Screw conveyor.
Mill C—Screw in a conveyor that was largely square—disassembled and cleaned weekly.
Mill D—45° gravity conveyor anterior to magnet-cleaned almost daily.
Mill E—The location where a tube-type conveyor became rectangular in cross section. The latter was at a 45° angle.

Approximately 9000 tons of animal feeds were manufactured weekly in these mills. Each mill produced from 1000 to 2700 tons. The physical form and type of feed produced in the five mills follow with the percentage of total production and tonnage.

<table>
<thead>
<tr>
<th>PHYSICAL FORM</th>
<th>WEEKLY PRODUCTION</th>
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<tbody>
<tr>
<td>MEAL</td>
<td>43%</td>
</tr>
<tr>
<td>PELLETS, CRUMBLIES, ETC.</td>
<td>57%</td>
</tr>
<tr>
<td>(Heat-treated)</td>
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<td><strong>TOTAL 9000</strong></td>
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</table>

<table>
<thead>
<tr>
<th>TYPE OF FEED</th>
<th>WEEKLY PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWINE.</td>
<td>54%</td>
</tr>
<tr>
<td>POULTRY.</td>
<td>21%</td>
</tr>
<tr>
<td>CATTLE.</td>
<td>22%</td>
</tr>
<tr>
<td>OTHER.</td>
<td>3%</td>
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<tr>
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<td><strong>TOTAL 9000</strong></td>
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</table>

MEAL FORM OF FEED BY TYPE AND WEEKLY TOTAL PRODUCTION IN THE FIVE MILLS

<table>
<thead>
<tr>
<th>FEED</th>
<th>WEEKLY PRODUCTION</th>
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</thead>
<tbody>
<tr>
<td>SWINE FEED</td>
<td>16%</td>
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<tr>
<td>POUULTRY FEED.</td>
<td>14%</td>
</tr>
<tr>
<td>CATTLE FEED.</td>
<td>11%</td>
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<td>MISCELLANEOUS FEED</td>
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<td><strong>TOTAL 3854 Tons</strong></td>
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Swine feed constituted 54% (4822 tons weekly) of the total production in the five mills. Thirty per cent (1467 tons) of the total swine feed produced was in the meal form. Sixty-six per cent (1241/1880 tons) of the poultry feed was manufactured in the meal form. Fifty per cent (997/2001 tons) of the cattle feed was a meal. Swine feed was produced as 67% (3207/4822 tons) concentrates and 33% finished feeds.
PROCEDURE

Eight (8) monthly visits were made to each mill from June 1967, through January 1968. The following samples were collected on each visit:

1. Two (2) samples of meat meal usually from the conveyor before going into the blender. Both meat meal and meat-and-bone meal were grouped as meat meal for this survey. The mills purchased about 407 tons of meat meal weekly.

2. Two (2) samples of ingredient accumulation from the feed conveyor tube between the blender and sacking operation. As nearly as possible, the same location was sampled in each mill. Within each mill, the same location was resampled at each visit.

3. Two (2) samples of dust accumulated on top of the bagger—the same location sampled each time. This bagger usually handled all or nearly all of the meal that was sacked.

4. Two (2) samples of swine concentrate meal containing meat meal taken from bags prior to shipment. No antibiotics were included in these rations.

5. Two (2) samples of cattle concentrate meal taken from bags prior to shipment. This cattle concentrate contained no animal proteins or antibiotics.

6. Starting on the fourth round in September, two (2) samples of spillage were collected under the bagger where the dust samples had been collected.

   The swine and cattle rations were almost always sampled on each visit to a mill. Each of these concentrates had passed through the conveyor and bagger that were being checked for contamination.

   Heat-sterilized tablespoons were used to collect the samples. Three to four spoonfuls were placed in a sterile, plastic bag which was sealed, identified, and dated. A spoonful was usually collected from each corner of an area 8x12 inches.

   Isolation and identification of the Salmonella organisms were made at the Regional Diagnostic Laboratory, Peoria, Illinois, according to the Recommended Procedure for the Isolation of Salmonella Organisms from Animal Feeds and Meat Byproducts, ARS-91-36, October 1962, and its addendum. Serotyping was conducted at the National Animal Disease Laboratory, Ames, Iowa.

   Personnel of the Illinois Division of Meat, Poultry, and Livestock Inspection collected the rendering plant samples as part of their routine surveillance program. These were cultured in the same laboratory using the same procedure. Samples were collected at 33 plants from March 1, 1967, through February 28, 1968. Three of the plants sampled may be considered blenders which purchased processed animal by-products.

RESULTS

Of 448 samples from the feed processing line in five feed mills (Table No. 1), 105 (23%) yielded Salmonella organisms. Typing of the isolates revealed twenty-seven serotypes (Table No. 2). Sixty-five per cent (52/80) of the meat meal samples were positive. Approximately 90% of the meat meal arrived at the mill in railroad boxcars and was purchased primarily from four plants. Three of the plants buy processed animal by-products for blending.

   Accumulations inside the conveyor tubes had a recovery rate of 18% (14/80). Dust on the five baggers had a recovery rate of 10% (8/80).
The recovery rate from swine concentrate meal was 26% (21/80), cattle concentrate meal 4% (3/78), and spillage under the baggers 14% (7/50).

Beetle larvae of the family Dermestidae were identified in four of the five mills in the survey. Dermestids are mostly scavengers and feed on a great variety of plant and animal products. These were found in dust and accumulation in hard-to-clean places on the floor. One of the six samples containing these larvae was positive for Salmonella. Each sample was divided into larvae and feed particles and cultured separately. All larva cultures were negative for Salmonella.

DISCUSSION

Meat meal was a primary source of Salmonella contamination for the feed mills. It was 67% (407 tons) of the total animal by-products used in the five mills. Apparently the meat meal was already contaminated when received.

The three blending plants providing the bulk of the meat meal had a recovery rate of 53% (28/53). Three serotypes were found most frequently in both feed mills and Illinois rendering plants. They were S. montevideo, S. senftenberg, and S. eimsbuettel. These common serotypes further exemplify the closeness of the problem between feed mills and rendering plants. The time of year did not significantly change the recovery rate of Salmonella from meat meal in the feed mill (Table No. 6).

The contamination rates of accumulations (18%) inside conveyors were associated with the level of animal proteins in feeds going through the conveyor. Mills C, D, E (Table No. 3) had the higher level of animal protein going through the conveyor and had more frequent contamination.

The contamination rate of dust on the bagger also varied with the protein level in feeds being sacked. Mills C and D (Table No. 4) had more animal protein in the rations and had more recoveries from the dust on the bagger. The one exception (Mill E) allowed the dust to collect the longest (one month) on the bagger and will be discussed later.

Conveyor accumulations and dust had similar recovery rates when the protein level of feeds being processed and frequency of cleaning were the same. Such a comparison can be made in Mills C and D (Tables No. 3 and 4). The baggers in these mills were cleaned weekly. The recovery rate of "fresh" dust from the bagger would probably indicate the level of contamination in recent accumulations inside feed conveyors and possibly other processing equipment.

The accumulation or dust had less contamination when it was in the sampling area the longest. The conveyor in Mill E yielded five positive samples and the dust one. Dust on the sampling area of this bagger was allowed to accumulate the longest (cleaned monthly), which may have resulted in a lower number of live organisms in the sample. The conveyor in Mill A had less contamination (0/16) than the dust (1/16) on the bagger. The conveyor accumulations built up to 1/8 of an inch during the month, while the dust was cleaned almost daily from the bagger. Any organisms in the sample from the conveyor were subject to a longer period of desiccation than those on the bagger.

The depths of accumulation and dust were not related to the frequency of Salmonella recovery from 25 gram samples (Tables No. 3 and 4). Greater depths of
accumulation in Mills C, D, and E did not significantly change the recoveries of Salmonella. The serotypes in accumulation and dust were similar to those in meat meal (Table 2).

Twenty-six per cent (21/80) of the swine concentrate samples were positive. Mills C, D, and E (Table No. 5) had higher protein levels in the rations sampled and had the most frequent contamination. Mills A and B which had lesser levels of animal protein had the least.

Salmonella contamination in the conveyor (18%) and dust (10%) was a potential source of Salmonella for feeds being processed. Four per cent (3/78) of cattle feed was positive. The cattle concentrates containing no animal proteins were produced with the same system (blender, mixer, conveyor, bagger) as the swine feed with meat meal. Cross-contamination was indicated by common serotypes between cattle feed and meat meal.

<table>
<thead>
<tr>
<th>CATTLE FEED</th>
<th>CATTLE FEED LOCATION</th>
<th>LOCATION</th>
<th>IDENTICAL SEROTYPE ISOLATED IN SAME PLANT</th>
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</thead>
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<tr>
<td>No. Positive</td>
<td>Serotypes</td>
<td>Same Plant</td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mill A</td>
<td>1</td>
<td>S. oranienburg</td>
<td>Meat meal at same time.</td>
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<tr>
<td>Mill B</td>
<td>1</td>
<td>S. cubana</td>
<td>Conveyor accumulation 5 mos. previously.</td>
</tr>
<tr>
<td>Mill C</td>
<td>2</td>
<td>S. schwarzengrund</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*S. simbury</td>
<td>Conveyor accumulation at same time.</td>
</tr>
<tr>
<td>Mill D</td>
<td>1</td>
<td>*S. eimsbuettel</td>
<td>Meat meal one mo. previously.</td>
</tr>
</tbody>
</table>

In Mills A and D, the two isolates, S. oranienburg and S. eimsbuettel, were found at the proper time and place relationship for meat meal to be the source of the cattle feed contamination. S. oranienburg was also isolated three weeks later from a bag of tankage in the blending plant where Mill A purchases most of its meat meal. S. oranienburg was isolated from only four (three meat meal, one cattle feed) samples in the feed mill survey. In Mill B, S. cubana in cattle feed was found previously in the mill's conveyor and in the meat meal of two other mills sampled.

Contamination tended to occur at the same time in more than one sampling area (Table 6). With the exception of meat meal, many of the areas yielding organisms were restricted to a relatively few of the forty times the mills were sampled. All areas were negative on twenty visits. One area was positive on seven visits, and multiple areas were contaminated on thirteen visits. These thirteen visits provided 81% (43/53) of the positive samples (meat meal excluded). This indicates day-to-day contamination may be infrequent, but when there is contamination, the level can be quite high.

Contaminated accumulations inside the conveyors were a potential source of Salmonella organisms for subsequent batches of feed. Salmonella organisms in dust may be incorporated in feeds during processing. The dust-collector system over the baggers and throughout the plant may be a source of contamination, particularly if the dust collections are saved for certain rations. Even feed heated with sufficient

*These two isolations were made from forty extra cattle feed samples collected during December and January.
temperature to kill Salmonella may become recontaminated by dust in the air used to cool the pellets. Spillage under the bagger, if salvaged, should be added to a ration that is sufficiently heat-treated.

The results suggest that the organisms do not persist and multiply within the mills, but that new serotypes are continually being re-introduced. The same serotype was isolated only three times from the same sampling area or material on successive visits. S. bredeney was found in meat meal and S. senftenberg was isolated twice from swine concentrate on successive visits.

The serotypes isolated from diagnostic cases of swine at the Regional Diagnostic Laboratory, Peoria, Illinois, during 1967, were as follows: S. anatum (1 isolate), S. bareilly (1), S. cholerae-suis var. kunzendorf (8), S. derby (8), S. enteritidis (1), and S. typhimurium (4). Four of these serotypes (S. anatum, S. bareilly, S. derby, and S. typhimurium) were also isolated in rendering plants, and two serotypes (S. anatum and S. derby) were isolated in feed mills. Fourteen of the twenty-three Salmonella isolates cultured from the swine cases were also found in rendering plants and nine in feed mills. Epidemiological studies should be done in this area.

The beetle larvae of the family Dermestidae were not related to incidence in the limited samples taken.

An explanation is needed for the apparent “build up” of contamination in animal by-products while in the channels of trade from the rendering plant, blender, and feed mill. The recovery rate was only 17% (106/625) in rendering plants, 53% (28/53) in three blending plants sampled, and 65% (52/80) from meat meal in feed mills. Eighteen per cent (299/1661) of the samples from rendering plants in the United States were positive in the Cooperative State-Federal Salmonella Control Program during October, November, and December 1967.

The culturing procedure used was a qualitative not a quantitative test for Salmonella organisms, so the number of organisms could actually remain the same or decrease. The increased frequency of contamination may be related to uniformly dispersing the organisms throughout the animal protein during blending or handling. Blending or mixing animal proteins (one highly contaminated) may cause a product to have a high recovery. Handling (processing, transporting, and storing) may disperse any organisms present throughout a batch. Blending and/or handling may change the contamination rate, especially when a part of the whole is highly contaminated. This may be observed when the recovery is compared with the same number of samples collected before and after the mixing. One can find only a 21% drop in recovery rates when comparing meat meal recovery, 63% (30/48) in Mills C, D, and E, with swine concentrate, 42% (20/48); yet there has been a dilution (1-6) of meat meal with other ingredients which have been relatively Salmonella-free. While blending and mixing may only disperse the contamination, it is the breaks in sanitation that give the organism an opportunity to enter the material after being sterilized in the rendering plant cooker or adequately terminally heated in a blending plant.

SUMMARY

The distribution of Salmonella along the feed processing line gave insight into the source and extent of Salmonella contamination.
The study revealed that meat meal was a primary source of Salmonella contamination for the mills and rations sampled.

Cross-contamination from meat meal to cattle feed was demonstrated by three of the five positive cattle feed and meat meal samples having common serotypes with two related in time and place.

With the exception of meat meal, contamination in sampling areas along the feed processing line was infrequent; however, when contamination was present, more than one sampling area usually contained Salmonella organisms.

These organisms did not persist and multiply within the sampling areas, but new serotypes were continually being re-introduced.

The mills need animal protein suppliers that have a clean product through sanitary practices and sampling for Salmonella. After the survey animal proteins that had been terminally heated at the blending plant were being received at three of the mills. Monitoring of incoming animal proteins by four of the mills have made them Salmonella-conscious. Such monitoring further defines the problem so the rendering and feed industry may continue to take the lead in solving this mutual problem.

Feed mills have two lines of defense against the Salmonella organism. The first is the purchasing program; the second is good housekeeping, or good manufacturing/sanitation practices, carried out within the mill.

The purchasing program for feed ingredients should include the following:

1. Purchase from suppliers having an appropriate Salmonella control program in effect in their plants and utilizing clean vehicles for transportation of ingredients to the feed mill.

2. Inspection of ingredients upon receipt for evidence of contamination by such Salmonella carriers as rodents, birds, and insects, as well as water damage; and,

3. Analysis of ingredients for Salmonella on a periodic or selected spot basis to ascertain suppliers ability to control Salmonella contamination.

The purpose of good housekeeping (sanitation guidelines) is to reduce the chance of the finished feed being contaminated. Any organisms penetrating the first line of defense should be contained to minimize dispersion through the mill, and thereby help prevent the possible contamination of ingredients and finished feeds. Practices which will contribute to production of uncontaminated feeds are dust control, keeping ingredients and finished feeds dry, periodic removal of accumulations occurring in the processing and conveying equipment, heat treatment (pelleting) and an insect, bird, and rodent control program within the plant and its surroundings.

REFERENCES

## TABLE NO. 1

**RECOVERY OF SALMONELLA FROM MATERIAL SAMPLED IN FIVE FEED MILLS IN THE MIDWEST**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>MILL A</th>
<th>MILL B</th>
<th>MILL C</th>
<th>MILL D</th>
<th>MILL E</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAT MEAL</td>
<td>*15/16</td>
<td>7/16</td>
<td>14/16</td>
<td>8/16</td>
<td>8/16</td>
<td>52/80 (65%)</td>
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<tr>
<td>ACCUMULATION Conveyor</td>
<td>0/16</td>
<td>2/16</td>
<td>4/16</td>
<td>3/16</td>
<td>5/16</td>
<td>14/80 (18%)</td>
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<tr>
<td>DUST</td>
<td>1/16</td>
<td>0/16</td>
<td>3/16</td>
<td>3/16</td>
<td>1/16</td>
<td>8/80 (10%)</td>
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<tr>
<td>SWINE CONC.</td>
<td>1/16</td>
<td>0/16</td>
<td>5/16</td>
<td>6/16</td>
<td>9/16</td>
<td>21/80 (26%)</td>
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<tr>
<td>CATTLE CONC.</td>
<td>1/16</td>
<td>1/16</td>
<td>1/14**</td>
<td>0/16</td>
<td>0/16</td>
<td>3/78 (4%)</td>
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<tr>
<td>SPILLAGE UNDER BAGGER</td>
<td>0/10</td>
<td>2/10</td>
<td>1/10</td>
<td>2/10</td>
<td>2/10</td>
<td>7/50 (14%)</td>
</tr>
<tr>
<td>TOTALS</td>
<td>18/90</td>
<td>12/90</td>
<td>28/88</td>
<td>22/90</td>
<td>25/90</td>
<td>105/448</td>
</tr>
</tbody>
</table>

*No. Positive / No. Samples

**Cattle feed samples not available during October.

( ) - Percent positive
**TABLE NO. 2**

**FREQUENCY OF OCCURRENCE OF SALMONELLA SEROTYPES ISOLATED FROM FIVE FEED MILLS IN THE MIDWEST**

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>MEAT MEAL NUMBER</th>
<th>CONVEYOR ACCUMULATIONS NUMBER</th>
<th>DIST NUMBER</th>
<th>SWINE CONCENTRATE NUMBER</th>
<th>CATTLE CONCENTRATE NUMBER</th>
<th>SPILLAGE NUMBER</th>
<th>TOTAL NUMBER</th>
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<td>1</td>
</tr>
<tr>
<td>west-hampton</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>worthington</td>
<td>2</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>untypable</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>77</strong></td>
<td><strong>18</strong></td>
<td><strong>8</strong></td>
<td><strong>23</strong></td>
<td><strong>3</strong></td>
<td><strong>8</strong></td>
<td><strong>137</strong></td>
</tr>
</tbody>
</table>

*27 samples yielded more than one serotype*
## Table No. 3

**Relation Between Salmonella Recoveries from Accumulation and the Depth of Accumulation and Animal Protein Level of Feeds Going through Conveyor Tubes in Five Feed Mills in the Midwest**

<table>
<thead>
<tr>
<th></th>
<th>Mill A</th>
<th>Mill B</th>
<th>Mill C</th>
<th>Mill D</th>
<th>Mill E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recovery of Salmonella</strong></td>
<td>0/16*</td>
<td>2/16</td>
<td>4/16</td>
<td>3/16</td>
<td>5/16</td>
</tr>
<tr>
<td><strong>Depth of Accumulations in Sixteenth Inches</strong></td>
<td>1-3</td>
<td>2-10</td>
<td>2-12</td>
<td>4-14</td>
<td>4-24</td>
</tr>
<tr>
<td><strong>Percent of Feed with Animal Protein</strong></td>
<td>50%</td>
<td>64%</td>
<td>93%</td>
<td>75%</td>
<td>79%</td>
</tr>
<tr>
<td><strong>Percent of Animal Protein in Most Rations</strong></td>
<td>5%</td>
<td>7%</td>
<td>10%</td>
<td>9%</td>
<td>8%</td>
</tr>
</tbody>
</table>

*No. positive / No. samples

## Table No. 4

**Relation Between Salmonella Recoveries from Dust and the Depth of Dust, Protein Level Going through Bagger, and Frequency of Cleaning in Five Feed Mills in the Midwest**

<table>
<thead>
<tr>
<th></th>
<th>Mill A</th>
<th>Mill B</th>
<th>Mill C</th>
<th>Mill D</th>
<th>Mill E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recovery of Salmonella</strong></td>
<td>1/16*</td>
<td>0/16</td>
<td>3/16</td>
<td>3/16</td>
<td>1/16</td>
</tr>
<tr>
<td><strong>Depth of Dust in Sixteenth Inches</strong></td>
<td>3-6</td>
<td>4-8</td>
<td>4-8</td>
<td>4-12</td>
<td>8-24</td>
</tr>
<tr>
<td><strong>Percent of Feed with Animal Protein</strong></td>
<td>50%</td>
<td>25%</td>
<td>93%</td>
<td>72%</td>
<td>70%</td>
</tr>
<tr>
<td><strong>Percent of Animal Protein in Most Rations</strong></td>
<td>5%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Frequency of Cleaning Top of Bagger</strong></td>
<td>1-2 days</td>
<td>Weekly</td>
<td>Weekly</td>
<td>Weekly</td>
<td>Monthly</td>
</tr>
</tbody>
</table>

*No. positive / No. samples
TABLE NO. 5

SALMONELLA CULTURE RESULTS ON ANIMAL FEEDS
SAMPLED IN FIVE FEED MILLS IN THE MIDWEST

<table>
<thead>
<tr>
<th></th>
<th>MILL A</th>
<th>MILL B</th>
<th>MILL C</th>
<th>MILL D</th>
<th>MILL E</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE</td>
<td>1/16*</td>
<td>1/16</td>
<td>1/14**</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>CONCENTRATE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Meal) HAS NO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANIMAL PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWINE</td>
<td>1/16</td>
<td>0/16</td>
<td>5/16</td>
<td>6/16</td>
<td>9/16</td>
</tr>
<tr>
<td>CONCENTRATE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Meal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANIMAL PROTEIN</td>
<td>2.6%</td>
<td>6.8%</td>
<td>20.0%</td>
<td>16.9%</td>
<td>15.6%</td>
</tr>
<tr>
<td>LEVEL IN SWINE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Number positive / Number samples
**Cattle feed samples not available during October

TABLE NO. 6

STATISTICAL DATA ON SALMONELLA RECOVERIES
FROM SAMPLES AS TO TIME, PLACE, AND CULTURE RESULTS
FROM FIVE FEED MILLS IN THE MIDWEST

<table>
<thead>
<tr>
<th></th>
<th>PLANT A</th>
<th>PLANT B</th>
<th>PLANT C</th>
<th>PLANT D</th>
<th>PLANT E</th>
<th>TOTALS</th>
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<tr>
<td>Accumulations</td>
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</tr>
<tr>
<td>MEAT MEAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52/80</td>
</tr>
<tr>
<td>ACCUMULATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14/80</td>
</tr>
<tr>
<td>DUST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5/80</td>
</tr>
<tr>
<td>SWINE CONC.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21/80</td>
</tr>
<tr>
<td>CATTLE CONC.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3/78</td>
</tr>
<tr>
<td>SPILLAGE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7/50</td>
</tr>
</tbody>
</table>

- Black - 2 samples positive, 2 samples collected
- Lines - 1 sample positive, 2 samples collected
- Clear - No samples positive, 2 samples collected
- - No samples collected
THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

by

A. F. Ranney* D.V.M., M.S.

Presented at the Seventy-second Annual Meeting
United States Livestock Sanitary Association
New Orleans, Louisiana
October 10, 1968

We are still battling bovine tuberculosis. The fight is not over, as some may think. We are considerably short of our goal of eradication.

The Tuberculosis Eradication Program may be judged in several ways. In past presentations to this Association, we have usually given an evaluation of the overall program. This year, we will segment the program to better define the complexities with which we are faced.

There are three major groups of herds, each of which should be dealt with in a somewhat different manner. Specifically, we have one group which we are confident is affected with bovine tuberculosis. We have a second significantly larger group under quarantine, from which we have taken tuberculin reactors in which no tuberculous lesions have been found. Considerable professional judgment is required to properly service and classify herds in this second group. The third group is all the remaining herds in the Nation. These are the tested herds reported to be "clean" and the untested herds. Many of the untested herds are under limited market cattle identification and lesion traceback surveillance.

The first group of herds; those known to be affected with bovine tuberculosis, numbered 74. The second group; those under quarantine, numbered 497. We believe that as many as 10 percent in this second group are also affected with bovine tuberculosis. Infection in the third group; or the remaining herds in the Nation, can only be estimated. According to informed sources, it is possible that as many as 600 *M. bovis* herds are in this group.

One way of arriving at this estimate is based on the number of cattle condemned for tuberculosis on regular kill in fiscal year 1967. This was compared with the ratio of cattle condemned to reacting cattle at the inception of the program when the problem of no-gross-lesion reactors was less significant. Several other methods were used, and all resulted in estimates of between 600 and 700 infected herds.

Let us summarize this further. Nationally there are 74 herds to which we are giving special attention. There are probably about 50 no-gross-lesion herds not yet specifically identified as *M. bovis* herds. Last, but definitely not least, there are an estimated 600 other tuberculous herds which have not been pin-pointed.

*Chief Staff Veterinarian, Tuberculosis Eradication, Animal Health Division, Agriculture Research Service, United States Department of Agriculture.
Many times in the past we have discussed the problems associated with herds having no-gross-lesion reactors. Through applied and intensified epidemiology, we believe that we are making progress with this difficult situation. I choose, today, to dwell on the problems that pertain to the other two major groups: those with infection indicative of \textit{M. bovis}, and those which have not been detected.

The decline in the number of herds with known bovine tuberculosis, as illustrated in Figure 1, suggests progress. We must ask ourselves, however, do the figures reflect existing conditions.

Figure 2 shows that 74 herds affected with bovine tuberculosis during fiscal year 1968 were located in 21 states and in Puerto Rico. No one can accurately predict where in the United States the next herd with infection will be located. It will be noted that 24 herds, or approximately one-third of the 74 herds, are located in Louisiana, where concentrated or selected area testing is being carried out in Cameron Parish. Here infection has been found in several community pastures which are used by many herd owners.

There has been a general decrease in the percentage of infected herds detected as a result of routine testing as compared to herds tested following traceback procedures. This decrease for the years 1962 through 1968 is shown in Figure 3, with a corresponding increase in percentage of infected herds detected by traceback testing. This traceback versus routine testing is further illustrated by Figure 4. It is apparent from this graph that over the six-year period the number of infected herds found by traceback has changed very little while there has been a noticeable decrease in the number found as a result of routine testing procedures.

As pointed out in the report to this association last year, the degree of emphasis placed upon a procedure for finding infection will have a bearing on the resulting data.

The important question, however, is how many undetected infected herds remain and continue to spread infection.

Of the 4.6 million cattle tested in fiscal year 1968, 4.41 million, or approximately 99 percent, were routinely tested, while 59,858, or about 1 percent, were tested after traceback procedures.

As illustrated on the chart, Figure 5, the one percent tested after traceback to find over one-half of the 74 infected herds is very impressive data. However, it is obvious that if no routine testing had been done we would have missed, for the time being at least, almost one-half of the infected herds.

It may be observed from Figure 6 that there has been a considerable decrease in the percentage of routine tests applied in 1968 as compared to 1963. The big decrease in area testing (61 percent) is undoubtedly a major factor responsible for the decline in the number of infected herds found following routine testing.

As illustrated in Figure 7, there was a very slight increase in the number of animals tested after tracing lesion cases reported from regular-kill meat inspection in 1968 as compared to 1963. The smaller number of reactors reported in 1968 than in 1963 and the decline in number of herds detected with \textit{M. bovis} infection contributed to the 77 percent decrease in tests made after tracing reacting animals.

In view of the above data and the fact that routine testing has consistently detected early infection more effectively than traceback testing, we should again point out this year that the development of our market cattle identification and
TRACEBACK PROGRAM HAS THUS FAR FAILED TO COMPENSATE FOR THE DECLINE IN AREA TESTING. UNLESS OR UNTIL OTHER MEANS OF DETECTING EARLY INFECTION ARE MORE PRODUCTIVE, ROUTINE TESTING - EVEN THOUGH RELATIVELY COSTLY - SHOULD BE MAINTAINED IN SELECTED AREAS AS AN ESSENTIAL PART OF THE PROGRAM.

HISTORICALLY, TUBERCULOSIS HAS BEEN MORE PREVALENT IN DAIRY-TYPE THAN IN BEEF-TYPE CATTLE. THIS IS PARTLY DUE TO CLOSER ASSOCIATION OF DAIRY CATTLE AND THE MORE EXTENSIVE MOVEMENT FROM HERD TO HERD. BECAUSE GREATER ATTENTION HAS FOR MANY YEARS BEEN GIVEN TO THE DISEASE IN DAIRY CATTLE AND POSSIBLY AS A RESULT OF REDUCED AREA TESTING, TUBERCULOSIS WAS REPORTED IN MORE HERDS OF BEEF-TYPE CATTLE IN 1968 THAN IN HERDS OF DAIRY-TYPE CATTLE. THIS IS SHOWN IN FIGURE 8.

WE CONTINUE TO BE PLAUGED WITH RECURRENT HERD INFECTION. THIS YEAR 29 OF 74 HERDS WITH M. BOVIS INFECTION ARE KNOWN TO HAVE HAD INFECTION IN A PRIOR FISCAL YEAR. SOME HAVE CONTINUOUS OR RECURRENT INFECTION OVER A PERIOD OF MANY YEARS. EMPHASIS PLACED ON TERMINOLOGY, SUCH AS "RED FLAG" FOR HERDS IN WHICH ANIMALS DISCLOSE LESIONS OF TUBERCULOSIS AFTER REPEATED TESTS, HAS CALLED ATTENTION TO THE NEED FOR THE LIQUIDATION OF KNOWN M. BOVIS HERDS. AT THE END OF FISCAL YEAR 1968 THERE WERE 24 HERDS LISTED IN THE "RED FLAG" CATEGORY LOCATED AS SHOWN ON THE MAP, FIGURE 9.

THE PROVISION OF INCREASED PAYMENT OF INDENMTY ON EXPOSED NONREACTING CATTLE HAS STIMULATED THE LIQUIDATION OF SOME M. BOVIS HERDS. THE MAXIMUM FEDERAL PAYMENT FOR AN EXPOSED ANIMAL WHEN LIQUIDATING AN INFECTED HERD HAS BEEN $100 FOR A GRDE ANIMAL AND $200 FOR A PUREBRED ANIMAL. THE OWNER MAY RECEIVE FEDERAL PAYMENT UP TO THESE AMOUNTS WITH A LIMITATION THAT JOINT STATE-FEDERAL INDENMTY PLUS SALVAGE DOES NOT EXCEED THE APPRAISED VALUE OF THE ANIMAL. FEDERAL PAYMENTS ARE LIMITED TO THE AMOUNT PAID BY THE STATE IN WHICH THE ANIMAL WAS CONDEMNED.

THE GRAPH, FIGURE 10, DEPICTS THE PERCENTAGE OF HERDS THAT ARE KNOWN TO HAVE BEEN INFECTED IN ONE OR MORE PRIOR YEARS AS COMPARED TO THOSE IN WHICH INFECTION WAS RECORDED FOR THE FIRST TIME LAST YEAR. THE FIGURE ON THE COLUMN TO THE RIGHT OF THE BARS IS THE PERCENTAGE OF TOTAL INFECTED HERDS LIQUIDATED OR SLAUGHTERED IN THEIR ENTIRETY. THE ACTUAL NUMBER OF HERDS ON WHICH THE ABOVE PERCENTAGE FIGURES ARE BASED IS RECORDED IN FIGURE 11. THE DATA ON THESE TWO GRAPHS AND THE RECORD OF "RED FLAG" HERDS STRONGLY SUGGESTS THAT GREATER EMPHASIS SHOULD BE PLACED ON THE LIQUIDATION OF KNOWN M. BOVIS HERDS.

THE 29 HERDS WITH INFECTION IN 1968 THAT HAD INFECTION IN ONE OR MORE YEARS PRIOR TO 1968 IS FURTHER ILLUSTRATED IN FIGURE 12 BY DEPICTING THE YEAR OR YEARS THAT INFECTION WAS REPORTED IN THESE HERDS. ONLY SEVEN OF THESE HERDS WERE LIQUIDATED AS SHOWN IN THE LAST COLUMN. THE DATA ON THIS CHART CERTAINLY POINTS OUT THE DANGER THAT EXISTS FROM HAVING INFECTED HERDS RELEASED FROM QUARANTINE BEFORE ALL OF THE INFECTION HAS BEEN REMOVED. THERE IS REAL CONCERN THAT INFECTION MAY YET BE SPREAD FROM SOME OF THE 5 HERDS THAT HAVE BEEN RELEASED FROM QUARANTINE AND THE 17 STILL UNDER QUARANTINE THAT MAY BE RELEASED BEFORE WE CAN BE SURE THAT ALL INFECTED CATTLE ARE DETECTED AND SLAUGHTERED.

THE MAP, FIGURE 13, SHOWS THE NUMBER OF HERDS LIQUIDATED IN EACH OF TEN STATES DURING THE PAST FISCAL YEAR. IN SOME INSTANCES ADDITIONAL HERDS MIGHT HAVE BEEN SLAUGHTERED IN THEIR ENTIRETY IF THE STATES INVOLVED WERE IN A POSITION TO MAKE GREATER INDENMTY PAYMENTS.

EPIDEMIOLOGICAL FIELD STUDIES WERE COMPLETED ON 2,241 CASES OF NONREACTORS SHOWING TUBERCULOUS LESIONS OR THORACIC GRANULOMAS ON REGULAR-KILL MEAT INSPECTION...
during fiscal year 1968. A considerable number of these cases, 1,301 or 58 percent of the total cases, were from the Southwestern States with a presumptive diagnosis of coccidiodomycosis. Work is in progress to evaluate the effectiveness of submitting these and other thoracic granulomas for laboratory examination.

There were 295 lesion cases from regular-kill meat inspection with a final classification "indicative of *M. bovis.*" These are shown on the map, figure 14. It is apparent that these cases involve movement from, through, or into almost every State.

The important fact here is how many were actually traced and identified with the source of infection. We note in Figure 15 that 238 or 81 percent were not identified with any infected herd, while only 57 cases or 19 percent were traced to infected herds. Of the 57 cases traced to infected herds last year, 32 percent were cases where *M. bovis* was not confirmed at the laboratory. While tissues from all cases do not have bacteriological examination, it is essential that cases indicative of *M. bovis* be given a thorough follow-through.

Now, let us consider the efficiency of traceback procedures applied to cases that were confirmed *M. bovis* in the laboratory. The organism was cultured in 74 or 25 percent of the 295 cases indicative of *M. bovis.* Of the 74 cases cultured *M. bovis,* only 39 or 53 percent were traced to infected herds while 35 or 47 percent were not identified with any infected herds, as illustrated in Figure 16.

These figures should make us take a good look and ask ourselves again whether the 74 herds with bovine tuberculosis as shown in Figure 1 reflect conditions as they exist in the field. Actually, they are only one specific part of the complex. The estimate of 650 herds in this country may be a much more realistic number of infected herds.

This is the picture as we see it now with regard to *M. bovis* infection in cattle. I have not discussed tuberculosis in other species of livestock, nor the interrelationship of the disease between species of livestock. We are most concerned about transmissible tuberculosis affecting swine and poultry. Tuberculosis must be eliminated from all livestock if we are to fulfill our commitment to the livestock industry and to the consumer. From an economic standpoint, it is poor judgment to neglect potential disease when we have the capability to eliminate it. From a public health standpoint, it is unthinkable.

We all recognize the fact that funds are limited for the work at hand. We need to concentrate our efforts where the most significant gains can be made. As we succeed in our primary goal to eradicate bovine-type tuberculosis in cattle, let us not forget that other entities of tuberculosis will significantly increase in importance, and that right now we should extend ourselves to educate the producers, the processors, and the consumers as to what we are doing and what we intend to do in recognition of both need and obligation.

Our expectations and our problems are illustrated by a quotation called "On Hazard and Hope" by John W. Gardner, former Secretary of Health, Education, and Welfare, as stated in *Time* magazine, August 23, 1968. He said, "The prospects never looked brighter and the problems never looked tougher. Anyone who isn't stirred by both of those statements is too tired to be of much use to us in the days ahead."

We will still battle; repeat what has been said before; but, we are not too tired to keep going until tuberculosis in livestock is eradicated.
**STATE-FEDERAL TUBERCULOSIS ERADICATION**

**Tuberculosis Eradication**

**HERDS REPORTED WITH TB INFECTION**

*Indicative of M. bovis*

- **1962**: 223 herds
- **1964**: 184 herds
- **1966**: 111 herds
- **1968**: 74 herds

*Figure 1.*

**BOVINE-TYPE TUBERCULOSIS IN CATTLE OUTBREAKS-FISCAL YEAR 1968**

*Figure 2.*
Tuberculosis Eradication

DETECTING HERDS WITH TB INFECTION

Testing:

<table>
<thead>
<tr>
<th>Year</th>
<th>Routine</th>
<th>Traceback</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>78%</td>
<td>22%</td>
</tr>
<tr>
<td>1964</td>
<td>64%</td>
<td>36%</td>
</tr>
<tr>
<td>1966</td>
<td>44%</td>
<td>56%</td>
</tr>
<tr>
<td>1968</td>
<td>47%</td>
<td>53%</td>
</tr>
</tbody>
</table>

Indicative of *M. bovis*

Figure 3.

Tuberculosis Eradication

DETECTING HERDS WITH TB INFECTION

Figure 4.
Figure 5.

TRACEBACK VERSUS ROUTINE TESTING

4.6 mil. CATTLE TESTED

1% Traceback Testing found

99% Routine Testing found

53% infected herds

47% infected herds

EXCLUDES RETEST OF HERDS UNDER QUARANTINE

FISCAL YEAR, 1968

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

Figure 6.

ROUTINE TESTING

Comparative Results, 1968 with 1963

DECREASE

1963

AREA 61%

HERD ACCREDITATION 24%

MILK ORDINANCES 38%

SALE, SHOW, IMPORTS, OTHERS 9%
TRACBEA M TESTING
Comparative Results, 1968 with 1963
DECREASE

1963
+1%

REGULAR KILL (ANH 6-35)

77%

REACTORS (ANH 6-4A)

23%

EXPOSED ANIMALS (ANH 6-4B)

Figure 7.

74 HERDS WITH TB INFECTION

Mixed 3%

39%

Dairy

58%

Beef

Indicative of M. bovis FISCAL YEAR 1968

Figure 8.
RED-FLAG HERDS (TOTAL 24)

Tuberculosis Eradication

JULY 1, 1968

HERDS REPORTED WITH TB INFECTION

Indicative of M. bovis

<table>
<thead>
<tr>
<th>Year</th>
<th>Slaughtered</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>16</td>
<td>45%</td>
</tr>
<tr>
<td>1966</td>
<td>9</td>
<td>25%</td>
</tr>
<tr>
<td>1967</td>
<td>16</td>
<td>37%</td>
</tr>
<tr>
<td>1968</td>
<td>22</td>
<td>39%</td>
</tr>
</tbody>
</table>

Infection reported in a prior F.Y. No record prior infection

Figure 9.

Figure 10.
**Tuberculosis Eradication**

**HERDS REPORTED WITH TB INFECTION**

Indicative of *M. bovis*

<table>
<thead>
<tr>
<th>Year</th>
<th>Herds Slaughtered</th>
<th>Indicative of</th>
<th>Slaughtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>141 22</td>
<td>63</td>
<td>78</td>
</tr>
<tr>
<td>1966</td>
<td>111 10</td>
<td>28</td>
<td>83</td>
</tr>
<tr>
<td>1967</td>
<td>14</td>
<td>82</td>
<td>54</td>
</tr>
<tr>
<td>1968</td>
<td>16</td>
<td>29</td>
<td>45</td>
</tr>
</tbody>
</table>

Infection reported in a prior F.Y. No record prior infection

U.S. DEPARTMENT OF AGRICULTURE

**Tuberculosis Eradication**

**RECURRENT TB INFECTION REPORTED IN 29 HERDS**

<table>
<thead>
<tr>
<th>INFECTED HERDS</th>
<th>FISCAL YEAR</th>
<th>HERD STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>6 1 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 1</td>
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</tr>
<tr>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 1</td>
<td></td>
</tr>
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<tr>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>29</td>
<td>17 5 7</td>
<td></td>
</tr>
</tbody>
</table>

*TB infection also reported in 1949-51*

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AGRICULTURAL RESEARCH SERVICE
Tuberculosis Eradication

16 TB INFECTED HERDS LIQUIDATED

Figure 13.

STATE-FEDERAL INDEMNITY PAID ON EXPOSED ANIMALS IN 10 STATES, FY 1968

TB TRACEBACK
295 LESION CASES—REGULAR KILL
(Indicative of M. Bovis)

Figure 14.

FISCAL YEAR 1968

Slaughter plant
Point of origin
**Tuberculosis Eradication**

**295 TB LESION CASES (regular kill)**

- 19% Traced to infected herds (57 cases)
- 81% Not identified with any infected herds (238 cases)

Figure 15.

Data from meat inspection (6-35 Reports), FY 1968

U.S. DEPARTMENT OF AGRICULTURE

**295 LESION CASES (regular kill)**

- **M. bovis**
  - Confirmed* (74 cases) 25%
  - Traced to infected herds (39 cases) 53%
  - Not identified with any infected herds 47%

* M. bovis Cultured

Indicative of M. bovis, Fiscal Year 1968

Figure 16.

U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
TUBERCULOSIS – ERADICATION OR PROCRASTINATION?

by Dr. F. J. Mulhern

You are all familiar with the reply of the person on welfare when the politician thought he was ungrateful for all he had done for him. He said, “Yes, but what have you done for me recently?” Today people are protesting against the status quo. Sure, among the protestors are too many communists, some unfortunate people of our society who may have trouble adjusting to their environment, but also there are those who are good, honest, conscientious citizens—and others who support their views who are not protesting—who are trying to tell us something. I think one message that they are saying is don’t be satisfied with what we are doing today because we have been doing it this way for the past ten or twenty years.

Each generation tries to make its mark, so to speak, so that its accomplishments can be identified. Contagious pleuropneumonia was eradicated before the turn of the century; cattle fever tick eradication began after 1900; there was the dramatic reduction in tuberculosis from 1917 to 1940; foot-and-mouth disease was eradicated six times since 1900; brucellosis has been dramatically reduced since 1937; vesicular exanthema was eradicated in the 1950’s, etc. But, like the welfare worker, the country is asking us what have you done for me lately. He could say, “Let’s take tuberculosis eradication.” The country was modified accredited, or declared to have less than 1/2 of 1 percent cattle infection, in 1940, and here it is 28 years later and it’s still not eradicated. Or in another way, from 1917 to 1940 (23 years) the most amazing job of bovine tuberculosis reduction was accomplished. But from 1940 to date—28 years later—we still don’t have the disease eradicated, and we haven’t even set a date when we think it will be eradicated.

Do we know how? Of course we know how. The Tuberculosis Committee has been telling us year after year how it can be done. We have become soft in our attitude towards tuberculosis eradication. We don’t have, or we don’t show, the attitude and enthusiasm that the pioneers in tuberculosis eradication had. They told some tales of cleaning out infected herd after infected herd—in some counties having as high as 85 percent herd infection. We should be ashamed to tell them that those of us who have carried on have allowed the last vestiges of this disease to exist for so long.

May I refer all in the audience who have interest in disease eradication to get a copy of Dr. Fred Soper’s reprint entitled “Problems in the Eradication of Tuberculosis.” which appeared in the June 1962 issue of the National Tuberculosis Association Bulletin. He said, “The acceptance of the concept of eradication in place of control forces a radical psychological and administrative change in attitude towards the existence of low incidence tuberculosis in a community. This change is based on the fundamentally different objectives of control and of eradication.

“In control, one may glory in the percent reduction of disease incidence when it is initially high, whereas in eradication any reduction short of the absolute leaves one preoccupied with the seeds of infection that remain.
"In control, one tends to lose interest in a disease at the point where, in eradication, many times the greatest difficulties are often encountered. In eradication there is no stopping period, no resting period.

"In control, some cases and a few deaths are permissible; in eradication, 'Any is too many.'

"In eradication, one learns what the final difficulties are going to be and the solution to these difficulties."

What are these difficulties and what can be done about them?

1. Some States do not have adequate laws, or there is failure to put them into immediate use when and where tuberculosis is found.

   Does each State have authority to quarantine infected herds? Are all exposed animals adequately quarantined immediately? Are all exposed animals promptly tested, branded, and reactors slaughtered without delay even when the owner is uncooperative or downright antagonistic? Does each State have authority to indemnify owners for exposed animals and stop this long history of repeated outbreaks in problem herds?

   We must be ready and willing to take immediate action when necessary. We don't wait until our house is on fire to buy fire insurance.

2. Only one-half of the bovine carcasses with tuberculosis lesions detected on regular kill meat inspection are identified with the herd of origin.

   Are meat inspectors aware of the important part they play in tuberculosis eradication? Is each meat inspector in each State on a first-name basis with at least one full-time field veterinarian? Is each meat inspector aware of the importance of submitting all thoracic granulomas to the laboratory? Do slaughtering plants maintain complete identification through slaughter?

   Does each State have a system to inform the meat inspector of the results of disease eradication efforts expended on each specimen he submits?

3. The traceback phase of disease eradication, so important after tuberculosis is detected, is often extremely limited through lack of proper identification.

   Is the animal identification system designed to work as well for tuberculosis as it does for brucellosis? Is the fliptag being used in 100 percent of the auction markets? Are the cattle dealers in each State required by law to keep records and furnish them to animal health people upon request? Do they? Are field people encouraged to pursue each case through traceback as if they were trying to locate the last infected herd in the United States? (This might be a place to ask about the lack of industry support to the identification phase of the program.)

4. The tuberculin test needs to be put to use in a proper relationship with eradication efforts.

   Is the tuberculin test being used as a screening device, i.e., a tool, along with good histories and sound professional judgment to locate herds that have a high probability of tuberculosis infection? Is retesting of suspects used to locate and brand animals likely to have tuberculosis, or is it used as an excuse not to brand animals at all? Is tuberculin testing directed first
5. There is a need for special consideration of herds with tuberculosis infection.

Is tuberculosis in cattle handled as an exotic disease? Is purchase of the entire tuberculosis herd an expected rather than accepted practice? Is there a system of careful follow-up on each infected herd? If an infected animal was missed, who will suffer the consequences? Why do we get herd histories that show deficiencies on the part of those charged with the responsibility of eradicating tuberculosis from these herds?

6. We must continue to maintain confidence in areas that we claim are free of tuberculosis.

Is the program administered in such a way to create States completely free of bovine tuberculosis? Is the surveillance system in a State efficient enough to be relied upon? Does it assure that the true status of bovine tuberculosis in a given area is known? What assurance is there that tuberculosis will not be introduced from another area? Will it be detected promptly if it is introduced from outside the area? This past year State and Federal Governments spent $11,113,000 for tuberculosis eradication.

We found 74 known infected herds. We could say we spent an average of $150,000 to find one infected herd. How long can we expect Congress to appropriate funds for a disease that could have been eradicated if we had worked a little harder? How long will State legislators continue to provide money for tuberculosis control when we say the disease can be eradicated?

We know what has to be done. We have to find every case of bovine tuberculosis in each State and pluck it out by its *roots*. That is eradication. The key to it is tracing the roots so that we don't miss any part of them. It can be done — it just takes effort, the will, and commitment to do the job.

If we would put the same effort that we have used to reduce diseases into tuberculosis eradication, the job would have been done long ago. Each year this program has been reviewed, and those struggling with the problem have seen the choices before us quite clearly — Eradication or Procrastination or, as I mentioned in the opening remarks, Eradication or Control.

We have lived with procrastination and control too long while we have talked about eradication. The younger generation is challenging our credibility gap. They are saying if we mean what we say, why don't we live it?

Let's look this problem straight in the face. The challenge facing those in regulatory and research today is eradication of diseases that have been reduced to levels that make it feasible. Bovine tuberculosis is one of those diseases.

I hope we haven't become so sophisticated with knowledge that we don't believe we can eradicate diseases. We must keep reminding ourselves that those who provided leadership on successful programs of the past were so naive that they never stopped to consider the possibility of failure.

The fascination that diseases can be eradicated and that we are on the brink of eradicating some of them, one of which is bovine tuberculosis, should capture the
attention of all of us. The leaders among us must show the way. Younger men within our ranks should see the opportunities that they have before them. In this next decade we are going to accomplish disease eradication goals once thought by many to be unrealistic. Will bovine tuberculosis be one of them? Where there is the will, we will find the way.

Talk Presented by Dr. F. J. Mulhern, Deputy Administrator, Agricultural Research Service, USDA, During Annual Meeting of USLSA in New Orleans, La., Oct. 10, 1968
THE DETECTION OF ANTIBODIES TO M. JOHNEI
BY IMMUNOFLUORESCENCE

by N. J. L. Gilmour, Ph.D, BVMS, MRCVS
Moredun Research Institute
Edinburgh, Scotland

The absence of an adequate test for pre-clinical *M. Johnei* infection has made control of the spread of Johne's disease almost impossible. Allergic tests have been shown to be positive sporadically in only a small number of infected animals. (Gilmour, Nisbet and Brotherston, 1965). The complement fixation (CF) test is a useful confirmatory test in clinical cases of Johne's disease but is of no value in pre-clinical diagnosis due to the failure of many infected animals to react, and to non-specific test reactions (Rankin 1961). Recent work on the serology of experimentally dosed sheep by Merkal, Larsen, Kopecky, Kluge, Monlux, Lehmann and Quinn (1968), suggest that agar gel diffusion precipitin tests may be of value in early detection of antibodies. The detection of antibodies by immunofluorescence has been investigated (Gilmour and Gardiner, 1968). It is the purpose of this paper to describe briefly the test and some of the results obtained so far.

Initial experiments were concerned with the development of a suitable antigen. Like Shepard and Kirsh (1961) I found that intact mycobacteria were unsatisfactory. However, the incorporation of *M. Johnei* into mouse peritoneal macrophages gave a suitable antigen. The antigen was further improved by the use of peritoneal macrophages which have incorporated the cell-wall-free residue of *M. Johnei* or other mycobacteria obtained by disruption mechanically with glass powder. Half ml volumes of antigen are inoculated into mice intraperitoneally. Twenty-four hours later the mice are killed and their peritoneums washed out with isotonic buffered saline. The peritoneal washings are standardised then films made on glass slides. The films are air dried and fixed in acetone at 4°C for 10 mins. The fixed antigen can be stored at -20°C for at least 4 weeks. Sera are tested by pipetting dilutions of the sera onto the antigen films which are then kept in a moist atmosphere for 1 hour. After washing, the films are exposed to fluorescein isothiocyanate conjugate prepared in rabbits against the globulin of the species whose sera are being tested, for half an hour. The slides are then washed and mounted. They are examined with UV light using exciter filter UG1/25 barrier filter GC9/1, a transmitted light condenser, a X40 objective and X8 eyepieces. The titre of the serum is the greatest dilution which gives definite fluorescence. Titres of 1-4 or greater are considered positive. Appropriate positive and negative sera are included in each test.

It was shown that the test would detect antibodies to *M. Johnei* in hyperimmunised cattle and in advanced disease in sheep. The next step was to investigate the kinetics of the appearance of antibodies following infection. Eighteen 6-week old calves were infected orally with a total of about 10^9 viable units *M. Johnei*. The results of FA and CF tests are summarised in Table 1. The first FA positive reaction occurred at 3 months after dosing. Thereafter there was a gradual increase in the number of positive tests. The highest number of CF positive
tests was at month 16 when 12 animals were positive. There were more than twice the number of positive FA tests than CF tests.

Between 21 and 24 months after dosing with *M. johnei* the 18 cattle were autopsied. The results are given in Table 2. The gross lesions were all confined to the terminal ileum, usually to last 2-3 feet and in most cases were not confluent. None of those with gross lesions was clinically affected. It is important to note that *M. johnei* was recovered from the small intestines of 16 of the 17 infected cattle but from the nodes of only 12. For the correlation of diagnostic tests with infection it is therefore necessary to examine both sites culturally. The animal which was not positive to the FA test at autopsy had reacted previously. There was little correlation between FA titres which ranged between 1/4 to 1/64 and autopsy findings, apart from the fact that there was a tendency for the higher intestinal viable unit counts to be associated with titres in the range 1/32 to 1/64.

FA tests were carried out on animals which had not been dosed with *M. johnei* (Table 3). The sheep were ones reared in isolation within the Institute and highly unlikely to have been exposed to *M. johnei*. There were 3 reactors at a titre of 1 in 4 or less. Sixty nine 1 year old steers which had been fattened intensively indoors were bled prior to slaughter. There were 5 reactors at minimal titres. The mesenteric lymph nodes and intestines of the 5 were examined culturally. *M. johnei* was not recovered but the avian tubercle bacillus was isolated from both these sites in 1 of the 5.

Because of the close antigenic relationship between *M. johnei* and *M. tuberculosis var. avium* cross reactions to those antigens in avian and *M. johnei* infected animals were thought to be likely to occur. Therefore, it was decided to follow the pattern of FA reactions in calves dosed with the avian tubercle bacillus. This experiment is still in progress. The results obtained so far are shown in Table 4. Ten 6 week old calves were dosed orally with ten weekly doses of a bovine isolate of the avian tubercle bacillus isolated and identified by Dr. Birn of the Central Veterinary Laboratory, Weybridge, England. They received a total of about $10^9$ viable units. FA tests have been carried out at monthly intervals after dosing. The titres prior to dosing may have been due to colostral antibodies. Peak titres were reached within the period 3-6 months after dosing followed by either a drop or maintenance of the same level. It is hoped to continue examinations of sera from some of these animals to see how long the titres persist while others will be killed for bacteriological and pathological examination.

When avian and *M. johnei* antigens were used in parallel on the sera there was almost complete cross reactivity, the same titres or within one dilution being obtained. Examples of tests for cross reactivity with avian, *M. johnei*, B.C.G., and *C. pyogenes* antigens are given in Table 5. Sera from 4 *M. johnei* infected calves 24 months after dosing and from 4 avian infected calves 9 months after dosing were tested against the 4 antigens. *C. pyogenes* was included since it is known to have common cell wall constituents with mycobacteria (Cummins 1962). Antibodies resulting from avian or *M. johnei* infections could not be differentiated. Lower titres were obtained with B.C.G. antigen and no titres with *C. pyogenes* antigen.

From the results obtained so far it appears that the FA test will detect antibodies in the sera of *M. johnei* infected animals. The antibodies detectable by this test appear earlier in the course of infection than do CF antibodies and in a
higher proportion of infected animals in the preclinical state of infection. The problem of cross reactivity with antibodies due to avian infections particularly, is a formidable one. Further studies are necessary on the temporal relationships between the presence of antibodies and infection in both avian and *M. johnei* infections. Persistence of antibodies may be characteristic of *M. johnei* infection while those resulting from avian infection may decline within a few months. Further work is necessary to define the conditions under which the FA test for *M. johnei* infection could best be used. It is hoped by means of an extensive survey of abattoir material, to investigate the incidence of reactors to avian and *M. johnei* antigens and correlate this with bacteriological and pathological findings in an effort to evaluate the test under field conditions.

REFERENCES

TABLE 1

Number of positive FA and CF tests in 18 Experimentally Infected Cattle

| Months after Dosing | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Total + Tests |
|---------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------|
| FA                  | 1 | 4 | 8 | 5 | 7 | 7 | 10 | 11 | 9  | 7  | 12 | 15 | 13 | 14 | 14 | 14 | 151           |
| Total Tests         |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 288           |
| CF                  | – | 1 | – | – | 2 | 1 | 2  | 1  | 7  | 8  | 7  | 12 | 8  | 3  |   |   | 60            |
| Total Tests         |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 288           |

= no reactors
### TABLE 2

Pathology, Cultural and Diagnostic Tests at Autopsy

<table>
<thead>
<tr>
<th>No. Dosed Lesions</th>
<th>M. johnei from</th>
<th>Positive to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gross Microscopic</td>
<td>Allergic CF FA</td>
</tr>
<tr>
<td>18</td>
<td>8 17</td>
<td>7 1 17</td>
</tr>
</tbody>
</table>

12 mesenteric lymph nodes
16 small intestines

### TABLE 3

FA tests on animals NOT likely to be infected with M. johnei

<table>
<thead>
<tr>
<th>No. Positive</th>
<th>Autopsy culture examined</th>
<th>Autopsy culture examined</th>
</tr>
</thead>
</table>
| 69 cattle    | 5                        | 5                        | 0 M. johnei
|              |                          |                          | 1 M. avium |
| 55 sheep     | 3                        | Not done                 | Not done   |
DETECTION OF ANTIBODIES

### TABLE 4

FA tests in CALVES dosed with *M. avium*

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Months after dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4*</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>7</td>
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<td>8</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

* = reciprocal of titre

### TABLE 5

Cross reactions in FA tests on experimental cattle

Infected with:

<table>
<thead>
<tr>
<th>Antigen</th>
<th><em>M. johnei</em> (24 m.)</th>
<th><em>M. avium</em> (9 m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. avium</em></td>
<td>32* 8 16 16 16 16 8 8 8 16</td>
<td></td>
</tr>
<tr>
<td><em>M. johnei</em></td>
<td>32 16 16 16 16 8 8 -</td>
<td></td>
</tr>
<tr>
<td>B.C.G.</td>
<td>8 - 4 4 - 4 - 8</td>
<td></td>
</tr>
<tr>
<td>C. pyogenes</td>
<td>- - - - - - - -</td>
<td></td>
</tr>
</tbody>
</table>

* = reciprocal of titre
REPORT OF THE COMMITTEE ON TUBERCULOSIS AND PARATUBERCULOSIS


1. As in the past, a midyear meeting of the committee was held in April. The committee feels that these midyear meetings are advantageous in organizing work for the annual meeting.

2. Brief reports pertaining to tuberculosis problems were presented to the committee as follows:

(a) "PENNSYLVANIA PLAN FOR CHECKING COMPLIANCE WITH REQUIREMENTS FOR ACCREDITED HERD STATUS" - Dr. J. C. Shook, State Veterinarian, Pennsylvania.

(b) "TWO HERD OUTBREAKS IN MICHIGAN FOLLOWING TWO YEARS WITH NO M. BOVIS REPORTED" - Dr. C. L. Hendee, Federal Veterinarian, Michigan.

(c) "STATUS OF A COMPLEX TUBERCULOSIS PROBLEM IN NEBRASKA AND SOUTH DAKOTA" - Dr. M. D. Mitchell, State Veterinarian, South Dakota and Dr. E. H. Nordstrom, Federal Veterinarian, Nebraska.

(d) "REVIEW OF COMPREHENSIVE TUBERCULOSIS TESTING PROGRAM IN CAMERON PARISH, LOUISIANA" - Dr. F. E. Henderson, Federal Veterinarian, Louisiana.

(e) "PROGRESS IN SWINE IDENTIFICATION STUDIES" - Mr. Paul Zillman, Livestock Conservation, Inc., Chicago, Illinois.

(f) "TUBERCULOSIS IN WILD SWINE - CALIFORNIA" - Dr. J. H. Wommack, Federal Veterinarian, California.

(g) "CONVEYOR BELT TO MAINTAIN IDENTITY OF HIDES WITH CARCASS DURING INSPECTION" - Dr. R. W. Gerding, Federal Veterinarian, Colorado.

3. Report of the sub-committee on swine identification: A Swine Identification Workshop was held in Chicago, August 5 and 6, 1968, attended by representatives of the swine industry and allied interests. It was recommended that Livestock Conservation, Inc. establish a committee on swine identification to survey and develop a national plan for swine identification. Our committee recommends that this Association support this important effort.

4. Report of the sub-committee on the Liquidation of Herds Infected with M. bovis: This sub-committee presented a report to the committee at the midyear
LIQUIDATION OF HERDS INFECTED WITH *M. BOVIS*

As the number of herds infected with *M. bovis* decreases, the need for close attention to program details increases if we are to progress toward our objective of eradication.

By eradication of *M. bovis* in the bovine we mean the complete elimination of this type of tuberculosis from all domestic cattle so that it does not reappear unless introduced from the outside. We are continually confronted with the specter of the anergic animals which fail to respond to the intradermal test for tuberculosis but do have lesions of tuberculosis and are oftentimes spreaders of the disease. Such animals are detected only when slaughtered. This type of animal may be sold and resold and may enter several herds possibly infecting each with *M. bovis* even though the animal may be tested before each sale. Liquidation of known *M. bovis* herds will eventually eliminate this source of spread of the disease by eliminating the anergic animal.

Consideration should be given to the liquidation of herds with *M. bovis* infection. Experience to date in handling an outbreak of infection in a valuable bull stud suggests that liquidation of some special types of herds may be contraindicated. The following are some of the factors that should be taken into consideration when considering liquidation of a herd:

- a. The percentage of infection in the state
- b. Percentage of infection in the herd
- c. Spread of infection within the herd
- d. Length of time that the herd has been infected
- e. Value of the animals
- f. Control and quarantine measures
- g. Facilities on the premises
- h. Agreement on the part of the owner to carry out detailed program procedures for eradication

(Eventually, liquidation of each herd in which *M. bovis* is found would be mandatory, when such action is deemed advisable by State and Federal authorities after consideration of the above factors.)

With the low level of *M. bovis* infection, each State should have legal authority to liquidate an *M. bovis* herd when it is considered necessary. In cases where authority does not exist for the payment of indemnity for all animals in the herd appropriate authority should be obtained. It should be noted that the Federal government has provisions for payment of indemnity not to exceed $100 for a grade bovine animal or $200 for a purebred animal. There are now 35 states plus Puerto Rico and the Virgin Islands that have authority to pay indemnity for exposed animals as well as reactors for liquidation of herds.

It is recommended that the owner be required to sign an agreement which would include the following:

- a. Agree to furnish all available information as to the sales and purchases made after the date that the herd probably became infected;
b. Agree to complete annual herd tests for three years subsequent to restocking of the premises; and

c. Agree to carry out eradication procedures as outlined by the State or Federal officials.

The State and Federal officials should place in the agreement a list of the specific procedures that are to apply to the premises involved. Consideration should be given to other animals on the farm, such as swine, poultry, dogs, and cats, as well as drainage and requirements for cleaning and disinfecting. These important procedures are to be carried out prior to the payment of indemnity.

In looking to the future, and as we draw nearer to eradication, each state should have authority to order the condemnation of infected herds. It is proposed that the following be used as a guideline for requesting legislation for this purpose:

Be it enacted that:

Section 1. If the Commissioner (or secretary) of Agriculture (or Livestock Sanitary Board) finds any herd of cattle or swine to be infected with tuberculosis or brucellosis, he may, in his discretion, order the condemnation of such herd and compensation therefor shall be paid provided the State shall not pay more than fifty percent of the difference between the salvage and appraisal and further provided that such payment by the State shall not exceed the sum of $200 for a purebred nor $100 for a grade bovine animal nor shall it exceed $40 for a purebred nor $20 for a grade swine animal. Said compensation shall not be paid, nor shall the premises from which the herd was taken be restocked, until such premises have been cleaned and disinfected, and such premises have been inspected and approved by the Commissioner (Secretary of Livestock Sanitary Board) or his deputy or any authorized agency of the Commissioner (Secretary of Livestock Sanitary Board.)

Section 2. This act shall take effect from its passage.

5. Report of the special committee to consider changes in the Uniform Methods and Rules: Copies of the special committee’s report were sent to all Chief Livestock Sanitary Officials and Veterinarians in Charge with requests for comments prior to the October meeting. The following are the recommendations of our committee:

(1) Change the Uniform Methods and Rules - Tuberculosis Eradication (Effective December 13, 1967) as follows:

13(b) - page 7, delete entire paragraph and replace with the following:

“The cooperating State and Federal officials have records showing that during each of the last three successive years surveys show that not less than 10 percent of the cattle over two years of age or a total of 30 percent or more during three successive years have been identified through slaughter at an accredited establishment. This free status may be continued if records show that during six successive years 60 percent or more of the cattle over two years of age have been identified through slaughter at an accredited establishment.”

Paragraph 6 on page 2 - add the following:

“and which maintains its status in accordance with these Uniform Methods and Rules.”

One additional change was recommended at this meeting. Paragraph 6(d), page 4, referring to additions to accredited herds, change 30 days to 60 days. This change was made in line with the committee’s recommendation last year that States
consider accepting 60 day tests for the interstate movement of cattle.

6. Two resolutions were presented for the committee's consideration. Since the committee considers animal identification extremely important in disease control and eradication programs, it adopted and highly recommended these two resolutions and referred them to the Committee on Nomination, Resolutions, and Internal Affairs.

7. Improved methods of fecal culture make possible the identification of many cattle that are shedding *Mycobacterium paratuberculosis*. Removing these animals from the herd reduces the amount of infection on the premises and minimizes the chances of susceptible cattle in the herd becoming infected. This in turn decreases the extent of infection in the herd and aids in the control of the disease. It should be pointed out that some infected cattle may shed organisms only periodically or not at all. Therefore, cattle with negative fecal cultures should not necessarily be considered free of *M. paratuberculosis*.

It has been noted that in some instances phosphorous supplements have been added to rations of cattle in herds that are infected with *M. paratuberculosis* for the purpose of controlling Johne's disease. Information in the literature indicates that this would be of no value if the ration already contained adequate amounts of phosphorous. If mineral supplements are tried as a method of control it is recommended that this be done as a controlled and statistically valid experiment.

8. At present there are no commercial sources of supply for Johne's vaccine for sheep. The committee recommends that Agricultural Research Service, USDA make this vaccine available to State and Federal regulatory officials for use on a special study basis.
Strains of Leptospira pomona were selected and grown in a modified Tween synthetic medium (MTSM). When it was discovered that they were noninfectious for hamsters, guinea pigs, and swine, cultures of avirulent L. pomona in MTSM were given as immunizing agents to small numbers of hamsters, swine, and cattle. The experimental vaccine conferred adequate protection against challenge-inoculations of virulent L. pomona, and it did not interrupt pregnancy, cause nephritis, or initiate persistent renal infections. Since virulence was not regained during subcultures in medium supplemented with rabbit serum or two passages through hamsters, the available evidence indicated that the experimental vaccine was both efficacious and safe. Nevertheless, the extreme importance of the safety of any attenuated microbial vaccine prompted a more rigorous investigation of the stability of the noninfectious character of avirulent L. pomona. This report concerns further tests of safety in swine and attempts to alter avirulent L. pomona in the direction of greater virulence by serial transfers in vitro and by serial passages in cattle.

**MATERIALS AND METHODS**

Studies in vitro.—Leptospira pomona strain DM-2 was grown in MTSM (Table 1) on a shaker for 5 days to a nephelometric value of 297. One hundred milliliters was transferred to each of 4 sterile Erlenmeyer flasks and 10 ml. of a sterile solution (5%) of bovine serum albumin* in MTSM was added. Two flasks were stored at room temperature (21 to 23 C.) and two flasks were stored at 16 to 18 C. At intervals of approximately 1 month, the number of viable leptospires per milliliter was determined by inoculating 1 ml. from each flask into duplicate tubes containing 10 ml. of Stuart's medium, albumin-polysorbate 80 medium, or modified albumin-polysorbate 80 medium; further decimal dilutions were made in the same medium using separate pipettes for each dilution. The inoculated tubes were incubated at 28 to 30 C. for 30 to 60 days and observed for evidence of leptospiral growth.

The infectivity of avirulent L. pomona for hamsters was determined. Groups of 5 hamsters each were given ca. 1 x 10^6 or 1 x 10^8 viable avirulent leptospires by intraperitoneal injection. The hamsters were observed for 2 to 3 weeks when the survivors were killed and examined for evidence of renal leptospirosis by cultural techniques.


The author acknowledges the assistance of Dr. R. C. Cutlip of this laboratory for the preparation and examination of histologic sections.
Avirulent *L. pomona* strain Ohio was transferred from MTSM to albumin-polysorbate 80 medium and subcultured in the same medium at weekly intervals by adding 1 ml. of culture to 10 ml. of fresh medium. After 16 and 66 subcultures in complex medium, the virulence of the culture was determined in swine as well as in hamsters. Each of 3 pigs weighing 60 to 70 kg. was given 5 ml. of culture (ca. 2.5 x 10^9 organisms) by intramuscular injection; the exposure was repeated in 24 hours. Sixteen days later, the pigs were killed and examined for evidence of renal leptospirosis by cultural and hamster-inoculation techniques.

*Studies in vivo.*—The swine used in these studies were purchased and tested for leptospirosis as previously described. Cultures of avirulent *L. pomona* in MTSM (nephelometric readings of 22 to 28) were given by intramuscular injections to swine weighing 60 to 80 kg. Nine experiments were conducted with swine as summarized in Table 2. The volume of culture given to each pig and the
approximate number of leptospires used is given. In the last experiment, 7 susceptible (control) pigs were maintained in close contact with the 11 exposed pigs.

After exposure to avirulent *L. pomona*, the pigs were observed daily for clinical signs of leptospirosis and rectal temperatures were recorded. Samples of urine were tested for protein, ketones, glucose and occult blood,* and for leptospires by microscopic examination and by cultural and hamster-inoculation techniques. The pigs were killed 1 to 33 days after exposure. After a sample of blood was collected for serology, the kidneys were removed aseptically, examined for macroscopic evidence of renal leptospirosis,* and tested for leptospires. A portion of each kidney was stored in 10% formalin solution, stained with hematoxylin and eosin or silver (Levaditi's impregnation technique), and examined under a microscope.

Twenty-four to 48 hours after exposure to avirulent *L. pomona*, the pigs in experiments 1 through 6 (Table 2) were killed and examined for leptospires. Samples of blood and renal and hepatic tissues were obtained and tested for leptospires by cultural and hamster-inoculation techniques. The stability of avirulent *L. pomona* strain DM-2 was also tested by serial passages in cattle. The cattle were obtained from a herd maintained at the National Animal Disease Laboratory. Serums from the cattle did not agglutinate *L. pomona, Leptospira grippotyphosa* or *Leptospira hardjo* in the microscopic agglutination test.* Different volumes of culture in MTSM were administered (Table 3). At intervals thereafter of 0.3, 0.5, 1, 4, 6, 24, 30, 48, 54, 72, and 96 hours, samples of blood were obtained by venipuncture and 1 ml. was inoculated into duplicate tubes of Stuart's medium. After 0.5 ml. of the diluted blood sample was given to each of 2 hamsters by intraperitoneal injection, further decimal dilutions were made in 8 tubes of the same medium. The tubes were incubated and observed for evidence of leptospiral growth; the hamsters were subsequently examined for evidence of renal leptospirosis.*

Nine to 21 days after exposure to avirulent *L. pomona* (Table 2), the cattle were killed and were examined for evidence of leptospirosis* as described above for swine.

For exposure of the next cow in the series, the last culture obtained during leptospiremia was inoculated into tubes of Stuart's medium, and the tubes were incubated at 28-30 C. without shaking or aeration until the nephelometric readings were 22 to 28; the cultures were pooled and were administered to the next cow in the series.

Each culture was also tested for infectiousness in hamsters (see *Studies in vitro*) and for ability to grow in MTSM. One milliliter of culture from bovine blood and 1 ml. of virulent *L. pomona* (nonadapted to growth in MTSM) were each added to duplicate tubes of MTSM and incubated. When evidence of growth appeared, subcultures were made in the same way in MTSM for 5 or 6 subcultures.

**RESULTS**

*Studies in vitro.*—The survival of avirulent *L. pomona* during storage is shown in Fig. 1. After 12 months, the pH values at 17 C. and 22 C. were 6.4 and 6.6,

*Labstix Reagent Strips, Ames Co., Elkhart, Ind.*
respectively. After storage at 17°C and at 22°C for 5, 10, and 12 months, *L. pomona* was administered to hamsters, but leptospires were not recovered from the kidneys of the exposed hamsters.

After weekly subcultures for 16 weeks (ca. 80 generations) in medium supplemented with serum albumin, *L. pomona* was still noninfectious for hamsters or swine, since leptospires were not recovered from the kidneys of the exposed animals. However, after an additional 50 subcultures (ca. 320 generations), the culture was infectious for hamsters and swine. Leptospires were isolated from the kidneys of all exposed hamsters (<1 x 10^7 organisms per gram of renal tissue) and from 1 of 3 exposed swine (ca. 1 x 10^4 organisms per gram of renal tissue).

*Studies in vivo.*–The results of studies on avirulent *L. pomona* in swine are summarized in Table 2. The only clinical signs were moderate fevers (40, to 40.3°C) in 3 pigs in experiment 9 for 1 day only. Ninety-five samples of urine were collected and tested for the presence of protein, glucose, ketones, and blood with

![Graph showing the decline of leptospires over time](image-url)
negative results except that proteinuria (trace or + reactions only) was recorded for 1 day only in one pig only on postexposure days 4, 5, 6, 7, 9, 11, 15, 16, 17, 18, and 19. The urinary pH values ranged from 6 to 9 (average of 6.7), flocculent urinary sediments were not observed, and microscopic examinations for leptospires were negative.

Hemocultures performed 24 or 48 hr. after inoculation of avirulent *L. pomona* into 6 pigs (Table 2) yielded only 1 culture of leptospires. It did not initiate renal infections when administered to 5 hamsters. Attempts to recover *L. pomona* from porcine renal or hepatic tissue failed.

Table 3 summarizes the results of studies on the stability of avirulent *L. pomona* in cattle. If massive numbers of leptospires were given (ca. 5 x 10^10) by intravenous injection, recoveries were made for as long as 48 hours; the intraperitoneal route was less successful. By plotting the levels of leptospiremia against time, a “survival curve” for avirulent *L. pomona* in cattle by the intravenous route was derived (Fig. 2).

After the administration of avirulent *L. pomona* to cattle, clinical signs of leptospirosis were not detected and leptospires were not isolated from the urine of exposed cattle or from renal tissue obtained at necropsy.

The kidneys of cattle and swine were examined grossly for evidence of leptospirosis with negative results. Microscopic examination of stained histologic slides revealed only minimal tissue changes consisting of small accumulations of lymphocytes, a few juxtaglomerular lymphoid aggregates, proteinaceous material, and cellular debris. They were considered of little or no significance. The results of examination of silver-stained slides were negative except that a few, long, filamentous structures typical of leptospires were seen in sections from cattle no. 5595, 5782, 3931, and 5736. The available sera were then tested with additional antigens in the microscopic agglutination test; the results are shown (Table 4).

After each passage in cattle, the virulence of *L. pomona* was investigated by exposing susceptible hamsters. None of the cultures initiated renal infections in hamsters. All of the cultures grew during 6 to 7 subcultures in synthetic medium whereas a control strain of virulent *L. pomona* did not grow.

**DISCUSSION**

The present studies were designed to critically test the safety of an experimental leptospirosis vaccine by demonstrating the stability of the noninfectious character of avirulent *L. pomona in vitro and in vivo*. The results confirm and extend the earlier observations^4,6,7^ that *L. pomona* grown in MTSM is noninfectious for hamsters, guinea pigs, cattle, and swine, and in addition, they demonstrate that the avirulent character is stable.

Reversion to virulence did not occur when avirulent *L. pomona* was maintained in complex (i.e. serum protein-containing) medium without subcultures for 12 months. It did occur during growth in complex medium but only after many (< 16) subcultures. The dependence of reversion on numerous cell divisions (< 1.1 x 10^9) indicates that the process probably involves “back mutation” to some attribute which is not closely linked with the metabolism of *L. pomona* in complex medium. The genetic control of leptospiroplasm virulence has not been investigated.
The remarkable persistence of leptospires in vitro without subculturing may be ascribed to their "biochemical inertness". Compared to most heterotrophic bacteria, leptospires are biochemically inactive, and do not drastically change the pH values of complex mediums. In synthetic medium, however, the pH values drop during leptospiral growth; the changes recorded herein (7.4 to 6.4) are sufficient to account for the abrupt decrease in leptospiral viability after 10 months of storage. The survival of *L. pomona in vitro* was not enhanced by storage at 17°C as compared to storage at 22°C. The effect of storage at higher temperatures was not studied.

Although enormous numbers of avirulent *L. pomona* were given to cattle and swine, persistent infections were not produced and reversion to virulence did not occur. On the contrary, avirulent *L. pomona* disappeared rapidly from the blood of cattle (see Fig. 2) and recoveries were possible only if large volumes of culture were given and only during the first 48 hrs. postexposure.

The observation of filamentous forms resembling leptospires in the kidneys of 4 of the cattle cannot be explained with certainty at this time. Since serums from the cows did not uniformly agglutinate any antigen (Table 4), it is unlikely that the filamentous forms represent an infection with an extraneous leptospire. They may represent nonviable remnants of avirulent *L. pomona*, or they may be artifacts induced during the staining of renal tissues which were mildly inflamed by exposure to enormous numbers of avirulent *L. pomona*.

Because the virulence of leptospires for hamsters can be increased by serial passages through hamsters, the technique was applied to studies of avirulent *L. pomona* in cattle. During six serial passages through susceptible cattle, avirulent *L. pomona* was maintained in cattle blood or in medium supplemented with rabbit serum for 8 months with < 66 or 84 cell divisions before the final virulence-assay in cattle or hamsters, respectively. Nevertheless, virulence for cattle or hamsters was not regained. If the experimental leptospirosis vaccine contained a minute number of leptospires capable of infecting hamsters or cattle, or if the avirulent character was not stable, these serious defects should have been detected in the course of these trials, because in vivo conditions select for greater virulence in leptospires. However, not only was virulence not regained but two attributes of avirulent *L. pomona strain DM-2* were retained: susceptibility to the leptosiricidal effects of *pomona strain DM-2* were retained: susceptibility to the leptosiricidal effects of normal serum and the ability to grow in protein-free medium. It was considered that the conditions of these experiments provided a far greater opportunity for avirulent *L. pomona* to regain virulence than the organism is ever likely to encounter under conditions of field use. Therefore, it was concluded that the noninfectious character of avirulent *L. pomona* was stable.

**SUMMARY**

The stability of the noninfectious character of avirulent *Leptospira pomona* was investigated. Avirulent *L. pomona* was maintained in medium supplemented with bovine serum albumin without subcultures for 12 months when the cultures were still noninfectious for hamsters and swine. Other cultures were subcultured at
weekly intervals. After 16 weeks (ca. 80 generations), the cultures were noninfectious but after 66 weeks (ca. 320 generations), they caused persistent renal infections in all of 5 hamsters and in 1 of 3 exposed swine.

As many as 30 or 250 billion viable, avirulent leptospires were administered to 28 pigs or 11 cattle, respectively, but renal leptospirosis was not detected in the inoculated animals or in 7 susceptible swine maintained in contact with the inoculated swine. Avirulent *L. pomona* was serially passaged through six cattle. None of the cattle became infected. The culture was still noninfectious for hamsters and capable of growth in protein-free medium.

**REFERENCES**


**TABLE 1. CHEMICALLY CHARACTERIZED LEPTOSPIRAL GROWTH MEDIUM**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amounts are in mg. per 100 ml. distilled water; pH 7.4.</th>
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<tr>
<td>KH2PO4</td>
<td>10.0</td>
<td>Nicotinic acid 0.002</td>
</tr>
<tr>
<td>MgSO4·7H2O</td>
<td>40.0</td>
<td>d1-Calcium pantothenate 0.002</td>
</tr>
<tr>
<td>CaC12·2H2O</td>
<td>1.4</td>
<td>Pyridoxine HC1 0.002</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.55</td>
<td>HOEDTA 2.0</td>
</tr>
<tr>
<td>MnSO4·H2O</td>
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<td>(NH4)2SO4 25.0</td>
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<tr>
<td>ZnSO4·7H2O</td>
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<td>Tween 60 2.0</td>
</tr>
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<td>CuSO4·5H2O</td>
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<td>Tween 80 60.0</td>
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<tr>
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<tr>
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<tr>
<td>Biotin</td>
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<td></td>
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Amounts are in mg. per 100 ml. distilled water; pH 7.4.
### Table 2: Protocol and Results of Studies on Stability of Avirulent *Leptospira pomona* in Swine

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Pig No.</th>
<th>Exposure ml. of culture</th>
<th>No. organisms injected ($\times 10^3$)</th>
<th>Clinical signs</th>
<th>Leptospiremia (hours)</th>
<th>Leptospiruria Culture</th>
<th>Leptospiruria Hamster</th>
<th>Days from exposure to autopsy</th>
<th>Renal leptospirosis Culture</th>
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<th>Gross Renal lesions</th>
<th>Microscopic titer**</th>
<th>MA titer***</th>
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Cultures of avirulent *L. pomona* in synthetic medium were administered to swine as shown, and they were observed for clinical signs of leptospirosis. After tests for leptospiremia and leptospiruria, renal tissue was examined for leptospires by cultural and hamster-inoculation procedures and for lesions of leptospirosis.

*ND = not determined; - = negative results; + = positive results; ** Log 10 of titer.
### TABLE 3. PROTOCOL AND RESULTS OF STUDIES ON STABILITY OF AVIRULENT *LEPTOSPIRA POMONA* IN CATTLE: SERIAL ATTEMPTS

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>Animal No.</th>
<th>Exposure (ml of culture)</th>
<th>Donor animal</th>
<th>No. organisms injected ((x 10^{10}))</th>
<th>Route</th>
<th>Clinical signs</th>
<th>Leptospiremia Culture Hamsters</th>
<th>Leptospiruria Culture Hamsters</th>
<th>Days from exposure to autopsy</th>
<th>Renal leptoospirosis Cultures Hamsters</th>
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<th>MA titer</th>
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<td>9</td>
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<td>- + ND</td>
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 Cultures of *L. pomona* in synthetic medium were administered to cattle. At intervals thereafter, hemocultures were made and 0.5 ml of blood was administered to hamsters. Two weeks later, the hamsters were examined for leptospires. Cultures from the end of leptospiremia were grown in Stuart's medium and passed through more cattle as shown. The cattle were observed for clinical signs (fever and anorexia), and urinary shedding. At necropsy, renal tissue was examined for leptospires and gross and microscopic lesions.
TABLE 4.-RESULTS OF MICROSCOPIC AGGLUTINATION TESTS FOR LEPTOSPIROSIS

<table>
<thead>
<tr>
<th>Antigen</th>
<th>5595</th>
<th>4932</th>
<th>5782</th>
<th>3903</th>
<th>3931</th>
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<td><em>L. grippotyphosa</em></td>
<td>-</td>
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<tr>
<td><em>L. mini</em></td>
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<tr>
<td><em>L. autumnalis</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<tr>
<td><em>L. bataviae</em></td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
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</tr>
<tr>
<td><em>L. hyos</em></td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>L. australis</em></td>
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<tr>
<td><em>L. pyrogenes</em></td>
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<td>-</td>
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<tr>
<td><em>L. hardjo</em></td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3055</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Serums were obtained from cattle no. 3903 and 3931 before exposure and from other cattle at necropsy and tested. The results are shown as logarithms of the reciprocal of highest dilution that reacted.
The role of wildlife in the spread of disease to livestock has always been a topic of interest. With the increase of the deer population in Kansas in recent years and the increasing reports of deer and cattle grazing in common areas, the question of the role of the deer in the spread of leptospirosis in cattle herds became more important.

For the continued propagation of deer on grass lands, a compatible understanding between wildlife conservationist and livestockman was of utmost importance. In order to maintain deer herds in numbers necessary to allow regulated hunting seasons, information regarding the deer as a reservoir of infection to cattle needed to be determined. The livestock people of Kansas have been most cooperative and additional information on leptospirosis in Kansas deer has been of interest to all groups concerned. In other states several surveys on the prevalence of leptospirosis agglutination titers in deer serums have been reported.1,2,3,4,5,6

A survey project was initiated involving the Kansas Forestry, Fish and Game Commission, the College of Veterinary Medicine and deer hunters. The survey included the findings of a three-year study of serums of deer that were killed during the hunting seasons. The results of serum titers are included in this report for the years 1965, 1966 and 1967. The primary objective of the survey was to determine the incidence of leptospirosis agglutination titers in deer that were killed in Kansas during the hunting seasons.

MATERIALS AND METHODS

Plastic blood collection tubes were mailed to all hunters at the time of issuing hunting permits. Instructions were included on the method of collecting blood and information about the importance of the hunter aiding the project. Blood samples were collected by fish and game authorities at check points and each tube was identified by location of the deer kill. Blood tubes were collected each evening at each check station and were transported to the Veterinary Diagnostic Laboratory. On arrival at the laboratory all samples were centrifuged and serums were identified and stored at 4 degrees Centigrade. Macroscopic agglutination tests, using the rapid plate method, were used to determine serum titers. Commercial antigens *were used to conduct the test. Serum dilutions ranging from 1:32 to 1:256 were used.

*From the Veterinary Diagnostic Laboratory, Department of Pathology, College of Veterinary Medicine, Manhattan, Kansas.
**From the Kansas Forestry, Fish and Game Commission, McPherson, Kansas.
***From the Kansas Forestry, Fish and Game Commission, Emporia, Kansas.

*Difco Laboratories, Detroit, Michigan
Leptospiral antigens used in the agglutination tests were *Leptospira ballum*, *L. canicola*, *L. icterohemorrhagiae*, *L. bataviae*, *L. grippotyphosa*, *L. pyogenes*, *L. autumnalis*, *L. pomona*, *L. sejroe*, and *L. hyos*. A serum dilution of 1:64 or higher that agglutinated on the plate test was considered a positive reaction.

RESULTS AND DISCUSSION

A total of 1,576 deer serums were examined over the three-year period. Ninety percent of the blood samples collected by the hunters were satisfactory for testing. Nine hundred sixty-two samples were examined in 1965, 282 samples in 1966, and 333 in 1967. Of the 962 samples examined in 1965, six were considered positive to leptospirosis. In 1966, all samples tested were negative for leptospirosis and, in 1967, 29 or 333 samples were positive. The combined results for the three-year survey was a two percent incidence of leptospirosis in the serums tested.

Table 1. Number of deer serums with agglutination titers to specific strains of Leptospira antigens.

<table>
<thead>
<tr>
<th></th>
<th>1965</th>
<th>1966</th>
<th>1967</th>
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</thead>
<tbody>
<tr>
<td><em>L. pomona</em></td>
<td>3</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td><em>L. canicola</em></td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td><em>L. icterohemorrhagiae</em></td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td><em>L. grippotyphosa</em></td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL POSITIVE BY YEAR</td>
<td>6</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>TOTAL SERUMS TESTED</td>
<td>962</td>
<td>282</td>
<td>333</td>
</tr>
</tbody>
</table>

Ferris\(^2\) reported that 15.92 percent of deer serums reacted to Leptospira antigens in a two-year study in Illinois. A survey in Minnesota\(^6\) indicated 16 percent of deer serums gave some degree of reaction. Reynolds\(^4\) examined 628 deer samples collected in Massachusetts and found all to be negative for *L. pomona* antibodies.

The deer population in Kansas has increased in recent years and evidence in this report suggests the incidence of agglutination titers for leptospirosis also increased. It is difficult to evaluate the possibility that deer could be a reservoir of infection. Possibly the increased numbers of deer have established larger deer herds and the exposure rate increases could be the result of infections occurring from deer to deer. The exposure rate of deer to cattle is not known and cannot be established with the information available at this time.

ACKNOWLEDGMENTS

The authors wish to thank Dr. D. O. Manley, Veterinary in Charge, A.N.H., Mr. A. G. Pickett, Livestock Sanitary Commissioner, and Dr. John Hudelson, State Veterinarian, for their cooperation in this survey.
The non-shaded areas represent the geographical areas that serums were collected from deer.

REFERENCES

REPORT OF THE COMMITTEE ON LEPTOSPIROSIS


The Committee reviewed the previous year's report and information that has become available during the current year. In domestic cattle and swine, the primary causes of leptospirosis are Leptospira pomona, L. hardjo, and L. grippotyphosa. The detection of infection with the latter serotypes is seriously hampered by the lack of adequate plate antigens similar to the currently available L. pomona plate antigen, and consequently, the incidence of infection with L. hardjo and L. grippotyphosa for many parts of the country is not known. In those areas where adequate testing is being done, the results indicate that L. hardjo and L. grippotyphosa may account for as much as fifty percent of the clinical manifestations of leptospirosis. The committee recommends that other states initiate routine sampling and testing of cattle and swine by the application of a combination of plate and microscopic agglutination tests to a statistically significant sample of sera perhaps similar to the present Market Cattle Testing Program for brucellosis.

The present L. pomona bacterins are widely used. They are important in reducing the economic losses due to L. pomona infections. Due to the increasing clinical, serologic and bacteriologic evidence of L. grippotyphosa and L. hardjo infections, the committee recommends that licenses be issued to interested biological companies to produce a polyvalent bacterin (L. pomona, L. grippotyphosa and L. hardjo). It is further recommended that the immunogenicity for each of the serotypes included in the polyvalent bacterin be at least equivalent to that in currently available L. pomona bacterins. The efficacy tests currently in use may not be applicable to all bacterins and new methods may be desirable, such as tests for protection against the renal carrier/shedder condition in a suitable test host.

A definite diagnosis of leptospirosis requires the isolation and serologic identification of the causative leptospire. The isolation of leptospires from cattle and swine is now routine in some states where new mediums, some available commercially, are used along with chemicals which inhibit contaminating bacteria (5-fluoruracil) and simplified procedures for collecting and inoculating urine or tissues into cultural media. Newly isolated leptospires can be tentatively identified by the use of known antisera in the microscopic agglutination test. Positive identification may be made by the WHO/FAO Leptospirosis Reference Laboratory located at the Walter Reed Army Institute of Research, Washington, D. C.

The results of studies with chemotherapeutic agents indicate that the carrier state may be eliminated in cattle and swine.

In conjunction with bacteriologic and serologic tests, the use of antibiotic
agents may permit the continued use of valuable breeding stock which may have low, persistant serologic reactions, or provide an additional safeguard when cattle or swine are introduced into valuable, leptospirosis-free herds.

The committee wishes to reiterate the opinion expressed in previous reports that leptospirosis is not amenable to eradication because of the numerous serotypes encountered, the difficulty of detecting carrier animals and the wide range of domestic and wild animals hosts. Therefore, the Committee emphasized that effective control depends upon the development of adequate prophylactic and therapeutic measures, and that adequate financial support for research is necessary for the development of these control measures.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this committee be continued.
FOOT AND MOUTH DISEASE IN GREAT BRITAIN

A. Gwyn Beynon, M.R.C.V.S.D.V.S.M.

This paper is intended to give an account of the severe epidemic experienced in Great Britain during the end of 1967 and the early part of 1968.

The methods we employ in dealing with the disease are, I think, well known to you. They consist of the destruction of all infected animals and those which have been in contact with them, followed by a thorough disinfection of the premises and a rest period of 4-6 weeks before restocking is allowed. At the same time a ban is imposed on the movement of susceptible animals in the vicinity of disease outbreaks.

Major epidemics have been experienced during this century, especially during the years 1922/24, 1941/42, 1951/52, 1960 and 1967/68. Following some of these major incidents independent committees have been appointed to enquire into the methods which were used and have made detailed recommendations on policy. All have supported the stamping out policy. Following the 1922/24 epidemic, salvage of carcasses was discontinued but due to exigencies of war and the inevitable shortage of meat, it was resumed from 1939 to 1954. Since 1955, however, all affected and in contact animals which have been slaughtered have been disposed of, either by burial or burning. This followed the recommendations of the Committee of Enquiry which reported in 1954. This Committee also recommended that the size of an Infected Area should be reduced from 15 miles to 10 miles around the Infected Premises and this procedure has been followed since that time.

Between 1962 and 1965, Great Britain experienced its longest period of freedom since 1908 and no outbreak occurred for nearly 3 years. No outbreak has occurred in Scotland since 1961. This satisfactory situation was attributed to three factors.

(1) The ban on the importation of pork and pork offals from South American countries in 1961.
(2) The greatly improved disease situation in those countries on the western seaboard of Europe, which had enabled them to introduce a stamping out policy in addition to vaccination.
(3) The improved disease situation amongst the cattle population in Argentina following an intensive vaccination campaign.

This period of freedom was broken in the Spring of 1965 when a single outbreak occurred in the south eastern part of the country. In 1966 there were 34 outbreaks - 32 of these occurred in one confined area in the county of Northumberland and virus type 01 was involved.

During this series foot and mouth virus was isolated from a human patient on premises where disease was confirmed 8 days previously. Paired blood samples from the patient showed a marked increase in the antibody level 26 days after onset of symptoms but swabs taken 6 days after appearance of symptoms and on subsequent occasions failed to detect virus. This was the first authenticated case of foot and mouth disease in a human patient in Great Britain. It seems that the duration of infectivity in the human patient is very short and that it would
certainly be necessary to avoid contact with animals during this period.

Later, two associated outbreaks were confirmed in the south of England and in which an exotic virus type A22 was isolated. This strain had been responsible for the epidemic in the near East but the method of introduction into Great Britain was not discovered.

In 1967, there were 33 outbreaks up to the time when the major epidemic began in October; 29 of these outbreaks occurred in one series in the south of England and 4 cases in the Midlands. In both these series the disease first appeared in swill fed pigs and type 01 virus was involved.

The Recent Epidemic

After a period of 7 weeks' freedom, disease was confirmed on clinical findings in Owestry, Shropshire, on 25th October, 1967. 2,364 outbreaks occurred in this epidemic, during which virus type 01 was isolated from more than 100 field samples of material submitted to the Animal Virus Research Institute at Pirbright.

Although 18 counties became involved the disease was largely confined to six in the north west Midlands and adjacent Welsh border where the epidemic commenced and where 2,228 (94.25%) outbreaks occurred. Intensive dairy farming has been traditional in this area, with a very high density of stock of over 60 cattle per 100 acres in addition to sheep and pigs, compared with the national average of 31 cattle per 100 acres.

The initial case was confirmed in pigs on a mixed farm with a total stock of 71 cattle, 47 sheep and 66 pigs, of which 28 pigs were found to be affected, 26 had recent lesions and in 2 sows lesions were estimated to be at least 4 days old; the cattle and sheep on this premises were healthy at the time of slaughter. During the first week of the epidemic there were 23 outbreaks of which 14 were in the vicinity of the initial case and the remaining 9 cases were scattered in a north-easterly direction. One outbreak occurred 90 miles to the north in Lancashire and the remaining 8 cases were distributed 10-30 miles from the initial case in the Cheshire/Shropshire Plain. During the second week, 104 outbreaks occurred, all north-east of Oswestry and were distributed within a well defined triangular area bound on the west by the Welsh hills with its apex near the initial case and extending north-east 40 miles across Cheshire and Shropshire.

The prevailing wind at the critical time of 24th-26th October had been towards the north east but the occurrence of such a massive fall-out over such long distances was difficult to explain when no older case was found near to the initial outbreak and in none of the early cases had there been large numbers of cattle affected at the time of diagnosis. The build up of infection in the main area of disease incidence increased from 104 outbreaks in the second week of the epidemic to 490 in the fifth week of the epidemic, of which 98% were located in the north-west Midlands.

Thereafter there was a gradual decline in outbreaks; the weekly totals of confirmed cases fell from 218 in the seventh week to 88 in the tenth week, and overall 95% of them were located in the north west Midlands.

During the first 4 weeks of 1968 there were 138 outbreaks and in the subsequent 4 weeks there was a marked drop to 24 outbreaks, and the overall improvement in the disease position continued with the main weight of infection still being in the north-west Midlands. The last case in February occurred on the
21st and it was indeed disappointing when on 18th March, after an interval of 25 clear days, a further outbreak occurred in Shropshire on a restocked farms, 24 further outbreaks occurred in the Cheshire Plain; the last case was confirmed on 4th June. Of these 24 outbreaks, disease had recursed on 14 premises following restocking.

Apart from the main area of high disease incidence, 136 outbreaks occurred in 12 other counties, 2 to the north and the remaining 10 counties to the south and east of the principal focus of disease. The disconcerting feature of these cases was that many of them were 30 miles or more from the main weight of infection and could not be directly linked with it. In only 3 of these counties (Lancashire, Derbyshire and Worcestershire) did local spread result in more than 10 secondary outbreaks and in 11 initial outbreaks no secondary spread occurred.

As the disease position improved a restocking plan was prepared in areas where the weight of infection was not unduly high, and this followed the normal procedure of withdrawing restrictions four weeks after the disinfection, or six weeks after slaughter of the last case in the area concerned. In the areas of high disease incidence, restocking was controlled in that the restrictions were retained on the individual premises and animals were moved under licence and subject to veterinary inspection 7-14 days after movement. This operation was phased, the criteria used being freedom from disease during the preceding 21 days within 10 miles. Despite the recurrence of disease on a small number of these premises, by the end of May, 1968, 2,099 premises had been restocked. The restrictions on individual premises were withdrawn after 6 weeks had elapsed from the restocking date. During this restocking plan, Infected Area restrictions were retained but gradual contraction around the periphery took place and the last Infected Area restriction was removed on 25th June, 21 days after the last outbreak in the country.

As well as imposing Infected Area restrictions in the vicinity of disease, because of the explosive nature of the epidemic Controlled Area restrictions on the movement of livestock were applied at an early stage. These restrictions prohibit the holding of store markets and allow the essential movement of animals under licence only.

Initially this applied to counties adjacent to Infected Areas but by mid-November they were extended to the whole of England and Wales, and one week later to Scotland. These restrictions remained in force until the end of January, when they were removed from Scotland and the North of England. As the disease situation improved further reductions in the extent of the Controlled Areas were made until on 4th March the remaining Controlled Area was released from restriction.

The total number of animals slaughtered on the 2,364 Infected Premises was 370,630, comprising 189,413 cattle, 82,305 sheep, 98,878 pigs and 27 goats of which 8,250 cattle, 2,253 sheep and 329 pigs were affected with the disease at the time of slaughter. In addition, 21,085 cattle, 22,200 sheep, 15,985 pigs and 12 goats were slaughtered on other premises as dangerous contacts to infection involving a further 1,518 owners and the total compensation amounted to over £26 m.

During the latter part of November, 1967, stocks of FMD vaccine were
acquired and stored in this country. This step was taken as an insurance measure and it was considered prudent to arrange for a second line of defence, in view of the alarming spread of the disease, albeit in a limited geographical area. There was also the possibility of a fresh strike in another part of the country, which might well have brought about a situation in which our veterinary resources would not be sufficient to cope. However, there came a time when it was apparent that continued rigorous application of the policy was becoming effective and it was not found necessary to use the vaccine.

In order to deal with an epidemic of this magnitude, 348 British Government whole-time veterinary staff were employed exclusively on foot and mouth disease duties, and these were augmented by 497 temporary veterinary inspectors (mainly practising veterinary surgeons) and 84 veterinary surgeons from overseas (20 from private practice in Eire, and Government veterinary surgeons from Australia (14), Canada (12), New Zealand (7 including 5 from the New Zealand Meat Board), Northern Ireland (14), Erie (26), and U.S.A. (11).

In early December, 1967, over 750 veterinarians were operating from 24 control centres and during the epidemic a total of 38 control centres were manned. In addition to lay staff from within Animal Health Division, other branches of the Ministry of Agriculture supplied staff, and in the West Midlands, this amounted to 700 additional staff, many of whom were trained to carry out non-veterinary duties such as supervising disinfection of Infected Premises and milk depots, thus relieving veterinary manpower for other work. The military authorities also came to our aid by providing troops for disinfection duties.

The Mode of Introduction of the Virus

The ultimate success of any eradication policy based on a "stamping out" policy must depend to a great extent on the discovery and elimination, wherever possible, of the source of infection.

Outbreaks are classified as primaries and secondaries. The former classification is applied when there is no known connection with an existing centre of infection in the country, and secondary cases are these originating from known outbreaks. The origin of primary outbreaks must usually be based on circumstantial evidence. Bound up with the question of establishing an origin is the need for the virus to be typed and subtyped.

Between 1954 and September, 1967, 1,002 outbreaks occurred in Great Britain, of which 179 have been primaries and have been attributed to the following sources:-

<table>
<thead>
<tr>
<th>Source</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continent of Europe</td>
<td>41</td>
<td>(22.9%)</td>
</tr>
<tr>
<td>(Windborne infection or bird movement)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imported meat/bones/meat wrappers</td>
<td>97</td>
<td>(54.2%)</td>
</tr>
<tr>
<td>Pirbright Research Institute</td>
<td>1</td>
<td>(.6%)</td>
</tr>
<tr>
<td>Obscure</td>
<td>40</td>
<td>(22.3%)</td>
</tr>
</tbody>
</table>

In many of those cases classified as obscure, imported meat or windborne infection might have been responsible for the introduction of the disease.
Origin of Infection in the Recent Epidemic

The investigations carried out lead to the conclusion that the initial case in this epidemic was, in fact, a primary outbreak and after consideration of all possible sources of infection, including the possibility of virus from a previous outbreak remaining in the country, the possibility of windborne infection and bird movement from the Continent, movements of animals and persons, materials used on the farm and from imported meat, it was concluded on circumstantial evidence that imported frozen lamb was responsible.

These investigations brought to light the fact that lamb carcasses from the same consignment as those implicated in the initial case were distributed over the area where outbreaks occurred during the early weeks of the epidemic. In view of the build-up within an incubation period, over distances in excess of those normally attributed to local spread, there appeared to be a strong possibility that multiple primary strikes had occurred in this area. Enquiries revealed that a number of these early outbreaks involved farms where there was a possibility that imported lamb had been purchased. Affected animals were invariably cattle at pasture and it is of interest to note that pigs were rarely found affected during the whole epidemic. This may have been due to the virus strain being one that was not easily adapted to pigs.

Further investigations were pursued with regard to the origin of outbreaks well away from the main centres of infection. In a number of outbreaks, it was possible to establish a link with imported meat and in particular with lamb. Most of these enquiries showed that the imported meat had been supplied either to the household itself for human or animal food or to neighboring houses, and from these sources it was possible for it to come into contact with the stock on the farm under investigation.

The pattern of bulk distribution of imported meat during the period July-December 1967 was also investigated and in particular lamb. Apart from deliveries to the major urban areas the main areas of infection did appear to receive a greater concentration of this type of meat than other rural areas. However, large quantities of imported meat were distributed to the south of England and south Wales where foot and mouth disease did not arise.

While these enquiries were proceeding the Government imposed a ban on the importation of all meat except from countries with a long history of freedom from the disease. At the same time wholesalers voluntarily agreed to hold in cold store stocks of frozen imported meat from those countries subject to this ban. Chilled beef however could not be held and was distributed in the London area where the risk of infection being carried to susceptible animals was minimal. Subsequent legislation was introduced to prohibit the removal from cold store of all imported meat; this enforced the voluntary ban then operating. Following the publication of the findings implicating imported lamb, other types of imported meat were released from cold store on licence but restrictions were retained on lamb, mutton and offal of South American origin.

In March 1968 a Veterinary Mission visited the Argentine and other South American countries with a view to improving the present sanitary arrangements and other possible safeguards. Their report is now under consideration by the Government.
Epidemiology

Whilst the meat origins investigation was proceeding, epidemiological studies were also being carried out in an endeavour to explain this unprecedented spread of infection.

Field and laboratory observations suggest that a combination of factors all played their part. The virus type involved appeared to be one which possessed some unusual features. These included an exceptional capacity for spread in cattle and an ability to survive outside the host for a longer period than is usual. The results of experimental work also suggest that excretion of virus occurred at an earlier stage in the disease than is generally accepted. It is well known that an infected animal may shed virus for 24-48 hours before the appearance of lesions but following experimental work with O1 virus, Burrows (3) has recorded that in cattle and sheep, excretion of virus occurred in some instances up to 5 days before symptoms appeared. In the pig, excretion of virus up to 10 days prior to development of lesions was observed. These observations suggest that notwithstanding the very high standard of reporting of a suspicion of disease by the farmer or his veterinary surgeon, there would be greater opportunity for dispersal of virus from the initial and other cases than had previously been though possible in Great Britain. Further, this danger was enhanced by the fact that although in many cases only one animal was affected with the disease at the time of diagnosis, considerable numbers of contacts could be showing symptoms within a matter of a few hours.

The pattern of distribution of the first 89 cases, which occurred within 10 days of slaughter of the initial case, suggest that wind borne dissemination did occur and a study of the meteorological evidence supports this contention. In fact during the first three weeks of the epidemic the prevailing wind was from the south west. The limits of wind borne spread are difficult to define but distances up to 20 miles are not unrealistic.

Accepting that the weather conditions favoured the drift of infection in a north easterly direction, the vast majority of secondary outbreaks in the north west Midlands were caused by local spread. The predominant factor in this spread was undoubtedly the concentration of livestock, in particular milking herds out at grass. Stock on neighboring farms were closely associated with one another and there were few natural breaks, such as arable land. The same virus subtype in less densely stocked areas (e.g. east Midlands) produced little or no local spread. Movement of birds - particularly starlings - may also have contributed to the local spread of disease. Rats may also have been responsible for mechanically carrying virus from one premises to another but laboratory examination of rat blood sera failed to reveal any FMD antibodies and we have no evidence that rats became infected with the virus. Although naturally occurring disease in hedgehogs is well authenticated, in this series of outbreaks no incidence of disease in this species was recorded. Deer were present in certain areas where foot and mouth disease occurred but there was no evidence of their involvement in this epidemic. The possibility of dissemination of virus by watercourses was investigated but it was concluded that this was not a material factor in the spread of disease.

The part played by milk as a means of spread of infection was also the subject of a special study. The large number of dairy farms on which disease was confirmed in conjunction with the fact that the milk of infected cows may contain virus up to
48 hours before lesions are evident, produced a situation where a considerable volume of potentially dangerous material was leaving farm premises before the imposition of Infected Place restrictions.

The dangers associated with the movement of infected milk have been appreciated for many years and in Infected Areas a standard drill of disinfection is applied to milk churns and vehicles. Churns are collected at the roadside. Bulk milk tankers pose a special problem and steps were taken to disinfect the milk hose and coupling after each farm collection. A filter containing sodium carbonate was fitted to the vacuum pump to reduce the risk of aerosol spread.

Experimental work carried out suggests that the risk of aerosol spread of virus from a bulk tanker was probably minimal. It was very difficult to differentiate outbreaks due to milk collection vehicles from those due to local spread but records show that on 15 occasions these vehicles were the most likely origin and 7 of these were linked with churn collections and 8 with milk tankers.

Milk products used for animal feeding were the cause of 9 outbreaks during the current series. Eight of these were linked with the feeding of skim milk to pigs and in six of the cases the skim milk in question originated from farms in "clean country" which had been processed at a dairy receiving milk from an Infected Area. The other two outbreaks were attributed to a dairy which handled large quantities of milk produced in the main disease area. One outbreak was attributed to a dairy employee feeding churn washings to his pigs.

The movement of persons was probably the cause of 10 secondary cases. In this connection, the practising veterinary surgeon can be placed at considerable risk especially when it is known that animals may excrete the virus for some time before the appearance of clinical signs. It should be recorded that the general farming public responded well to the advice not to visit farms.

The movement of animals resulted in only one outbreak of disease during the epidemic and contamination of a lorry which carried stock caused 2 outbreaks. This small number is accounted for by the early imposition of large Infected and Controlled Area restrictions, reducing animal movement to a minimum.

Recrudescence of Disease

In the 1922-24 series of outbreaks of foot and mouth disease the recrudescence of disease on numerous premises following restocking was a serious problem. It has again been a problem but to a lesser extent. Although the disinfection procedure which was carried out was similar to that practised in the past, twelve cases have occurred which could be attributed to this cause. One of these premises was in the south west Midlands out of a series of 33 outbreaks. The remaining 11 cases were out of a total of 2,099 restocked premises in the area of high disease incidence and were not limited to any one distinct locality. Six of these cases could be attributed to feeding contaminated hay, and the other six cases could have been due to -

1. use of contaminated straw as a bedding;
2. feeding contaminated silage;
3. contaminated manure heap;
4. contaminated sacks;
5. a contaminated trailer being used for feeding hay to outlying stock;
6. use of a contaminated knife for paring sheep's feet.
The shortest period after restocking to confirmation of disease was 5 days; the longest period was 55 days. The shortest period after completion of disinfection to reappearance of disease was 41 days and the longest was 145 days.

Disease has also reappeared on 6 other restocked premises but in these cases, the origin of infection can be attributed to local spread from other Infected Premises.

Research

It is axiomatic that before a control and eradication programme can be undertaken it is necessary to have full knowledge of the infective agent and its behaviour in the susceptible host. In Britain, the Animal Virus Research Institute at Pirbright has a world-wide reputation for its work on foot and mouth disease virus and there is no need to elaborate its considerable contribution to world knowledge. The Institute of course carries out research in other viruses which are the cause of animal disease throughout the world. It examines field samples as an aid to diagnosis and in positive cases it reports the type and subtype.

Committee of Inquiry

The very large number of outbreaks which occurred during the recent epidemic and the large number of animals which had to be slaughtered before the disease was finally eliminated has caused considerable concern in Great Britain and a Government Committee under the Chairmanship of the Duke of Northumberland has been appointed "To review the policy and arrangements for dealing with foot and mouth disease in Great Britain and to make recommendations."

This Committee's conclusions will be awaited with considerable interest.

REFERENCES

Foot and Mouth Disease Weekly Outbreaks
25th Oct. '67 - 12th March '68

No. of Outbreaks

Week No. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Oct. 25 31 7 14 21 28 5 12 19 26 2 9 16 23 30 6 13 20 27 5 12

Foot and Mouth Disease Weekly Outbreaks

FOOT AND MOUTH DISEASE IN GREAT BRITAIN
Foot and Mouth Disease
Great Britain

Distribution of Outbreaks
25\textsuperscript{th} Oct. 1967 – 4\textsuperscript{th} June 1968
Foot and Mouth Disease
Weekly Outbreaks
12th March '68 - 4th June'68

Outbreaks attributed to Recrudescence
Other Outbreaks

FOOT AND MOUTH DISEASE
Classification of Origins
January 1954 - September 1967

<table>
<thead>
<tr>
<th>TOTAL OUTBREAKS</th>
<th>1,002</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMARY CASES</td>
<td></td>
</tr>
<tr>
<td>Continental origin (by birds or wind-borne)</td>
<td>41</td>
</tr>
<tr>
<td>Imported meat/bones, meat wrappers</td>
<td>97</td>
</tr>
<tr>
<td>Pirbright Research Institute</td>
<td>1</td>
</tr>
<tr>
<td>Obscure</td>
<td>179</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SECONDARY CASES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Local spread</td>
<td>517</td>
</tr>
<tr>
<td>(ii) Movement of -</td>
<td></td>
</tr>
<tr>
<td>Animals</td>
<td>(a) 187</td>
</tr>
<tr>
<td>Vehicles</td>
<td>6</td>
</tr>
<tr>
<td>Persons</td>
<td>(b) 74</td>
</tr>
<tr>
<td>Milk Products</td>
<td>(c) 15</td>
</tr>
<tr>
<td>Things</td>
<td>(d) 4</td>
</tr>
<tr>
<td>Total</td>
<td>823</td>
</tr>
</tbody>
</table>

(a) 133 via a market, 49 via a direct sale, (c) 15 via skim milk
(b) 46 via V.S., 28 others (d) 4 via slaughterhouse refuse, 1 via egg boxes and trays.
FOOT-AND-MOUTH DISEASE
Classification of Origins of Infection
25th October 1967 - 4th June 1968

<table>
<thead>
<tr>
<th>PRIMARY CASES</th>
<th>SECONDARY CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,364 - X</td>
</tr>
</tbody>
</table>

1. Known Sources

<table>
<thead>
<tr>
<th>Source</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Animals</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(b) Vehicles</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>(c) Persons</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>(d) Milk Products</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>(e) Hay</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Recrudescence</td>
<td>12</td>
<td>51</td>
</tr>
</tbody>
</table>

2. Local Spread

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,313 - X</td>
<td></td>
</tr>
</tbody>
</table>

FOOT-AND-MOUTH DISEASE
Stock Slaughtered
October 1967 - June 1968

<table>
<thead>
<tr>
<th>Number of infected premises</th>
<th>2,364</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cattle</th>
<th>Sheep</th>
<th>Pigs</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Animals Slaughtered (affected with F.M.D.)</td>
<td>8,250</td>
<td>2,253</td>
<td>329</td>
<td>-</td>
</tr>
<tr>
<td>Number of Contact Animals Slaughtered (not affected)</td>
<td>202,248</td>
<td>102,252</td>
<td>114,534</td>
<td>39</td>
</tr>
<tr>
<td>Total Animals Slaughtered</td>
<td>210,498</td>
<td>104,505</td>
<td>114,863</td>
<td>39</td>
</tr>
</tbody>
</table>
Foot-and-mouth disease has not gained entry into the United States since 1929; however, many factors are changing which increase the possibility of the disease getting in.

1. World trade is rapidly expanding. We are importing more than one billion pounds of animal products, such as hides, a year. This does not include live animals or meat. All animal products coming in are not legal. Unfortunately, we know of incidences where meats and meat products are smuggled into the country.
2. International travel is on the increase. From 1962 to 1968, passengers entering the United States from foreign countries have increased from 160 million a year to 218 million a year. There are many documented cases where man has spread foot-and-mouth disease by carrying the virus on his clothing and footwear. For example, in previous United States outbreaks almost 1/5 of the herds infected were attributed to spread of the virus by man. We know of many recent cases where American cattlemen visited livestock premises in foot-and-mouth disease endemic countries, returned to the United States, and were in contact with livestock a few hours later without cleaning and disinfecting their clothing and footwear. We have gotten away with it these times, but when will it be the means by which foot-and-mouth disease is introduced into the United States?
3. With the ever-increasing speed of transportation, travel time is being reduced. At the turn of the century, a trip from another continent to the western hemisphere was accomplished by a 30-day or so boat trip. Animals exposed just prior to shipment developed the disease before reaching this country and were destroyed. This served as an important factor in reducing the risk of entrance of foot-and-mouth disease. Today, a six-hour plane trip offers little, if any, protection.
4. Natural geographical barriers are rapidly being penetrated. The last remaining barrier between the foot-and-mouth disease endemic countries of South America and the United States is the Darien rain forest which encompasses the border between Colombia and Panama. Civilization is slowly encroaching on the Darien jungle. The Pan American Highway, when completed, will pass through it and will greatly increase the risk of entry of foot-and-mouth disease to Panama, Central America, and North America.

We must face reality. Foot-and-mouth disease can get into the United States as it did in 1902. We must plan What We Are Going To Do When It Happens. We have three alternatives:
1. Do Nothing
2. Control the disease—which would mean utilization of vaccines, or
3. Eradicate.
1. If we do nothing, foot-and-mouth disease will rapidly spread over the entire country. In Mexico, the disease spread up to 500 square miles a day. It has been estimated that foot-and-mouth disease increases the cost of producing a given quantity of meat and milk by about 25 percent in those countries where foot-and-mouth disease is endemic. The United States is the world's largest producer of animal protein and milk, and the cost of allowing foot-and-mouth
disease to become endemic here would be staggering, probably in excess of $5 billion a year.

2. Another alternative is to control the disease by vaccination. Turkey, Israel, Argentina, and most European countries have a government subsidized vaccination program. These vaccination programs have greatly reduced losses from foot-and-mouth disease. For example, in 1967 Germany reported only 3,350 outbreaks; Turkey, 2,173 outbreaks; and Argentina reported 4,634 outbreaks. However, vaccination programs are expensive. We estimate that a national vaccination program for the 200 million livestock in the United States would cost almost $1 billion a year. Of course, the disease could not be eradicated in this manner, so the cost would occur annually.

3. The third alternative, and the one we consider to be the most feasible economically, is to eradicate the disease. Foot-and-mouth disease has been introduced into the United States and eradicated six times in this century, and three times prior to that. The first outbreak during this century occurred in 1902 and was confined to the New England States—Massachusetts, New Hampshire, Vermont, and Rhode Island. The last outbreak occurred in 1929 in California. The 1929 outbreak was introduced by ships' garbage. During the 1924 outbreak, the disease gained entry into the deer population in the Stanislaus National Forest in California. Some thought that the widespread infection in wildlife would make eradication impossible. It did pose a monumental problem. More than 22,000 deer had to be destroyed, but the outbreak was contained in California and eradicated.

The most extensive United States outbreak occurred in 1914. This outbreak was first detected in the vicinity of Niles, Michigan, in October 1914, after it had evidently been under way since the preceding August. Unfortunately, the mild form and atypical appearance of the first cases permitted the disease to spread unrecognized to a considerable number of herds of cattle and hogs. Some of the latter reached and presumably infected the stockyards at Chicago. From Chicago, the infection was disseminated to other stockyards and to points north, east, south, and west by shipments of livestock, especially stockers and feeders. Twenty-two States and the District of Columbia were infected before the disease could be eradicated.

The cost of eradication when it happens here is difficult to predict and would vary greatly, depending on factors such as (a) the virulence of the virus, (b) location of outbreaks, (c) rapidity of reporting, and (d) whether diseased animals get into marketing channels.

The most recent examples of foot-and-mouth disease eradication costs are the 1946-1954 Mexican outbreak, the 1952 Canadian outbreak, and the 1967-1968 British outbreak. In the 1946-1952 and 1953-1954 Mexico outbreaks, over one million head of livestock were destroyed before the disease was eradicated. The United States cooperated with Mexico in this campaign at a cost to the United States of over $137 million.

The 1952 Canadian outbreak occurred during mid-winter in the province of Regina in Central Saskatchewan. Snowfall was so heavy that movement of man and animals was severely curtailed resulting in a very slow spread of the disease. Canadian officials estimated cost of eradication to be approximately $1 million with only 42 herds destroyed. Because of climatic differences, it seems likely that
WHEN IT HAPPENS HERE 213

the cost of eradicating a similar outbreak in the United States would have been
much greater.

The 1967-1968 outbreak in the United Kingdom resulted in the destruction of
approximately 2 percent of their cattle, 12% percent of their swine, and ½ of 1
percent of their sheep. If the United States suffers an outbreak of that magnitude,
our eradication costs will be approximately $3 billion. So, many factors will
influence costs of eradication; however, two of the most important factors are the
time which elapses between the first occurrence of disease and start of an efficient
eradicaton program and whether or not diseased animals enter marketing channels.
Our expected range of foot-and-mouth disease eradication cost is from $1 million
to $3 billion depending on the circumstances involved, but even if costs reach the
upper limit, it will be cheaper to eradicate than to spend up to a billion dollars a
year to vaccinate.

If we intend to eradicate foot-and-mouth disease when it happens here, there
will be a need for large numbers of veterinarians, livestock inspectors, and other
personnel and a vast quantity of equipment and supplies. Today we have 760
veterinarians and 790 livestock inspectors employed by the Animal Health Division
and a comparable number employed by the different States. During the height of
the recent British outbreak, over 10,000 persons (including approximately 900
veterinarians) were engaged in the eradication program. Large quantities of
equipment and supplies for digging burial pits, burning carcasses, and cleaning and
disinfecting must be immediately available for each farm found infected. An
infected animal has been likened to a manufacturing plant in that it is continuously
producing more virus; therefore, in order to prevent the further multiplication and
spread of virus, infected animals should be slaughtered and their carcasses disposed
of as rapidly as possible.

Eradication will require that every segment of the livestock industry—owners,
markets, packing plants, dealers, and regulatory officials—cooperate in the
detection of disease and the control of its spread. For instance, we checked
movements of livestock from three Federally inspected stockyards for a period of
one week. During that period, livestock moved into 42 of the 50 states.

When an outbreak occurs, livestock movements will have to be rigidly
controlled. We know that foot-and-mouth disease is readily spread by movements
of exposed and infected animals. We must have the legal authority, manpower, and
money to accomplish complete control of livestock movements. Adequate funds,
both State and Federal, must be immediately available to assure prompt and
vigorous action to eliminate the outbreak. The success of eradication is dependent
upon early detection, rapid destruction, and disposal of infected animals. We must
preplan to assure that we have these capabilities.

We have a basic plan for conducting an eradication program and a few
experienced veterinarians. However, if the outbreak is large, we will be woefully
short of personnel. Each State has an emergency animal disease eradication
organization with personnel specifically assigned. Test exercises are conducted
annually to assure capability to deal with any foreign animal disease outbreak. A
national organization has been established with headquarters at Beltsville, Mary-
land. A special ready room has been developed and equipped with a communication
system. Large maps of the United States and county maps for each State are part of
the ready room equipment. Personnel have been assigned to each specific duty at
the ready room, and test exercises are conducted to train new members of the
organization and sharpen the skills of the older members.

A tri-agency agreement between the U.S. Department of Agriculture, the
Department of Defense, and the General Services Administration has been signed,
which provides that the military will furnish and deliver certain supplies,
equipment, and services as required by the national interest. The General Services
Administration has assembled and stored in seven different locations, supplies
necessary to initiate eradication procedures. These supplies, which include
disinfectants, rubber clothing, tools, spray pumps, and other essential equipment,
can be airlifted to any part of the United States in a few hours.

We have 65 ANH veterinarians who have received special training in the
diagnosis of foreign animal diseases and also a number of veterinarians who worked
in the Mexican campaign. These men will form a sound nucleus around which we
can build an eradication organization.

We have talked about the successful foot-and-mouth disease eradication
programs that have occurred, and we have talked in terms of preparedness in
handling an outbreak when it occurs. The experience gained by the 12 ANH
veterinarians who visited England and assisted them in their eradication program
has pointed out many weaknesses in our plans and in our preparedness. Except for
the experience in Mexico and to a much lesser degree in Canada, we have not had
an opportunity to apply foot-and-mouth disease eradication principles for almost
40 years. One of the explanations of the great capability of England in combatting
this disease is that they have had outbreaks over the years by which they could
refine and improve their techniques. More important, it has kept them abreast of
the drastic steps that are necessary to be successful and has kept those officials who
have responsibility for such endeavors alert to the importance of recognizing the
threat and be prepared to move rapidly and with decision.

We had experience in this country in the 1950's in our vesicular exanthema
eradication efforts. Before we talk with too much assurance of how well equipped
we are to deal with foot-and-mouth disease, let's ask ourselves how we are doing
with some of the other animal diseases that are present in this country. Particularly,
let us look at the handling of hog cholera outbreaks that are occurring in those
areas of the country where emergency measures should be applied, the elimination
of those outbreaks that remain or re-enter after the first push is over.

We currently have hog cholera outbreaks of some scope in two States that have
reached free status under the program. Both of these appear to involve spread of
the disease through marketing channels. In both instances, the disease was
apparently not reported until after it had a chance to start moving. These aren't the
only times this sort of thing has happened in States that were well on the road to
eradication or had reached free status, and this isn't something that only concerns
these States. It concerns all of us, and it may well raise a serious question as to
whether our livestock industry, and those of us in regulatory veterinary medicine,
have the determination to conduct a stamping-out program of any magnitude.

What makes us think we can face up to the unpleasant decisions and actions
involved in stamping out foot-and-mouth disease if we can't face up to it in the case
of hog cholera? In the hog cholera program, we have a built-in model to test how
we really could be expected to perform if confronted with an outbreak of a serious exotic disease. When we talk about our plans for emergency disease organizations, let's remember that the proof is in the doing, and that we have a test right now that faces us.

Foot-and-mouth disease or many other exotic diseases of livestock pose a tremendous threat to the total economy of our country. With all of our preplanning and attempts at preparedness to overcome such a threat, we must recognize that we still have much to do. When foot-and-mouth disease gains entrance into this country, we can but assume the worst. If it should get into our marketing channels before recognition, we know it would become widespread. The impact on the livestock industry and the total economy of this country would be disastrous. Assuming even a proportion of the magnitude of the English outbreak of 1967-1968, we can anticipate severe curtailment of the activities of the livestock industry—which would result in reduction or temporary elimination of the income of thousands of people associated with the livestock industry, including the transportation, marketing, slaughtering, processing—the whole gamut of meat and milk production of this country. Not only must we as animal health regulatory officials be prepared to step in promptly with decisive action, but we must concentrate on informing all segments of the livestock industry of their responsibilities in such endeavors. Failure to recognize responsibility in reporting suspected conditions, indecisive action even by one day, will cost the economy of this country many thousands and maybe millions of dollars.

We must be prepared “When it happens here”.
REPORT OF THE COMMITTEE ON
FOREIGN ANIMAL DISEASES

N. M. Konnerup, Silver Spring, Maryland, 
Chairman; W. T. Berry, Jr., Morrilton, 
Arkansas; D. E. DeTray, Beltsville, Maryland; F. P. Gluckstein, Bethesda, 
Maryland; T. Griffin, Silver Spring, Maryland; L. Griner, San Diego, California; D. W. 
Johnson, Frederick, Maryland; K. L. Kuttler, College Station, Texas; F. D. 
Maurer, College Station, Texas; R. M. McCully, Onderstepoort, South Africa; N. L. 
Meyer, Hyattsville, Maryland; T. G. Murnane, Washington, D. C.; A. B. Park, 
Iowa; T. Rea, Phoenix, Arizona; R. C. Reisinger, Bethesda, Maryland; H. G. 
Wixom, Sacramento, California.

1. Changes in the world animal disease situation:
A number of changes in the worldwide animal disease situation has occurred 
since the 1967 meeting. The extension of destructive animal diseases into new areas 
poses problems even though the new extension covers only a small area and is not 
therefore classed as a major epizootic.

2. African horsesickness (AHS):
The report of the Foreign Animal Diseases Committee at the 1967 meeting of 
this association pointed out that AHS had been diagnosed in Spain. The disease was 
first reported in mid October 1966 in the province of Cadiz in southern Spain. The 
exact mode of introduction into Spain is not known.
The mortality in horses was reported as nearly 100 percent. The total number of 
equidae dead or slaughtered was not reported, but their worth was given as 
3,563,210 pesetas ($2,494,247 U.S.).

It was reported that an AHS infected zone was designated and depopulated by 
slaughter of all equidae. A vector control program was conducted. Vector Control 
stations were established and all vehicles entering or leaving the infected zone were 
treated. Prohibitions were established against stray dogs and capture of stray 
and loose dogs was accomplished. A zone of vaccination was established around the 
infected zone and the following were reported to have been vaccinated:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horses</td>
<td>72,777</td>
</tr>
<tr>
<td>Mules</td>
<td>325,318</td>
</tr>
<tr>
<td>Asses</td>
<td>116,941</td>
</tr>
</tbody>
</table>

Restocking of equidae in the affected area was prohibited for three months. The 
economic losses in Spain were reported as follows:

A. Value of the dead or slaughtered equidae 3,563,000
B. Value of the actual work and of travel involved in carrying out the order of 
the absolute immobilization of the susceptible equidae in the suspect 
zone 12,500,000
C. Evaluation of the damages due to the retardation of agricultural work as a 
result of the immobilization in the suspect zone 2,000,000
D. Damages caused to agricultural work and the work involved in the complete 
slaughter of equidae in the infected zone and the prohibition or repopulation 
during six months 3,600,000

216
The Spanish reported that African horsesickness has been eradicated. New cases have not been reported in Spain since the disease apparently disappeared during the winter of 1966-67.

African horsesickness is an excellent example of a disease on the move – truly an emerging disease.

3. **Foot-and-mouth disease (FMD)**:

Foot-and-mouth disease is considered to be the most important single disease of livestock. Its direct and indirect economic effect is probably greater than all other animal diseases combined.

The most significant outbreak during last year occurred in Great Britain. The details of that outbreak are the subject of a paper given at this meeting. Additional information on the worldwide FMD situation will be reported by the Vesicular Diseases Committee of this association.

4. **African swine fever (ASF)**

African swine fever is another animal disease on the move.

The disease reoccurred in Italy in the spring of 1968. This reoccurrence followed a long and costly eradication program which involved the slaughter of approximately 100,000 swine. The causative agent in the Italian outbreak appears to be similar to the attenuated virus identified in Spain and Portugal.

The presence of ASF in Italy, Spain and Portugal poses a serious threat to all of southern Europe. In the absence of an effective vaccine, there are only two alternatives:

1. Live with the disease; or
2. Eradicate by slaughter.

Since ASF has the potential of decimating the swine population in affected countries, prompt eradication of outbreaks in newly infected areas seems essential.

Swine breeders and veterinarians should be made aware that ASF is apparently on the move and that it has the potential of economic devastation to the United States swine industry.

5. **Bovine tuberculosis**:

Although tuberculosis is not a foreign animal disease, the Foreign Animal Diseases Committee believes it appropriate to point out some significant overseas control measures.

A few countries have resorted to BCG vaccination. The results of BCG vaccination should be carefully watched. It is possible that there will be a sudden increase in the percentage of infection, particularly if other control procedures are dropped.

South Africa has initiated a tuberculosis control system based on slaughter, disinfection of premises, regular testing, and therapeutic treatment of exposed animals.
6. *Contagious bovine pleuropneumonia (CBPP)*:

During early 1967, contagious bovine pleuropneumonia occurred in France for the first time since 1920. The disease occurred in an isolated mountain community near the Spanish border. All herds in the area were placed under strict isolation and serologic survey was started. From January to April 1967, 701 cattle on 16 farms were slaughtered. On July 26, 1967, the disease was declared eradicated. In October 1967 the disease was again diagnosed in the previously infected area. The infected herd was slaughtered and it is now planned to maintain the area under quarantine until January 1969.

Contagious bovine pleuropneumonia control in Australia has made further progress. The disease has not been found in any part of western Australia or the Northern Territory during 1967. Several other areas have enjoyed longer periods of freedom from the disease. As a result, several areas have been declared free.

A suspected outbreak of CBPP was reported in Ecuador, but the disease has not been confirmed. The presence of CBPP in this hemisphere would constitute a potential threat to the United States.

7. *International support of foot-and-mouth disease programs*:

Substantial monetary support is being provided to several countries, particularly South America, for the purpose of foot-and-mouth disease control. In the opinion of the committee scientifically sound foot-and-mouth disease control planning should be a prerequisite to receive a loan. The foot-and-mouth disease control recommendations provided by the expert committees, international agencies, and United States Government officials apparently have been totally ignored with respect to what countries are eligible for loan consideration.

8. *Garbage feeding*:

The problems which result from the use of garbage for swine feed are common to all countries that permit the use of garbage for that purpose. Only a few northern European countries have taken steps to eliminate or adequately control garbage feeding. Although specific information is not in hand, there is no indication of plans for the extension of garbage feeding control regulations into new areas.

With the introduction of African swine fever into Europe, there is an ever increasing threat of extension of the disease to new areas in Europe and even North America through the practice of feeding raw garbage to swine. The threat of other diseases and parasitic infestations in those countries is identical to and probably more critical than that faced by the United States prior to the development of our program requiring the cooking of garbage used for swine feed.

9. *Rinderpest*:

The rinderpest campaign in Africa has made further progress. The major foci of infection remain in Sudan, Ethiopia, and Somalia. The persistence of reservoirs in wildlife is still unexplored and should be the subject of a major investigation.

10. *Duck virus enteritis (duck plague) in the United States*:

Duck virus enteritis (DVE) was diagnosed for the first time in the United States in January 1967, in Suffolk County, Long Island, New York. A more complete description of DVE is contained in the report of the Committee on Transmissible Diseases of Poultry.
This incident exemplifies the vulnerability of our livestock and poultry to foreign diseases.

It is suggested that all livestock regulatory officials be alert to this disease of waterfowl and that all outbreaks be quarantined and appropriate eradication measures be instituted promptly.

11. **Committee recommendations:**

   A. More stringent regulations and better enforcement of regulations governing the importation of foreign breeding stock and exotic animals, especially laboratory and zoological specimens;

   B. More investigation of major disease outbreaks in foreign countries by USAID - USDA - DOD. Such investigations would assist in the preplanning of disease control programs in the US by providing information on the epidemiology of foreign animal disease.

   C. More effective collaboration of United States agencies in animal disease control programs overseas.

   D. More emphatic influence on the World Bank/IDA, IDB, and the IADB on animal disease control systems pertaining to eligibility for loans as referenced in item 7 of this report:

   E. The endorsement of a policy for the rational development of vaccine and biological production to meet world requirements.

Foreign Animal Disease Committee

After review of these recommendations I believe they should be called recommended goals. For that reason I am going to request that the committee consider methods of implementation and come back next year with specific plans.

Dr. Norvan Meyer
Acting Chairman
EQUINE PIROPLASMOSIS

A. A. Holbrook and Wayne M. Frerichs

A talk to be presented to the Equine Diseases Section of the United States Livestock Sanitary Association
New Orleans, Louisiana

Equine piroplasmosis is found around the world on both sides of the equator. It is found as far south as Cape Town, South Africa and as far north as Moscow, Russia. Australia and New Zealand are the only large land masses free of the disease. The disease is caused by two species of tick-borne protozoan parasites of the genus Babesia. Babesia caballi, the first species to be diagnosed in the United States, was found in the blood of a horse in Dade County, Florida, in August of 1961\(^{5}\). The tropical horse tick, Dermacentor nitens, found on infected horses in Florida, was shown to transmit \(B. \text{ caballi}\) transovarially\(^{4}\). Control of \(D. \text{ nitens}\) by means of intensive spraying along with drug treatment of infected horses have held \(B. \text{ caballi}\) infection to an enzootic area in southern Florida.

In March, 1965, the first \(B. \text{ equi}\) infection in the U.S. was diagnosed in a Thoroughbred horse in Dade County, Florida. A second case of \(B. \text{ equi}\) infection was found in August, 1967, when a horse belonging to the U.S. Olympic jumping team returned from a tour of Europe. Equine piroplasmosis had been diagnosed previously in this horse at Nice, France, two days after leaving the U.S. A third case of natural infection with \(B. \text{ equi}\) was found in a horse in Indian River County, Florida, during an outbreak of equine piroplasmosis in which most of the horses were infected with \(B. \text{ caballi}\). Serum from this horse gave a positive reaction with \(B. \text{ equi}\) antigen in the complement-fixation test, and a negative reaction with \(B. \text{ caballi}\) antigen. Blood from this horse, when injected into susceptible ponies, produced only \(B. \text{ equi}\) infections. At the present time, we do not know which ticks act as vectors of \(B. \text{ equi}\) in the U.S. nor do we know the distribution of the disease caused by this parasite.

Most of the experimental infections produced with either parasite in our Laboratory would go unrecognized if we were not expecting infection to occur and, consequently, recording temperatures twice daily and observing stained blood films every day. When large numbers of parasitized RBC's (\(10^8\) or more) are injected, as is done for producing complement-fixation antigens, the incubation period is short, i.e., 16-40 hours, and the horses die in 3 to 7 days. The clinical manifestations are numerous and varied. The most commonly observed clinical signs are inappetence, constipation, colic, pneumonia, encephalitis, and signs referable to the urinary system. The body temperature becomes very high, especially on the day the horse becomes moribund. When a relatively small number of parasitized RBC (\(10^4\) or fewer) is given, the incubation period is prolonged to 10-28 days with only slight loss of appetite, only slightly elevated (101-102 F.) body temperature, and extremely low parasitemia that lasts for only 3 to 10 days. Almost all horses in this group recover but remain carriers for varying periods of time. When a moderate
number of parasitized RBC's (approximately $10^6$) is given, the incubation period is usually 3 to 10 days, and the horses do not appear to be very sick until the 8th to 10th day after parasites first appear. At this time, antibodies detectable by the complement-fixation test first appear, and the horse undergoes a hypersensitivity reaction, accompanied by the clinical syndrome usually described for equine piroplasmosis—anemia, icterus, edema and lethargy. Most horses in this group live, but the recovery period is prolonged.

The aromatic diamines, such as phenamidine and Diampron, are very effective in reducing the severity of clinical signs produced by infection with either parasite. Treatment on two successive days will almost invariably eliminate \( B. \ caballi \) from the infected horse, but this regimen is less efficient in removing \( B. \ equi \), and more extended treatment periods are often required.

The complement-fixation test\(^{(1)} \) has been accepted by the Animal Health Division of the USDA and the State of Florida as an official diagnostic test for equine piroplasmosis. The complement-fixation titers generally last longer than the carrier state, i.e., the ability to transmit infection when blood from the carrier horses is injected into susceptible horses, disappears before the complement-fixation titers do.

\( Babesia \ equi \) develops from an anaplasmoid body to a spherical (signet ring) form much like the analogous stages of \( B. \ caballi \). Subsequently, this spherical form may develop in either of two ways. The chromatin material either organizes into 4 or 5 masses at the periphery of the sphere, and these chromatin masses form the nucleu of 4 or 5 piriform bodies, or the spherical body first divides into two spheres, each of which then divides into two small piriform bodies. The development of \( B. \ equi \) in ticks is not known at present because the tick vectors of \( B. \ equi \), at least in the U.S., are unknown.

The development of \( B. \ caballi \) in the RBC of the horse\(^{(2)} \) and in the tropical horse tick, \( D. \ nitens \)(\(^{(3)} \)), was followed by studying fixed preparations stained with Giemsa's or acridine orange, as well as by using phase contrast microscopy to study preparations containing living parasites. Within the erythrocyte, the parasite develops from a small anaplasmoid body, consisting mostly of nuclear material, into a larger amoeboid sphere which divides into two large piriform bodies by a process resembling budding. Two nuclei develop in each of the piriform bodies. Usually one nucleus is extruded from the blunt end as an anaplasmoid body. Most of the parasites within the RBC ingested by the tick apparently are destroyed. However, some of them survive and form small round bodies floating free within the gut contents of the tick. These spherical bodies give rise to large clavate (club-shaped) bodies which penetrate the large epithelial cells of the tick gut. In these cells, the clavate bodies form many vermicular bodies by a process of multiple fission. These vermicules are able to infect other tissues of the tick where they undergo a secondary cycle of multiple fission producing more vermicules. Vermicules are produced in all tissues of the female tick, its eggs, and the larvae, nymphs and adults of the next tick generation, except for salivary glands. In the salivary glands of the larvae, nymphs and adults of the next generation, small piriform bodies somewhat larger than the piriform bodies found in the equine erythrocyte are produced. These piriform bodies in the salivary glands are infective for the horse, and are able to enter the blood stream of the horse while the larvae, nymphs and
adults of the tick are feeding, thus starting the cycle anew.

REFERENCES

REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES


Charged with certain responsibilities in the fields of equine infectious anemia, equine piroplasmosis, African horse sickness, and equine respiratory diseases, as outlined in last year's report, the Committee on Infectious Diseases of Horses has striven to meet these goals. While there are areas in which the Committee has not completed its work, it must be recognized that in certain of these fields of endeavor the magnitude of the project has not permitted our doing so. However, initial efforts have been instituted in each of these areas and, if continued in orderly progression as contemplated, is expected to culminate in the development of uniform standards and guidelines of sound scientific value in the future handling of the aforementioned diseases.

EQUINE INFECTIOUS ANEMIA

Equine infectious anemia is recognized by horse owners, trainers, and veterinarians throughout the nation as a most devastating and costly disease to our horse industry. During fiscal year 1968, 452 cases occurring in 306 herds were reported in the United States. (See map attached.) Considerable effort has been expended this past year in the investigation and control of equine infectious anemia; however, a dependable diagnostic test other than horse inoculation has not been developed, thus limiting regulatory actions that can be taken against the disease.

Although at least nine research institutions throughout the country are directing efforts toward the development of a practical definitive diagnostic tool, a break-through has not as yet been realized therein. In the area of promising developments in this direction are studies being conducted in connection with the viral inhibition test, and the standard serological test to soluble antigen.

The 1967 report of this Committee included amendments to the Prospectus on Equine Infectious Anemia, and in this connection your Committee this year submits the following observations and amendments:

1. Under the first section of the Prospectus, the definition of an Infected (positive) horse should be amplified by adding the following verbage: "In the light of present knowledge, once a horse has been determined as positive through animal
REPORT OF COMMITTEE

inoculation procedures, it should continue to be so regarded and not retested. Although upon subsequent inoculation tests the titer of the virus might be so low as to be undetectable, such condition would have to be considered transitory since the animal in question remains a carrier.”

2. As presently written, the Prospectus requires the challenge of a test animal if negative at the end of 60 days. In discussions by the Committee, it developed that a better procedure than challenging is the euthanasia of such a negative animal following this observation period, utilizing histopathic evidence for completion of the picture. It is noted that even the mildest long term cases display more histopathology than acute cases, and generally such histopathic evidence supports the clinical evaluation. For this reason an amendment to this effect is herein recommended.

In addition to the development of a practical diagnostic tool, research continues in the following areas:

1. Perfection of methods of separation and purification of the causative virus and study of the immune response in chronically affected and/or carrier horses.

2. Determination of the role of prenatally infected foals in the expression of clinically typical equine infectious anemia.

3. Studies to determine the modes of transmission, pathogenesis, and chemotherapy and/or other methods for the control or eradication of equine infectious anemia.

EQUINE PIROPLASMOSIS

Recent evidence shows that equine piroplasmosis caused by Babesia caballi is endemic in Puerto Rico, an item of great importance due to increased trafficking of equidae between that country and the United States. Among eight horses proposed for shipment to Florida, seven reacted positively to the complement fixation test for equine piroplasmosis and were considered affected with the disease. In further Puerto Rican studies, in a group of 74 civilian owned horses at Fort Buchanan, 59 were positive to the complement fixation test, and at Roosevelt Roads Naval Station, 82 civilian owned horses of 84 bled were positive to the test. A horse recently imported from Puerto Rico to New Jersey has been found to have a positive reaction to the complement fixation test. In summary, during fiscal year 1968, among 281 equidae tested in Puerto Rico, 209 were found to be affected with Babesia caballi by the CF test.

Of 875 horses tested in South Florida by the complement fixation method, 90 were found to have had experience with Babesia caballi and two with Babesia equi.

From the foregoing it may be seen that the CF method of detection of equine piroplasmosis has increased in use and shows great promise of being a reliable diagnostic aid. As such, your Committee, recognizing the need for an officially acceptable diagnostic method, herein recommends that the Conference of Veterinary Laboratory Diagnosticians be requested to evaluate the efficacy of this test method for the detection of equine piroplasmosis.

As proposed in last year’s report, this Committee has prepared and submits herewith for adoption “A PROSPECTUS ON EQUINE PIROPLASMOSIS WITH GUIDELINES.”
AFRICAN HORSE SICKNESS

Much deliberation was afforded to the subject of African Horse Sickness in our Committee meetings this year, with particular reference to safe and acceptable methods of importing horses from foreign countries. The Committee feels that the present criteria upon which horses are accepted into the United States leave much to be desired and further “in depth” studies are being instituted in developing necessary precautionary procedures on future importations so as to preclude African horse sickness gaining a foothold within the United States. Such procedures will be included in the Prospectus on African horse sickness, now about 90% complete, to be considered for adoption at next year’s meeting of this group.

EQUINE RESPIRATORY DISEASES

The development of a Prospectus on Equine Respiratory Diseases as viewed by our Committee is a monumental undertaking. However, it is expected that work will be initiated by Committee members and experts in this field during the forthcoming year. It is anticipated that worthwhile information should be ready in embryonic form for Committee consideration during the meeting next year and that a completed Prospectus with Guidelines will be presented for your consideration two years hence.

IMPORT-EXPORT TASK FORCE

During the latter part of 1967, an intra-departmental study group of the United States Department of Agriculture proposed regulations relating to the importation of various species of animals and animal products into the United States. Included therein were recommendations involving horses and other equidae. The Committee on Infectious Diseases of Horses was asked at this meeting to review the proposals of this group and to comment on these recommendations. However, it was the consensus of the Committee that there were such wide ramifications in the report as to require more serious study than time afforded at this meeting, and they are now being studied by the Committee members and appropriate action will be taken as early as is possible.

Respectfully submitted,

C. L. CAMPBELL, Chairman
REPORTS OF INCIDENCE OF EQUINE INFECTION ANEMIA
IN THE UNITED STATES

FISCAL YEAR 1968

H-HERDS
C-CASES

TOTAL:
HERDS = 306
CASES = 452

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
A PROSPECTUS ON
EQUINE PIROPLASMOSIS
WITH GUIDELINES

1. DEFINITIONS FOR THE PURPOSE OF THIS REPORT.

A. *Equine Piroplasmosis*. An infectious hemoprotozan disease of solipeds characterized by fever, anemia, icterus, and other clinical signs arising from hemolytic anemia caused by babesia organisms (*Babesia caballi* or *Babesia equi*).

B. *State Veterinarian*. The chief livestock regulatory official—usually an employee of the State Department of Agriculture or State Livestock Sanitary Board and responsible for the control and eradication of animal disease within his state.

C. *Commission Veterinarian*. One employed by the State Racing Commission or Board for the purpose of advising and assisting with veterinary matters pertaining to the Commission and to direct the veterinary functions and activities of the Commission.

D. *Advisory Committee*. A group of consultants composed of practicing, regulatory, or other veterinarians who may be appointed by the State Veterinarian to assist him in an advisory capacity in dealing with equine piroplasmosis.

E. *Acceptable Tests for Diagnosis.*

1. **Direct Blood Smear.** Microscopic identification of babesia organisms by stained whole blood smear. (Organisms are usually only demonstrable for 1-3 days during the febrile stage.)

2. **Serological Test.**

   a. **Complement-fixation test.** Serological determination of chronic cases. (A titer of above 2+ in a 1:5 dilution is considered positive in the CF test.)

   b. **Subinoculation test.** Inoculation of 500 cc. of fresh whole blood (*properly collected in an anti-coagulant bottle*) into a splenectomized horse or pony or two intact horses or ponies. (Blood smears and temperatures accomplished daily on test horses. If negative for a period of 45 days, the test animal will be challenged with known infected blood to determine its susceptibility.)

F. *Equine or Horse*. When referred to in this prospectus, may be any member of the family of horses, mules, donkeys, asses, or zebras.

G. *Infected (Positive) Horses*. A horse that has a positive blood smear or reacts positively on *serological test* (CF or other acceptable test) for equine piroplasmosis or will infect test horses with equine piroplasmosis.

H. *Suspect horse*. One showing clinical signs or a reaction on a serological test
lower than that considered to be a reactor located on a premise where equine piroplasmosis has been diagnosed.

I. Adequate Vector Control. All equines or other animals located on premise that may be infested with *Dermacentor nitens* are properly inspected and the false nostrils, tail, and ears are treated with 1% lindane in cottonseed oil and the entire animal is then sprayed thoroughly with 0.5% toxaphene solution. This procedure is repeated approximately every 21 days until all animals are free of the *Dermacentor nitens* for a period of six months.

J. Drugs or Treatment for Equine Piroplasmosis:

Treatment for *Babesia caballi* (in order of drug preference):
- Diampron 5 mg/lb intramuscular for 2 consecutive days
- Berenil 5 mg/kg intramuscular for 3 consecutive days
- Phenamidine 4 mg/lb intramuscular for 2 consecutive days

Treatment for *Babesia equi*:
- Diampron 5 mg/lb intramuscular in divided doses for 6 consecutive days
- Phenamidine 4 mg/lb intramuscular in divided doses for 6 consecutive days

The needle should be left in after injecting the above amounts and then saline or dextrose solution should be flushed into the needle to prevent irritation when withdrawing it.

K. Means of Identifying Infected or Positive Horses.

Equines in this category should be permanently identified with a lip tattoo placed in the upper lip. This tattoo should consist of the last numeral of the year, followed by the letter "P", and then with the numerical number of the respective case which this animal represents, such as 8P1 or 8P10, etc. The code number of the respective state could be placed directly under the above tattoo to identify the state.

2. INFORMATION ACCEPTED AS FACT PERTAINING TO EQUINE PIROPLASMOSIS.

A. Responsibility for Control.

(1) The individual State Department of Agriculture or Livestock Sanitary Board is legally responsible for the control of equine piroplasmosis within their respective state.

(2) The U. S. Department of Agriculture has certain regulatory jurisdiction over horses entering the United States or known affected animals being moved interstate. Subchapter C, Part 71.3, of the Code of Federal Regulations, prohibits the interstate movement of any equine known to be affected with equine piroplasmosis.

(3) Individual states may enter into a cooperative program with USDA, ARS, Animal Health Division, to sustain an endemic outbreak and to eradicate the disease.

B. Health Certificates.
A health certificate is current and valid only at the time a horse is physically examined and the certificate issued.

Health certificates for interstate shipment on horses originating from an area where equine piroplasmosis is endemic (quarantined areas where the disease exists) should have negative results from an acceptable serological test for equine piroplasmosis.

The health certificate will contain certification stating that the horse is free of ticks and has been treated with an approved tickicide.

C. The Protozoa.

1. Babesia caballi is principally vectored by ticks from the carrier horse to a susceptible one in the United States. It has been proven that the Tropical Horse Tick, Dermacentor nitens, is a vector of this disease in South Florida. It is also possible that other blood-sucking insects might also be vectors.

2. It is not known what species of ticks found in the United States can naturally transmit Babesia equi.

3. Babesia caballi and Babesia equi can be transmitted from a carrier horse to a susceptible one by direct transfer of whole blood.

D. The Disease.

1. Equine piroplasmosis has been diagnosed in Florida, Georgia, and Puerto Rico, but should be suspected in other areas of the country where the Tropical Horse Tick is known to exist or where affected equines may have been transported.

2. Clinically it resembles other diseases and it is impossible to make a definite diagnosis without laboratory tests.

E. Spread of the Disease.

1. Infected horses may remain carriers for several years. (The original case in the United States was a carrier for four years, and may have remained longer had he not been treated.)

2. Tropical Horse Ticks fed on infected horses remain infective down through the fourth generation by trans-ovarian transfer. (The Tropical Horse Tick is known to survive for a period of eight to nine months without feeding.)

3. The direct transfer of infected blood, as in blood transfusions, would be the only other known method of spread in the absence of tick vectors.

4. The dual presence of both the vector and the carrier would be paramount in the spread of this disease.

F. Control Measures.

1. Control depends upon identification of infected or carrier horses and the treatment with approved babesicidal drugs (as recommended in 1. J.) to eliminate the carrier status. Premises on which infected or carrier animals are located should be placed under state quarantine. Procedures for release from this quarantine are listed below in 3. D., page 8. Endemic areas should be identified and so designated with
regulations to be applied for such endemic areas.

(2) Control of known tick vector. (The eradication of the carrier status in infected horses by drug therapy in conjunction with vector control as recommended in Section I. page 3, would lead to the eventual elimination of the disease. Vector control without identification and treatment of carriers would not be a good control measure, and conversely, identification and treatment of carriers without vector control would be an equally poor situation since the treated carriers would be susceptible to reinfection.)

3. **PROCEDURE FOR THE UNCONDITIONAL RELEASE FROM QUARANTINE OF EQUINES KNOWN TO BE INFECTED WITH BABESIA CABALLI AND ALSO THE PREMISE ON WHICH THE ANIMAL IS LOCATED.**

A. All positive (CF and blood smear) equine should be tick-free and all equine on premise should be under adequate vector control and subjected to CF test for equine piroplasmosis.

B. All positive equine will be properly identified and treated with 5 mg/lb body weight intramuscularly for two consecutive days with Diampron (Amicarbalide Isethionate) 500 mg. per 1 ml or other recommended babesicidal drug.

C. Equine that have met the above requirements may be considered for release from individual quarantine 45 days thereafter, *if recommended by the appropriate state livestock sanitary official, and the cooperating Animal Health Division Veterinarian in Charge.*

D. Premises on which a positive equine was located, and the procedures above have been followed, will remain under quarantine until all equine on the premise have been free of *Dermacentor nitens* for a period of six months. During this period all equine are to be inspected and sprayed (0.5% toxaphene) and the false nostrils, tail, and ears (1% lindane) hand-treated every 21 days. Test horses should be used at the end of six months to determine if premise is free of *D. nitens.*
4. **THE FOLLOWING ARE METHODS THAT MAY BE CONSIDERED IN HANDLING EQUINES AND PREMISES RELATIVE TO EQUINE PIROPLASMOSIS (B. CABALLI).**

<table>
<thead>
<tr>
<th>Situation</th>
<th>Steps to Release Quarantined Herds or Premises</th>
<th>Steps to Qualify Movements</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) No ticks</td>
<td>No restrictions</td>
<td>No restrictions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) EP affected and/or CF positive animals but no <em>D. nitens</em> found.</td>
<td>Give precautionary treatment for ticks and after two clean tick inspections at least 14 days apart. All serologically (CF) <em>B. caballi</em> positive animals to be treated* with Diampron (5 mg/lb body weight) for two consecutive days.</td>
<td>From premises:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-Inspected Tick-free and precautionary tickicidal treatment. and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*It may be necessary to immediately treat acute cases.</td>
<td>CF-Complement-Fixation negative (a CF negative test is not valid unless blood sample taken at least 42 days since most recent tick infestation on premise). To avoid delay, exposed CF negative animals can be released if treated in the same manner as CF positive animals.</td>
</tr>
</tbody>
</table>
or

CF positive, tick-free and maintained in tick-free environment and treat with 5 mg/lb body weight of Diampron for two days and hold for 72 hours before moving.

and

CE-Clinical examination reveals no evidence of EP.

S-CF positive horses can be moved intrastate directly to slaughter (movement and slaughter controlled) without chemotherapy. They will have to qualify the same as other horses insofar as ticks are concerned (T).

To premises:

T-(same as above)

and

Negative to CF tests

(C) *D. nitens* infected but no affected and no CF positive animals.

1. All equidae CF test negative.

From premises:

T-(same as above)

and

CF-(same as above)

and

CE-(same as above)

and

2. Maintain herd tick-free for six months and inspect and spray (0.5 toxaphene) (and hand treat false nostrils and ears (1% lindane) every 21 days.
3. A resident horse can be used as a tick-free test candidate (inspected and found tick-free test 21 days after last tickicidal treatment). If test horse remains D. nitens-free for 30 days, premises quarantine released.

4. Complement-fixation retest all animals in herd immediately prior to release of premises quarantine.

1. After two clean tick inspections at least 14 days apart.* All serologically (CF) positive animals to be treated with 5 mg./lb body wt. of Diampron for two consecutive days.

2. Maintain herd tick-free for six months. Inspect and spray (0.5 toxaphene and hand treat false nostrils and ears with 1% lindane) every 21 days.

3. A resident horse can be used as a tick-free test candidate (inspected and found tick-free) and/or CF positive and D. nitens infected.

S-(same as above)

To premises:

T-(same as above)

and

CF-(same as above)

(D) EP affected and/or CF positive and D. nitens infected.

QUARANTINE-Equidae and premises
free at least 21 days after last tickicidal treatment). If test horse remains *D. nitens* free for 30 days premises quarantine released.

4. Complement-fixation retest all animals in herd immediately prior to release of premises quarantined.

*It may be necessary to immediately treat acute cases.*

5. **PROCEDURES PERTAINING TO RELEASE OF QUARANTINE OF EQUINES POSITIVE ON SEROLOGICAL TEST FOR B. EQUI SHOULD BE OBTAINED FROM THE EQUINE DISEASE STAFF, ANH, HYATTSVILLE, MARYLAND.**

6. **AREAS OF RESPONSIBILITIES AND FUNCTIONS.**

A. Responsibility for a central headquarters office for the purpose of
   (1) collecting and disseminating all pertinent information, and
   (2) coordinating and analyzing all test and control procedures on a nationwide basis has been assigned to:
   Equine Diseases Staff
   Animal Health Division
   Agricultural Research Service
   U. S. Department of Agriculture
   Hyattsville, Maryland 20782
   Headquarters Office Telephone,
   Area Code 202, 388-8628

B. State Veterinarian or Chief State Livestock Regulatory Official (State Department of Agriculture or Livestock Sanitary Board)
   (1) Responsible for the control of contagious diseases of livestock within his state including disease among horses at race tracks.
   (2) May appoint an "Advisory Committee" from practicing or other regulatory veterinarians including the Commission Veterinarian to assist him in an advisory capacity in case of a suspected outbreak of equine piroplasmosis at any race track.
   (3) Positive horses should be removed from race tracks and placed under quarantine on another premise and placed under adequate vector control until such time as the infected horse can be declared not infected.
All other exposed equines should be checked for possible vectors, with repeated clinical examinations followed by serological test for equine piroplasmosis.

(4) Will be responsible for adequate permanent identification of positive horses (lip tattoo or brand).

(5) Will be responsible for conducting babesicide treatment, animal inoculation, and/or serology to determine carrier status after use of babesicidal drugs.

(6) Will report the results of each test on each horse to the USDA Central Office.

(7) Will maintain a list of all infected horses.

(8) Will declare equine piroplasmosis as a required reportable disease in states where such is not already the case so that all cases will be reported and recorded in each state.

C. State Racing Commissions and Commission Veterinarians

(1) Should see that adequate vector control as presented by the State Veterinarian is enforced at race tracks.

(2) Should insure that tattoo instruments and saliva collecting equipment are adequately sterilized under the supervision of the Commission Veterinarian (autoclave at least 15 minutes at 15 pounds or thoroughly wash clean and boil for at least 15 minutes prior to being used on any horse).

(3) Should enforce rules preventing the use of hypodermic syringes and needles on horses by other than veterinarians licensed to practice at the track.

(4) Should require provision and operation of adequate isolation facilities acceptable to the state veterinarian.

(5) The Commission Veterinarian should serve on the State Veterinarian’s advisory committee and will promptly report all cases of reportable disease suspected at the tracks.

(6) Should assist and support an accelerated national research program to combat equine piroplasmosis.

D. Race Track Management

(1) Should institute and carry out at all times adequate vector control measures.

(2) Should provide and maintain screened isolation facilities adequate to meet the needs of and be acceptable to the state veterinarian.

E. Owners, Breeders and Trainers

(1) Should realize that perfectly normal appearing healthy horses can be carriers for years without suspicion.

F. Practicing Veterinarians

(1) Will immediately report any horse suspected of being infected with equine piroplasmosis located anywhere within the state to the state veterinarian. If the horse is stabled at a race track, he will simultaneously report it to the Commission Veterinarian.
(2) Should continue the practice of using disposable hypodermic needles and syringes (one needle - one horse).
(3) Offsize needles and other surgical and medical equipment which must be reused should be sterilized either by thorough cleaning and boiling for at least 15 minutes or autoclaved at least 15 minutes at 15 pounds.

7. PROCEDURE IN EVENT OF SUSPECTED OUTBREAK:

A. Any practicing or other veterinarian suspecting a horse at a track of being affected with equine piroplasmosis because of either clinical signs or tests will promptly report the case to:
   (1) the State Veterinarian; and
   (2) the Commission Veterinarian.

B. The State Veterinarian and his advisory committee will then determine which horses will be subjected to further testing. Contributing factors, as well as the amount of testing required, may vary from track to track; however, such determinations should be so practicably encompassing as to afford the necessary protection to the health of other horses stabled there.

C. Horses suspected of being infected because of clinical signs will be moved into isolation until such time as further laboratory tests determine the status.

D. A horse which has been proven positive shall remain quarantined until released by the state veterinarian.

8. REGULATORY AND RESEARCH OBJECTIVES.

A. To establish the incidence of equine piroplasmosis in the United States.

B. To further knowledge of vector transmission in order to determine if vectors other than *D. nitens* are involved.

C. To identify the principal vector of *B. equi*.

D. Continue to evaluate any additional tests for their diagnostic capability.
REPORT - PHARMACEUTICAL COMMITTEE


Believing that a regularly revised "Compendium on Veterinary Drugs" would provide information necessary to the veterinary profession for proper drug usage which would assist in improved animal health, this committee reaffirms its earlier recommendations through the USLSA to the AVMA and the Bureau of Veterinary Medicine, F&DA that immediate action be taken to provide such a compendium.

Following the careful studies made by the National Academy of Science under contract with and recommendations to the Bureau of Veterinary Medicine, most drug labels will, in the near future, be revised to more nearly indicate safe, effective dosages and indications. Equally important will be emphasis on proper withholding claims to assure lack of drug residues in meat, milk, and eggs.

Those in the veterinary profession responsible for recommendations on the prevention, control and treatment of disease by biologicals and/or drugs should be aware of the best agent(s) available, their proper usage, singly or in combination and equally important whether residues may persist. Frequently, biologicals have contained antibiotics in their processing. Are they present in the product as administered? Do residues persist in the vaccinated animal? Are they hazardous to the species of animal being treated? to the ultimate consumer of the meat, milk, or eggs?

Scientists in the USDA, ARS and Bureau of Veterinary Medicine, F&DA have been surveying food products for the presence of antibiotic and food residues. Perhaps other naturally occurring feed contaminants consumed by the animals pose an equally important residue problem - for example, the mycotoxins. These agents have been demonstrated to cause abortion in animals, cause stunting, liver dysfunction and death. These toxins or their metabolites have been demonstrated in the milk of animals consuming contaminated feeds.

It is suggested that many of the older effective drugs now successfully used by the profession may result in residue problems and contamination of our food supplies. Therefore, it is recommended that funds should be provided to the Bureau of Veterinary Medicine to either establish contracts with Universities or private industry or for facilities, staff, and operational expenses to determine proper withdrawal periods for these agents. Priority for evaluation of these agents would be based on potential hazard and how extensively the agents are used.

Both industry and government are to be commended for their continuing efforts to acquaint the profession, producers, and public with the importance of...
proper drug usage, careful observance of approved label indications, contraindications, and withdrawal periods. Proper recognition of the importance of adverse drug reports by producers, industry, and governmental agencies is to the mutual benefit of all. Although the drug has been carefully evaluated on safety and effectiveness by the manufacturer, it is possible that not all conditions or multiple disease states could be anticipated. The manufacturer can adjust or modify the label recommendations to prevent further recurrence of the adverse reaction.

It should be stressed that proper drug usage entails a careful review of the case history including what drugs may have been used previously, insecticides as well. Prior treatments may have altered certain body enzyme systems, which, along with the disease process, may predispose to increased hazard of drug therapy even when used at the listed dosage.

Finally, may we reiterate the importance of expansion of the opportunities for graduate research training programs in pharmacology and toxicology to provide qualified, competent staffs for our colleges, industries, and governmental agencies.
EPIZOOTIOLOGIC PATTERN OF FOWL CHOLERA IN TURKEYS IN MISSOURI

L. D. Olson, E. L. McCune and R. E. Bond*
Columbia, Missouri

During the past 10 years fowl cholera in turkeys has been on a steady increase in the United States. This is a presentation of some observations on the pattern of the disease in Missouri.

EPIZOOTIOLOGIC OBSERVATIONS

Each year since 1959 there has been an increase in the number of turkeys involved in outbreaks of fowl cholera in Missouri (Table 1). From the same table it can also be inferred that the value of fowl cholera bacterins is questionable. The counties in Missouri with the highest number of outbreaks of cholera were those with the highest number of turkeys (Fig. 1). So far in 1968 there has been a total of 96 outbreaks of the disease, and yet the turkey population is down 20% from the record of 11.5 turkeys raised in the state in 1967.

Taking into consideration the fact that many outbreaks are diagnosed in the field or by several commercial poultry diagnostic establishments in Missouri, and consequently not reported to our laboratory, it is estimated that over 1 million turkeys were exposed to the disease in Missouri in 1968. Even with moderately effective antibiotic treatment, it is estimated that between 5 and 10 percent died. Then adding to this, the cost of vaccination for cholera which is approximately 10 cents per bird, it is not difficult to realize that this disease is costing the turkey growers of Missouri over a million dollars a year.

Fowl cholera is primarily a problem during the months of July through October (Fig. 2). This is at a time when turkeys are approaching market age and the grower is reaching his maximum investment in terms of labor and feed.

The average age of the turkey infected for the period 1959-66 was 20 weeks (Fig. 2). For 1967 it was 18.2 weeks, and for this year it is 16.3 weeks. Outbreaks in Missouri have been reported in turkeys as young as 3 weeks old.

Many of the epizootics in Missouri are of a chronic nature with the disease smoldering over a period of weeks. Once a flock is infected, it is thought the causative organism, Pasteurella multocida, is spread from turkey to turkey by pecking the infected mucous discharge from the mouths of the sick birds. Some isolates of P. multocida appear to have a predisposition for the air spaces in the head, whereas others seem to localize in the lungs.

During the past 2 summers it has been observed that the number of cases of fowl cholera in turkeys diagnosed at both the laboratory at Columbia and at Springfield markedly decreased following a decrease in atmospheric temperature (Fig. 3). This was particularly true in August 1968.

*Department of Veterinary Pathology (Olson and Bond) and Veterinary Microbiology (McCune), School of Veterinary Medicine, University of Missouri, Columbia, Missouri.
SUMMARY

Fowl cholera has become a disease of major importance to the turkey industry of Missouri. The value of bacterins in the prevention and control of the disease is questionable. During the last several years the average age of the infected flocks appears to be lowering. It has been observed during the past 2 summers that when the atmospheric temperature lowers, the incidence of cholera also decreases.

Figure 1:
FOWL CHOLERA CASES PER COUNTY FOR PERIOD 1959 - 1968
Table 1--Cases of Fowl Cholera Diagnosed in Turkeys at the School of Veterinary Medicine, University of Missouri, Columbia, Missouri

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fowl Cholera Cases</td>
<td>11</td>
<td>17</td>
<td>10</td>
<td>15</td>
<td>16</td>
<td>9</td>
<td>17</td>
<td>21</td>
<td>79</td>
<td>96</td>
</tr>
<tr>
<td>Number Birds Involved</td>
<td>44,000</td>
<td>48,800</td>
<td>37,200</td>
<td>56,875</td>
<td>79,900</td>
<td>42,400</td>
<td>112,300</td>
<td>139,395</td>
<td>602,233</td>
<td>884,289</td>
</tr>
<tr>
<td>Number Cases Vaccinated</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>Number Cases Not Vaccin.</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>16</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Number Cases Vac. Unk.</td>
<td>11</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>25</td>
<td>35</td>
</tr>
</tbody>
</table>

*Includes cases from State Diagnostic Laboratory, Springfield, Missouri
Fig. 2: NUMBER OF FOWL CHOLERA CASES PER MONTH FOR LAST 8 YEARS

- 1960-66 (avg. age: 20 wks)
- 1967 (avg. age: 18.2 wks)
- 1968* (avg. age: 16.3 wks)

*to October 24, 1968
Fig. 3: RELATIONSHIP OF ATMOSPHERIC TEMPERATURE TO THE INCIDENCE OF FOWL CHOLERA IN TURKEYS

1967

Avg. Weekly Temp.

No. of Cases of Fowl Cholera per Week

1968

Avg. Weekly Temp.

No. of Cases of Fowl Cholera per Week

May Jun Jul Aug Sep
SURVIVAL OF PASTEURELLA MULTOCIDA
IN SOIL, WATER, CARCASSES, AND IN THE MOUTHS OF
VARIOUS BIRDS AND MAMMALS

Leroy D. Olson and Ralph E. Bond*
Columbia, Missouri

*Pasteurella multocida can be isolated periodically from the palatal cleft of
chickens that have survived an acute outbreak. In some instances the source of
infection may be traced to birds that have survived an outbreak but are still
maintained on the premises as breeding stock. Yet, there are many outbreaks of
fowl cholera in turkeys on range where there is no indication as to the source of
infection and where the grower has depopulated the area of turkeys for one or two
years. The purpose of this study was to determine how long several strains of P.
multocida from turkeys would survive in soil, water, the carcasses of turkeys that
died of the disease, and in the mouths of various birds and mammals.

EXPERIMENTAL METHODS AND RESULTS

Isolates of Pasteurella multocida Used: Five isolates of P. multocida from
turkeys and one from chickens were used for this study. Isolate M8579 had a
predisposition for localizing in the air spaces of the head. Isolate M8754 was
obtained from an outbreak where septicemia was the primary lesion. Isolate M8954
was a relatively non-pathogenic organism. A pathogenic isolate from chickens,
X-73**, was used for the experiment on survival in water. Two additional isolates,
P-1089** and P-1340**, which were from turkeys, were used for the experiment on
survival in the mouth.

Bacteriologic Isolation: Swabs from the specimens tested were streaked on
dextrose starch agar***. Colonies of P. multocida were isolated by observing the
culture plate through a stereo-microscope with an obliquely transmitted light.
Smears of the microorganism from the cultural growth were stained with Gram’s
stain. Certain carbohydrates were used for biochemical identification.

Soil: Each of the three types of soil contained in 100 ml specimen jars were
inoculated with the 3 isolates of P. multocida and incubated at 4°C and 26°C
(Table 1). The soil had been previously sterilized by repeated autoclaving. The
survival time ranged from 6 to 21 days with no particular preference to soil type,
isoalte of P. multocida, or temperature.

Water: Ponds have been incriminated as a source of infections. The drinking
water from a group of ducks that had been experimentally infected orally with
X-73 was given to 8 three-week-old turkey poults. Pasteurella multocida had been
isolated from the water. None of the poults died, nor did any exhibit clinical signs
of the disease.

Carcasses: The carcasses from 30 sixteen-week-old turkeys that had died of
experimental fowl cholera were used for the study. All of the turkeys had been
inoculated via the eustacian tube, with 15 receiving M8579 and 15 receiving
M8954. The carcasses were placed in a shelter covered with hardware cloth on
December 1, 1967. Thereafter, one turkey from each isolate was removed each
SURVIVAL OF PASTEURELLA MULTOCIDA

week and the liver and air spaces of the head were cultured for *P. multocida*. Neither isolate could be obtained from the carcasses after 60 days.

Mouths of Birds and Mammals: Wild birds have been incriminated as carriers of *P. multocida*. Dogs frequently molest turkey flocks and have been suspected of carrying the organism in their mouths. Various birds and mammals were inoculated orally with differing amounts of the organism to determine how long the organism would survive in the mouths of those that lived (Table 2). As can be seen, the organism did not survive long in the mouths of any of the inoculated birds and mammals.

**SUMMARY**

The reservoir of infection in the instances where a farm has been completely depopulated of turkeys for one or two years has not been elucidated. *Pasteurella multocida* appears not to survive long in soil, water, or in the mouths of various birds and mammals. From the findings of this study the role of infected carcasses as a reservoir is questionable; however, removal of carcasses from ranges and the surrounding trees should still be practiced.

**REFERENCES**


*Department of Veterinary Pathology, School of Veterinary Medicine, University of Missouri, Columbia, Missouri.

**Compliments of Kenneth L. Heddleston, National Animal Disease Laboratory, Ames, Iowa.

***Difco Laboratories, Detroit, Michigan*

**TABLE 1**

SURVIVAL TIME (DAYS) OF *PASTEURELLA MULTOCIDA* IN SOIL

<table>
<thead>
<tr>
<th>ISOLATE NO.</th>
<th>CLAY</th>
<th>SOIL TYPE</th>
<th>SAND</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8579</td>
<td>40°C</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>26°C</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>M8754</td>
<td>40°C</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>26°C</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>M8954</td>
<td>40°C</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>26°C</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>SPECIES</td>
<td>ISOLATE AND NO. BACTERIA ADM.</td>
<td>NO. LIVED</td>
<td>REISOLATION FROM MOUTH</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------</td>
<td>-----------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Ducks</td>
<td>P-1340; 8x10^8</td>
<td>4</td>
<td>Not Reisolated</td>
</tr>
<tr>
<td>Pigeons</td>
<td>M-8954; 3x10^8</td>
<td>1</td>
<td>Not Reisolated</td>
</tr>
<tr>
<td></td>
<td>M-8954; 3x10^8</td>
<td>1</td>
<td>Not Reisolated</td>
</tr>
<tr>
<td>Japanese Quail</td>
<td>P-1059; 1x10^3</td>
<td>9</td>
<td>Reisolated for 1 wk.</td>
</tr>
<tr>
<td>Starling</td>
<td>M-8954; 1x10^7</td>
<td>1</td>
<td>Not Reisolated</td>
</tr>
<tr>
<td>Mice</td>
<td>M-8579; 1x10^7</td>
<td>2</td>
<td>Reisolated for 2 wks.</td>
</tr>
<tr>
<td></td>
<td>M-8954; 1x10^7</td>
<td>1</td>
<td>Reisolated for 2 wks.</td>
</tr>
<tr>
<td>Rats</td>
<td>M-8579; 1x10^7</td>
<td>2</td>
<td>Reisolated for 1 wk.</td>
</tr>
<tr>
<td></td>
<td>M-8954; 1x10^7</td>
<td>2</td>
<td>Reisolated for 1 wk.</td>
</tr>
<tr>
<td>Dog</td>
<td>M-8579; 3x10^8</td>
<td>1</td>
<td>Not Reisolated</td>
</tr>
<tr>
<td></td>
<td>M-8754; 7x10^8</td>
<td>1</td>
<td>Not Reisolated</td>
</tr>
<tr>
<td></td>
<td>M-8954; 3x10^8</td>
<td>1</td>
<td>Not Reisolated</td>
</tr>
</tbody>
</table>

TABLE 2

SURVIVAL OF PASTEURELLA MULTOCIDA IN MOUTHS OF VARIOUS BIRDS AND MAMMALS
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY


The Transmissible Diseases of Poultry Committee after due review of several of the serious disease problems wish to submit the following report:

I. Avian Leukosis Complex

The Avian Leukosis Complex, particularly Marek's Disease, has increased to the point where it is a devastating disease problem in poultry. The condemnation rate for avian leukosis complex in young chickens inspected by the U.S. Department of Agriculture indicates a dramatic rise beginning in 1963. Condemnations have increased at federally inspected plants from approximately 10 per 10,000 in 1961 to an increase in excess of 150 per 10,000 carcasses inspected. (see charts)

During fiscal year 1969, the Animal Health Division and the Animal Husbandry Research Division are making an attempt to conduct an experimental epidemiological evaluation to determine the pathogenesis and the influence of certain environmental factors upon Marek's Disease.

The committee recommends that funds be made available to the appropriate agencies to expand basic research and epidemiological studies to develop a control program in this highly contagious and important disease.

II. Duck Virus Enteritis (Duck Plague)

The committee commends the New York State Department of Agriculture and Markets, the Long Island Duck Research Laboratory, and the Animal Health Division of the U.S. Department of Agriculture for their activities regarding Duck Virus Enteritis.

The first diagnosis of duck virus enteritis (DVE) in the Western Hemisphere was in a concentrated duck producing area in Eastport, Suffolk County, Long Island, New York. DVE had been frequently recorded in the Netherlands where it first appeared in 1923. It has been suspected in France and China and known to occur in Belgium and India.

The farm on which DVE first appeared was depopulated with indemnity ($58,874.21) and cleaned and disinfected under State-Federal supervision. Suffolk County was placed under Federal quarantine (9 CFR, Part 83) to prevent the inter-state spread of DVE and to facilitate a State-Federal Program for the control and eradication of the disease.
CONDEMNATION RATES FOR AIRSACCULITIS AND LEUKOSIS IN YOUNG CHICKENS INSPECTED BY USDA

*Calculated from SRS POU-2-1
YOUNG CHICKENS INSPECTED AT SLAUGHTER BY USDA*

*From SRS POU-2-1
To determine the incidence of disease among ducks raised in Suffolk County where over 60% of all the ducks in the U. S. are produced, 1700 samples were collected from 13 farms on Long Island for serum neutralization test. To determine if DVE had occurred in other duck populations in the U. S. State-Federal animal health agencies conducted a serological survey among the commercial duck farms of Ohio, Indiana, Illinois, Michigan and California. Also, State-Federal wildlife personnel sampled wild waterfowl, especially along the Atlantic flyway. Sera from more than 4,600 ducks were subjected to the serum neutralization (SN) test at the Plum Island Animal Disease Laboratory (PIADL). During the survey PIADL determined that a virus neutralizing index of log 1.75 or greater indicated that the birds had been exposed to the virus of DVE.


Extent of the Outbreaks – The principal area involved in the outbreaks in the U. S. has been the highly concentrated Pekin duck raising area in Suffolk County, New York. Of the total of 41 farms, 12 have experienced the disease sometime during 1967-68. Even though the term ‘farm’ is used, many of the duck operations in Suffolk County are close together or even side by side and complicate the disease control and eradication picture. In addition to the Pekin ducks on Long Island, DVE was diagnosed in mallard, muscovy, black, bufflehead, and greater scaup ducks and in mute swans in other areas of New York State. DVE has also been diagnosed in wild waterfowl at the Patuxent Wildlife Research Center, Prince Georges County, Maryland, and on a premise at Warrington, Bucks County, Pennsylvania, where semi-wild waterfowl are raised.

General – DVE has been characterized as an acute disease of swans, ducks, and geese; however, chronic or latent infections must be considered as a possibility. The susceptibility of these species to DVE seems to vary with age, management practices, and the presence of concurrent disease agents.

The Agent – The DVE virus has been tentatively classified by PIADL as belonging to the herpes group. The virus was inactivated in 10 minutes at 56°C or 60°C and after 2 hours at 50°C. At 22°C it decayed slowly with total loss of infectivity after 30 days and in 9 days when dried over calcium chloride. The virus was stable at pH 5-9, but it was inactivated instantaneously at pH 3 and 11.

Epizootiology – The major factor of concern in the Control of DVE is that transmission is believed to result principally from ingestion of feed and water contaminated by cloaca discharge. No evidence of egg transmission has been indicated, except one Dutch report that the virus was isolated from an egg found in the cloaca of a duck dead from DVE. Cornell University and Animal Health Division personnel at the Long Island Duck Research Laboratory attempted to isolate the virus from embryos and candled-out eggs without success. There is no report of the virus being transmitted to other than waterfowl; however, in one of the vaccine studies the above personnel were able to detect a mild antibody titer in chickens in contact with ducks vaccinated with modified live DVE virus.

Several theories have been advanced to explain how the virus entered the U.S. The following theories have been examined:

1. Migratory birds - it has been postulated that wild waterfowl from the DVE
endemic area in Europe could have migrated north for the summer and exposed North American waterfowl in the arctic area or could have returned south themselves in the Atlantic Flyway. It is felt, however, that the introduction into the U.S. by this route is improbable.

2. Fomites - It is known that at least one of the duck farms employs Dutch nationals who usually make one or more annual trips to the Netherlands. They could have brought the virus into the U.S. inadvertently on clothing or other items. No active disease has been diagnosed on the farms where these people are employed. Even though fomites are a possibility, it is an unlikely explanation for the introduction of virus. However, it appears that the traffic of men and equipment between two of the Long Island duck farms is the most logical explanation for the spread of DVE in one case.

3. Imported Anseriformes - Several hundred exotic ducks, geese, and swans have been brought from DVE-endemic areas and introduced into Long Island and to other parts of the U.S. in recent years. Most of these birds are pinioned and are allowed to swim in outdoor ponds. These ponds are frequently visited by free-flying waterfowl in search of food. The free-flying waterfowl visit the Pekin duck farms and are a constant nuisance to the duck farmer. Occasionally, mallards mate with Pekin ducks. This method of introduction is more acceptable when the carrier state that has been experimentally produced by PIADL is considered.

Diagnosis - The systems usually appear in 3 to 7 days after exposure. Infected birds become listless, lose their appetite, develop a watery diarrhea with a great thirst. Occasionally droopy wings and swollen eyelids are seen. There is a marked drop in egg production with a slow return in infected breeder ducks. The mortality may reach 60% but is usually much lower. The post mortem lesions most often seen are necrosis of esophagus and cloaca with generalized hemorrhages. Thought to be diagnostic are the hemorrhages in the thymus gland; at the line of demarcation between esophagus and proventriculus; of the lymphoid rings of the small intestine; frequently in the female reproductive tract, and petechiation in the liver with focal necrosis. The spleen is normal or small in size and uniformly dark in color. Confirmation is based on virus isolation and SN test.

Experimental vaccines - At the Long Island Duck Research Laboratory (LIDRL) a modified live virus vaccine is being prepared. The seed culture used for this vaccine is a chicken embryo adapted strain obtained from the Netherlands. It was purified by cloning on tissue cell culture and SN and tested for virulence at PIADL. The vaccine virus harvest time is a minimum of 24 hours post inoculation to a maximum time of 5 days. The harvest materials are pooled into sterile jars, and 0.5 ml. sample of fluid is taken to check for gross contamination. All acceptable pools of membranes, embryos, and fluids, are ground to a fine pulp in a blender and pooled in a sterile carboy. The batches are run between 75,000 and 120,000 ml. Antibiotics are added to the pool as follows: penicillin, 900 units per ml; streptomycin, 500 micrograms per ml. of final product. Sterility tests for Salmonella and Mycoplasma are conducted on the final product by inoculating 1 ml. from each of the containers into thioglycollate, Sabouraud's medium, and brainheart infusion agar. The embryo lethal dose 50 is determined by titrating 1 ml. of each container in chicken embryos.

To date about 10 lots of modified live virus vaccine have been produced
attempting to meet the standards required by the Veterinary Biologics Division. The results appear encouraging at this time.

1. Potency Testing of experimental live vaccine:
   a. Virus content—Titrations were done in 9-11 day old embryos by the allantoic sac technique with appropriate ten-fold dilutions.
   b. Immunizing capability—In two or three weeks post inoculation, blood samples are drawn from ten vaccinates and ten controls prior to challenge with virulent virus. The challenge virus is a field isolate of DVE prepared to give an approximate 80% mortality in non-vaccinated ducks. Challenge inoculation was done by inoculating 0.5 ml. subcutaneously into each of the vaccinated ducks and into a similar number of non-vaccinated controls. For a satisfactory immune response, 90% of the vaccinates survive and 80% or more of the controls succumb. For the safety test, 20 day-old ducklings, susceptible to DVE and duck virus hepatitis were inoculated subcutaneously with 1 ml. each of the vaccine. Twenty (20) hatchmates were held separately as non-vaccinated controls. When more than 10% die due to causes obviously not related to the vaccine, the original test is repeated. If more than 5% succumb as a result of vaccination, the test is repeated or the serial discarded. For a purity test, at least 5 of the embryos that die after 48 hours incubation, the allantoic fluid is tested for hemagglutination activity against the fresh suspension of chicken red cells by the rapid plate method. For non-specific pathogens, 5 ducks susceptible to DVE and duck virus hepatitis are injected into the infraorbital sinus with 0.5 ml. of the prepared vaccine to indicate freedom from Pasteurella anatipestifer, pathogenic E. coli, and other upper respiratory agents.

2. Inactivated vaccines

Butterfield and Dardiri (PIADL) were able to inactivate DVE virus with .05% I-acetyiaziridine and produce what appeared to be a satisfactory vaccine; however, their results could not be reproduced at LIDRL and the trials with inactivated vaccines were dropped.

Present Status—Suffolk County continues to be under Federal quarantine 9 CFR, Part 83. Under provisions of this regulation, 19 Pekin and 2 aviculturist flocks have become “approved”, and are able to move domestic waterfowl and their products interstate. The approved Pekin flocks represent 54% of the market flocks and 38% of the breeder flocks. Some of the flocks are not approved because Part 83 restricts approval when modified live virus vaccine is used. There are 3 known infected premises in Suffolk County at this time.

Because breeders are highly susceptible to DVE during production, the industry feels the need to vaccinate breeders in advance of the breeding season. Detailed trials are underway to determine whether the vaccine virus will spread from vaccinates to non-vaccinates. The outcome of these trails will determine if unvaccinated market ducks may be raised on a premise where vaccinated breeders are kept without losing their “approved” status.

Under Part 83, viscera from unapproved flocks cannot move in commerce without being cooked. This poses a heavy economic loss on the growers as a ready
market for uncooked duck offal for mink food exists.

Large scale testing of blood samples from domestic and wild waterfowl was conducted outside New York to determine the incidence of Duck Virus Enteritis with negative results. This was an expensive and time-consuming endeavor. At the same time, it is necessary to continue surveillance work. Steps are being taken to enlist the support and cooperation of local diagnostic laboratories who frequently receive field cases of waterfowl for diagnostic purposes. These laboratories will be encouraged to determine if suspicious cases of DVE are in fact DVE. The U. S. Department of Agriculture will provide designated laboratories with DVE diagnostic antiserum for differential diagnostic purposes.

III. Pullorum Disease and Fowl Typhoid
(a) Report of Subcommittee for Eradication

The Chairman of the Subcommittee for the Eradication of Pullorum Disease and Fowl Typhoid sent a questionnaire to each State Veterinarian to ascertain the present status of the Pullorum-typhoid control program in each state. This information was prepared to be presented in St. Louis on January 7, 1968. Representatives were present from the following organizations: National Turkey Federation, Poultry Breeders of America, U.S.L.S.A., National Plans, A. A.A.P., and Animal Health Division and Animal Husbandry Division of A.R.S., U.S.D.A. There were no representatives from the American Poultry Hatchery Federation.

Summary of the Survey:

<table>
<thead>
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<th>Replies received</th>
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<tbody>
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<td>No official program</td>
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</tr>
<tr>
<td>(Nevada and Hawaii)</td>
<td>2</td>
</tr>
<tr>
<td>No replies</td>
<td>48</td>
</tr>
<tr>
<td>(New Mexico and Alaska)</td>
<td>2</td>
</tr>
<tr>
<td>National Plans only (NP)</td>
<td>38</td>
</tr>
<tr>
<td>NP + Equivalent State Program (SP)</td>
<td>8</td>
</tr>
<tr>
<td>100% Participation of Hatcheries</td>
<td></td>
</tr>
<tr>
<td>NP Chickens 16 states, Turkeys</td>
<td>23</td>
</tr>
<tr>
<td>NP + State program</td>
<td>7</td>
</tr>
</tbody>
</table>

Pullorum Disease and Fowl-Typhoid reportable

| Yes                                   | 33     |
| No                                    | 13     |

Pullorum Disease Outbreaks investigated that are reported

| Yes                                   | 42     |
| No                                    | 4      |

Pullorum Disease Outbreaks Quarantined

| Yes                                   | 30     |
| No                                    | 16     |

Importation Regulations

| Yes                                   | 33     |
| No                                    | 13     |
Regulations, Exhibition Birds

Yes  16 states
No  30 states

Outbreaks Pullorum Disease Reported (1966-67)

Yes  24 states
No  21 states
No information received  1 state
Number of outbreaks  71 C

Outbreaks Fowl Typhoid Reported (1966-67)

Yes  12 states
No  33 states
No information received  1 state
Number of outbreaks  23C

States having 100% participation (Chicken and Turkeys)

National Plans and State Program
North Atlantic (9)
6 States
East North Central (5)
1 State
West North Central (7)
4 States
South Atlantic (8)
4 States
South Central (8)
3 States
Western (9)
3 States

Theoretical Classification of States Under Proposed 5 Phase (U.S.L.S.A.) Program

North Atlantic
5 States Phase 2
1 State Phase 3
3 States Phase 4

East North Central
5 States Phase 2

West North Central
3 States Phase 1
4 States Phase 3

South Atlantic
5 States Phase 1
2 States Phase 2
1 State Phase 3
South Central
  3 States Phase 1
  3 States Phase 2
  1 State Phase 3
  1 State Phase 4

Western
  5 States Phase 1
  3 States Phase 2
  1 State Phase 4

(b) Uniform Methods and Rules

On September 12, 1968 a Midwest States Livestock Sanitary Board meeting held at Sioux Falls, South Dakota, passed a resolution requesting this Committee give serious consideration to modifying the Five Phase Program for the Eradication of Pullorum Disease and Fowl-Typhoid.

The committee endorsed the following Standard Procedures as recommended with one dissenting vote:

MINIMUM STANDARDS

The following standards are a combination of the recommendations for uniform methods and rules of the Committee on Transmissible Diseases of Poultry of the U. S. Livestock Sanitary Association, presented to the Executive Committee at Memphis, Tennessee, October 19-23, 1964, and of the American Association of Avian Pathologists Committee on Pullorum-Typhoid Eradication July 11, 1967, to provide the basis for the Division participation with States in cooperative Pullorum-Typhoid Programs.

General

To initiate the program, the Animal Health Division Veterinarian in Charge and the appropriate State official will provide, through the ANH Assistant Director, a joint statement covering authorities to conduct the program and plans for carrying out the program as follows:
A. The necessary State authorities as listed below exist. If any of the listed authorities do not exist, a statement should explain why they are not felt to be necessary for the program in that State.
1. Pullorum disease and fowl-typhoid are mandatory reportable diseases in the State.
2. The State has the authority to place and maintain quarantines (including quarantine of suspicious pullorum disease or fowl-typhoid cases).
3. All reports of pullorum disease and fowl-typhoid are investigated.
4. The State has an importation regulation requiring shipments of chickens, turkeys and hatching eggs to be from the U.S. Pullorum-Typhoid Clean or State equivalent status sources.
5. The State has a regulation requiring that chickens and turkeys going to public exhibition come from U.S. Pullorum Disease or Fowl-Typhoid Clean or State equivalent flocks or have a negative pullorum-typhoid test within the past 90 days.
B. The ANH Veterinarian in Charge and the appropriate State official will provide, through the ANH Assistant Director, a joint statement covering the following points:

1. The necessary State authorities as listed in A above, exist. If any of the listed authorities do not exist, the joint statement should explain why they are not felt to be necessary for the program in the State.

2. Plans (in outline) for carrying out steps included under Phase I below. If initial level of the program will extend to steps included under Phase II, Phase III, or Phase IV, plans (in outline) for carrying out these steps should be included.

The Division will either approve the joint statement as a basis for establishing a cooperative program or will, through discussion and correspondence, participate in modification of the joint statement until mutually satisfactory to the Division and the State, at which time approval will be made.

Definitions

A. State Livestock Sanitary Officials—The person(s) responsible in each State Government for the control and eradication of poultry diseases.

B. Division—Animal Health Division, Agricultural Research Service, USDA.

C. Recognized Laboratory—A laboratory approved by the state Livestock Sanitary Official for performing approved serological testing procedures and bacteriological culture techniques.

D. Area—State or group of States.

E. Flock—All domesticated and/or fancy birds on one premise.

F. Primary Breeding Flock—One that is maintained for the purpose of establishing, continuing, or improving parent lines which may include more than one generation of breeding stock.

G. Multiplier Flock—One that originated from a primary breeding flock and intended for production of hatching eggs.

H. Hatchery—Equipment on one premise operated or controlled by a person(s) for the hatching of chicken or turkey eggs.

I. Infected Flock—Flock in which one or more chickens or turkeys has been diagnosed as infected with pullorum disease or fowl-typhoid.

J. Authorized Testing Agent—A State or Federal employee or persons authorized by the State Livestock Sanitary Official to perform blood collection and testing under provisions of Phase II A, Phase III B.

K. Products—Chickens, turkeys, and chicken and turkey hatching eggs.

L. Testing Results

1. Negative—An approved testing procedure in which the serum-antigen mixture remains turbid without clumping.

2. Positive—An approved testing procedure in which there is distinct clumping of serum-antigen mixture.

3. Suspicious—An approved testing procedure in which there is partial or incomplete clumping of serum-antigen mixture.
Program Phases

I. Phase I - Preparation

A. A written agreement describing the function of each responsible agency participating in the State eradication program shall be initiated, that is, the official NPIP-NTP office and a State regulatory official shall have a written agreement on the responsibilities of each agency. A standard agreement form will be provided to the States in order that the agreements will be developed in a uniform manner.

B. The Extension Service will be consulted for educational purposes.

C. The role of diagnostic laboratories, testing laboratories, and accredited veterinarians will be established as it applies to the individual State.

D. Participation in the NPIP-NTIP or its State equivalent program will be eligible for participation in this phase.

II. Phase II - Reduction of Incidence

A. Flock Participation

1. Multiplier chicken and turkey flocks may participate in the program by serologically testing with no reactors at a rate that will give 95% assurance of disclosing 1% infection using the following table:

<table>
<thead>
<tr>
<th>N</th>
<th>d</th>
<th>n*</th>
<th>N</th>
<th>d</th>
<th>n*</th>
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<td>1</td>
<td>95</td>
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<td>10</td>
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<td>3,000</td>
<td>30</td>
<td>284</td>
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<tr>
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<td>210</td>
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<td>40</td>
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<tr>
<td>900</td>
<td>9</td>
<td>254</td>
<td>50,000</td>
<td>500</td>
<td>297</td>
</tr>
</tbody>
</table>

(* N = Flock size  d = Infected birds  n = Sample size)

2. Primary breeder flocks may participate in the program by serological testing with no reactors in the following manner, of all chicken or turkey breeders:

   a. Each chicken 22 weeks of age or older and each turkey 16 weeks of age or older, and before eggs are used for hatching purposes, will be tested each production year by an approved testing procedure conducted by an approved Veterinarian, State inspector, Federal inspector, or authorized testing agent.

   b. Flocks with no evidence of pullorum disease or fowl-typhoid for two consecutive tests, not less than 21 days apart, are eligible for participation for one year providing all eligible poultry on the premises have been included in such tests.

   c. Flocks originating from flocks that have obtained the status by (a) above will qualify with one clean test.
B. Maintaining Flock Status
   1. A flock of chickens or turkeys will maintain its negative status each
      year under this testing program if there is no serological or other
      evidence of pullorum disease or fowl-typhoid and all additions are of
      equal status.

C. Pullorum and Fowl-Typhoid Are Reportable Diseases
   1. State, Federal, commercial, industrial and private laboratories shall
      report immediately to the State Livestock Sanitary Official the name
      and address of the flock owner in whose chickens or turkeys they
      have diagnosed pullorum disease or fowl-typhoid.
   2. The flock owner of chickens or turkeys reacting to the serological
      tests shall submit reacting birds from this lot directly to a recognized
      laboratory for confirmation. If laboratory examination fails to reveal
      *Salmonella pullorum* or *Salmonella gallinarum* organisms, the flock
      shall be considered negative at the discretion of the State Livestock
      Sanitary Official.
   3. The number of poultry to be submitted to the laboratory for
      confirmation of serological tests shall be determined by the State
      Livestock Sanitary Official or its representative.
   4. The laboratory techniques used for the examination of tissues for
      pullorum disease or fowl-typhoid positives shall be as recommended
      by the Conference of Veterinary Laboratory Diagnosticians of the
      U.S.L.S.A.

D. Field Follow Up of All Isolations of Outbreaks
   1. Pullorum disease and fowl-typhoid eradication cooperative programs
      for chickens and turkeys shall be supervised by full-time State or
      federally employed veterinarians.

E. Quarantine of Infected Flocks and Their Products
   1. Flocks shall be quarantined until disposed of in one of the following
      manners:
      a. The flock may be moved to a State or federally inspected poultry
         processing establishment accompanied by a written certificate
         issued by State Livestock Sanitary Official or his representative.
      b. The flock may be destroyed under the supervision of a State or
         Federal Livestock Inspector.
      c. The positives may be removed from the flock and the flock
         serologically tested. If, as the result of two consecutive negative
         flock tests, the first not less than 21 days following the removal
         of positives, the second not less than 21 days thereafter, the flock
         shall be considered negative.
      d. If laboratory examinations fail to reveal *Salmonella pullorum* or
         *Salmonella gallinarum* organisms for serologically positive birds,
         the flock shall be considered negative at the discretion of the
         State Livestock Sanitary Official.

F. All incubating eggs from flocks found to be infected with pullorum disease
   or fowl-typhoid shall be removed from the incubator and destroyed prior
   to hatching.
G. Premises found to have housed incubated, brooded or ranged chickens or turkeys infected with pullorum disease or fowl-typhoid shall be cleaned and disinfected under regulatory supervision within 15 days following the depopulation. No infected premise shall be restocked with poultry or eggs for hatching purposes until the above cleaning or disinfection are completed.

H. Hatchery Inspections
1. The State Livestock Sanitary Official shall require that each hatchery be subject to periodic inspections. The inspector will consider:
   a. Hatchery sanitation practices
   b. Identification of all hatching eggs
   c. Disposal of hatching waste
   d. Cleaning, disinfecting and fumigating as recommended by the State Livestock Sanitary Official.
2. Only eggs from flocks that comply with the requirements described in Phase II above will be used for hatching purposes.

III. Phase III - Elimination of Outbreaks
A. It will be required that 100 percent of the eligible flocks and hatcheries in the area (State) participate in this Phase. Participation can be turkeys only, chickens only, or both. The flocks and hatcheries may be participating either in the NTIP-NPIP or State programs of comparable status.
B. Testing for Exhibition of Domestic Chickens and Turkeys
   1. Chickens and turkeys going to public exhibition must come from U.S. pullorum-typhoid clean or the equivalent flocks or have a negative pullorum-typhoid test within the past 90 days.
C. Chickens, turkeys, chicks, poults, and hatching eggs may enter a Phase III area or State providing they are not under quarantine and originate from an area or a State with comparable requirements or by 100% flock testing with no reactors by a registered testing agent.

IV. Phase IV - Protection for Reinfection
A. An area (State) will be declared Pullorum-Typhoid Free Chickens, Pullorum-Typhoid Free Turkeys, or Pullorum-Typhoid Free Chickens and Turkeys, when the following requirements have been met:
   1. All the chickens or turkeys or both within the area (State) have gone two years with zero infection.
   2. Pullorum disease and fowl-typhoid are not known to exist in the area (State).
   3. No other birds are raised on breeder premises unless they have been tested and found free of Salmonella pullorum and Salmonella gallinarum.
   4. Primary breeder and multiplier chicken and turkey flocks will be tested at a rate that will assure 95% confidence of detecting a 1% infection and found negative for pullorum disease and fowl-typhoid (see above table).
B. Hatchery Inspection
   1. The State Livestock Sanitary Official shall require that each hatchery
be subject to periodic inspection. The inspection will consider:
   a. Hatchery sanitation practices
   b. Identification of all hatching eggs
   c. Disposal of hatchery waste
   d. Cleaning, disinfecting and fumigation as recommended by the State Livestock Sanitary Official.

2. Only eggs from flocks that comply with the requirements described in Phase IV will be used for hatching purposes.

C. Additions to Pullorum-Typhoid Free Chickens, Pullorum-Typhoid Free Turkeys, or Pullorum-Typhoid Free Chickens and Turkeys.
   1. Chickens and turkeys, chicks, poults, or hatching eggs from a Pullorum-Typhoid Free Chicken, Pullorum-Typhoid Free Turkey, or Pullorum-Typhoid Chickens and Turkeys area (State) may enter another so designated area or one in the process of being designated as such with restriction.
   2. Chickens and turkeys, chicks, poults, and hatching eggs may enter a “Free Area” (State) providing it has been designated by a 100% flocks testing for 2 successive years by a registered testing agent. These flocks must have not been exposed to any known sources of infection and this must be verified by the State Livestock Sanitary Official in the State of origin or from the State where the stock may have been reared.

D. Maintenance of Free Areas (States)
   1. An area (State) may be maintained as Pullorum-Typhoid Free Chickens, Pullorum-Typhoid Free Turkeys, or Pullorum-Typhoid Free Chickens and Turkeys only if all requirements of A, B, and C are in effect and:
      a. No outbreaks other than primary outbreaks occur and such outbreaks are completely eliminated as described in Phase II, E, I, a, b. A primary outbreak is defined as one involving one owner whether or not occurring on one or more premises of said owner. Any spread from such a primary outbreak shall result in revocation of Pullorum-Typhoid Free Area (State) status.
      b. Authority is exercised to conduct traceback investigations and to conduct premise inspections.
      c. Blood testing of chickens and turkeys within the area (State) fail to reveal pullorum or typhoid.

E. As the Pullorum-Typhoid Eradication Program advances, it is probable that some of the USLSA-AAAP-ANH standards will be reconsidered or that other requirements will be added to further protect free areas (States). As these are adopted by the USLSA and approved by the Division, they will be transmitted through revision or additions to these standards.

The Committee recommends that the subcommittee for eradicating pullorum disease and fowl-typhoid continue through 1969.

The Committee directs attention to Resolution of the 1967 Committee adopted by the Executive Committee and sent to the Secretary of Agriculture. To
the best knowledge of this Committee no action has been taken by the U.S.D.A.

The Committee directs attention to the fact that nationally the Poultry Industry is rapidly developing many of the foundation breeding flocks with MG negative status. The program approved by the National Turkey Improvement Plan at the last annual meeting makes for "growth" along this line.
The management technique of "systems analysis" has been most closely identified with the Department of Defense and with military applications. There is a current interest in extending the points of view and techniques of the "systems approach" to the domestic problems that confront federal, state and local governments. There have already been studies in the fields of communication, transportation, law enforcement, urban development, health and water resources. The application of these techniques may not be imminent in Veterinary Medicine, yet there is reason to believe that the discipline will not remain untouched by these techniques which have been called everything from "quantified common sense" to the "greatest advance in the art of government in nearly a century."

There is no dearth of definitions for "systems analysis." Entoven(1) defines systems analysis "as nothing more than quantative or enlightened common sense aided by modern analytical methods." The approach to the problem involves the identification of the problem and defining the objectives which must be achieved to solve that problem. The approach further depends on providing the decision-maker with the information that will best help him to select the preferred way of achieving the objective. The process requires that the alternative ways of achieving the objective be identified. Then the estimate of the benefits to be derived and the costs of each alternative are estimated in quantitative terms. The aspects of the problem that cannot be quantified are stated explicitly. The goal is to choose the alternative that makes the best use of the limited resources available to the decision-maker.

There are, of course, critics of this approach(2, 3). These reservations cannot be explored in detail. In essence, the critics note that many social and political decisions must be made on broad judgmental grounds. There are few standards or objective criteria that express human values in a meaningful way. The critics are concerned that the quantitative standards which are more readily available often tend to dominate decision-making to the exclusion of the non-measurable and usually more important considerations.

The Methodology

The essential components of the systems approach have already been mentioned. These may be listed as:

1. goal setting (objectives, requirements, constraints)
2. evaluation of alternatives (modelling, simulation, cost-effectiveness)
3. data collection (historical surveys, description of the environment, the structuring of relationships)

The process of decision-making involves the application of goal-setting, alternative generation, evaluation and data collection at successively deeper levels of analysis. The interactions among these phases, along with human judgmental decisions, yields adjustments that ultimately converge to a "best" decision. It is important to think of the system approach as both sequential and repetitive, since the end product of an analysis may lead to the modification of the original objective.

Applications in Veterinary Medicine

Kaplan(4) has referred to studies in the World Health Organization on the possible applications of systems approaches to international health problems and particularly to problems of developing countries. The hypothetical example is described for a decision on the choice between a brucellosis control or eradication campaign. The model for such a decision should include cost items such as the finding of carriers (the detection of the last few percent of carriers in an eradication program may involve enormous costs) and the costs of inoculation, manpower and material resources. The logistics and costs of implementation including social costs would also be considered. These costs would cover only the introductory calculations for review. The benefits to be expected in human health, livestock health, economy and their spillover effects would also be calculated for the choice of the most acceptable alternative.

There is a real example of a similar problem posed by the recent epidemic of foot-and-mouth disease in Britain. The 1967 outbreak was of major proportion. The British Government attempted to shut off imports of meat from countries where the disease was endemic. The purpose was for disease control; the effect went far beyond livestock health and created serious diplomatic and economic problems for Britain. The decision alternates are 1) to maintain the policy of slaughtering infected animals and 2) adopting a policy of vaccination control. The model for such a decision must take account of the cost of a slaughter program (including payment for slaughtered animals at market prices) as constrained with the enormous costs and imperfections of a vaccination program. Further costs of a vaccination program would be the restriction of export of pedigree animals to disease-free areas such as the United States.

There are important costs in maintaining a slaughter policy, including the requirement that meat imports be banned from countries where the disease is endemic. Such imported meat is relatively inexpensive compared to meat from British domestic and other sources. There are also the more difficult social costs to consider. A slaughter policy can be disastrous to the individual whose farm is affected. Yet, the farmers as a group may still be desirous of maintaining the more strict method rather than make an irrevocable decision, despite the changing demographic shifts and mobility which increase the difficulty of preventing the spread of the virus. Walsh(5) notes that the slaughter policy may grow less effective and more expensive in time. The problem is amenable to a systems approach and a decision based on the "best" alternative. Decisions on the eradication of tuberculosis could also be analyzed from a systems approach. It is important to distinguish between the reduction of the incidence of the disease to a level
acceptable to public health authorities, and eradication, which is the total elimination of the etiologic agent from a region.

Apart from the methodological aspects, "systems" are important as a point of view, albeit the idea is not new. The point of view reaches back to a recognition of the Buddhist doctrine of interrelatedness: "The truth is that everything causes everything else. We do not speak, therefore, of one think causing another." Of course, it is not possible to deal with all our problems as an "integrated whole." At some point the limits of the "sub-system" must be specified. The foot-and-mouth disease problem in Great Britain is a sub-system which is directly related to the disease problem in a number of South American countries. Ruderman\(^6\) indicates that for a developing country which earns most of its foreign exchange from exports of livestock products, there is reason to assign a higher priority to veterinary programs than human health programs even without consideration of the contribution of the livestock economy to protein consumption within the countries. The Inter-American Development Bank recently made loans totaling 5.1 million dollars for coordinated and multi-lateral efforts to control and eventually eradicate foot-and-mouth disease in Chile and Paraguay. Similar loan requests totaling approximately 30 million dollars are now in an advanced state of preparation for other countries in South America. The disease causes livestock losses of 400 million dollars a year in South America. However, a computation of actual losses would have to include additional losses in export revenues that otherwise could accrue to meat producing countries where the disease is in evidence. Benefits include increased meat production through weight gains, higher milk production, increase in the net worth of the cattle and increase in fertility. These are costs and benefits that can be expressed for this livestock disease program in comparison with another livestock disease program, and a choice made between them. Insofar as a choice between a livestock disease program and a direct human health program, there are considerably greater difficulties. What is the economic value of human life? There are some answers to this question\(^7\). Beyond this, there are difficulties in the measurement of the intangible aspects of human life, including the value of good health.

The recent passage of the Poultry Products Inspection Act has excited some controversy and some expressions of doubt as to the effectiveness of present poultry inspection procedures, especially for the control of salmonellosis. It has been suggested that there be more extensive use made of microbiological testing. There can be little doubt that the development and use of microbiological criteria for Salmonella organisms in poultry meat would be useful. However, there is a definite requirement to assess the costs and effectiveness of such a procedure and compare among alternate ways of providing laboratory procedures and with other procedures in poultry inspection.

The analysis of these issues requires more than an exploration of the microbiological testing procedures. There is a requirement for studying meat hygiene as a system, i.e. as a set of inter-related components. The probabilities that a microbiological contaminant will gain entrance to a food depends on a number of inter-related factors, including, but not limited to:

a) agricultural production and technology
b) food handling methods (including human factors of care or carelessness)
c) food processing  
d) patterns of distribution and transportation  
e) microbial ecology  

The food-contaminant sub-system is only part of a larger system of control strategy and public and governmental response. A model of the food-contaminant system may give the necessary information for choosing the point in the chain where the control strategy is optimal. However, the control strategies themselves must be considered in relation to the political and social environment. There are usually a number of alternate approaches to food control, including field-inspection, technological fixes, laboratory testing, sampling vs total inspection procedures. These choices cannot be isolated from the evaluation of possibilities for action of the agency responsible for the program, the demands of the consumer, and the situation of the food industry. The mechanism of control is determined by the objectives of a food program, which in turn depend on political and social factors as well as health factors. These factors are intimately related to considerations of public expectation, law, economics and administration. All these systems components should be considered in assessing the cost-benefit ratios of microbiological analysis in poultry inspection.

Microbiological criteria are not a new topic of discussion. National and international scientific groups\(^8,9\) have reported extensively on methodology and proposals have been advanced for numerical criteria for a number of food products. Yet, the basic issues have been rarely touched. This follows from a neglect of the point of view which is characteristic of systems analysis. The question has been explored in the past primarily from the microbiological point of view. Little emphasis has been given to defining the objective of the microbiological procedure and translating the general statement of the objective into a quantifiable “objective function.” That is a statement of the relationship of finding a Salmonella organism or a specified number of Salmonella organisms to the program objective. Further, the economic, administrative and operational considerations related to the microbiological criterion have been conscientiously ignored.

Some elements of the system that should be considered include:

I. The formulation of the objective of the microbiological criterion
   (calculation of the benefits to be derived)

   The objective usually turns on phrases which are not wholly operational. The objectives are expressed in terms such as “freedom from pathogens” or “the protection of public health.” Such phrases need further definition and clarification before they can be translated into meaningful microbiological criteria. In other instances the objective has been based on the “principle of expediency.” That is, the result that may be obtained under conditions of good sanitation practice.

II. Economic factors (calculation of costs), including:
   (a) Costs to the governmental agency for
      1) operation of laboratories
      2) procurement of samples
      3) administrative costs
      4) training of personnel
      5) opportunity costs (money spent on the laboratory program is not available for an alternate activity in food control or to some other
program).

(b) Costs to the food industry
1) including costs similar to 1-4 above, and
2) increased processing costs, as well as increased cost for raw materials.
3) possible costs due to trade barriers.
(c) Costs to industries (including those in developing countries) that are technologically unable to meet the required standards in their food exports.

III. Administrative factors
There are variations in the administrative application of microbiological standards. The stringency of the application may vary from recommendations without sanctions, purchase specifications, import standards or legal standards. Governmental food control administrators have supported the development of microbiological criteria because these seem to offer a means for making decisions in difficult judgment situations. Actually, the use of standards to reject a food lot or to impose sanctions gives rise to considerable legal and administrative difficulties. The legal aspects of these questions are largely unexplored, and still have to be tested in the courts.

The most critical problems may not be related to laboratory methodology or the sampling procedures; but in the manner in which the food control administrator actually uses the decision criterion in field application. Many program administrators will have a limited knowledge of the variabilities involved in sampling and in the microbiological process. The standards user may have a higher confidence in the validity of the standard than may be justified. This may lead to an over-rigidity in the application of the standard. Further, judgments are more likely to be based on single or few samples rather than multiple samples taken at random.

IV. Legal and political factors. These include:
(a) the willingness of the political authorities to impose a legal standard
(b) the capability of the political and administrative authorities to enforce the legal standard
(c) the acceptance by the courts of the premise that microbiological counts in excess of a stated limit are prima facie evidence of a public health hazard.

V. Other factors
(a) problems relating to the varibility of the microbiological process. These include:
1) complications due to the fact that food processing represents an open system. The end-point specification may not express the actual food history. End-point quality standards are more successful with closed systems, as milk or water. The changing microbial ecology is more complex in the open system.
2) variance in results from laboratory to laboratory in the performance of the same test.
3) lack of significant correlations between different tests which supposedly measure food plant sanitation.
The variables listed above help point to the kinds of data needed to make a judgment on the desirability of requiring a routine laboratory test for Salmonella in raw poultry. The following questions may be asked:

1) What are the benefits of sampling raw poultry products for Salmonella? The Third Report of the Joint FAO/WHO Expert Committee on Zoonoses\(^{(10)}\) does indicate the public health significance of Salmonella carriers in poultry, and the chance for increase in numbers of the organism in transport, slaughtering and processing. The chain of transmission to humans is based on the probability of Salmonella organisms contaminating the environment rather than through the direct consumption of undercooked poultry. In fact, food-borne infections from poultry meat are almost always the result of contamination after cooking or through processed poultry products.

It does not take a systems analyst to see the effect of sampling raw poultry on the whole preceding chain of poultry raising and feeding. Would the public health objective be achieved more logically through end-point samples of processed poultry products and a strong educational campaign to instruct persons on the proper handling and cooking of raw poultry in homes and food service establishments? The reply may be that the objective is to minimize the prevalence of Salmonella in the community. This is a different objective and should be defined operationally to include the total ecological system that contributes to the problem. However, in choosing this latter objective, the caution raised by Katz and Koprowski\(^{(11)}\) is pertinent. They noted that “complete eradication of an infectious agent is in our view not yet accessible to the logical approach and, if pressured, may lead to unforeseen disaster rather than to the benefit of mankind.”

2) The operational definition of the objective should lead to a decision on the acceptance of a tolerance criterion or a zero tolerance for the organism. This question raises issues which are critically important, but beyond the scope of this discussion.

3) There is a further requirement to specify the frequency and pattern of samples. In dealing with a closed system such as water safety, it has been stated that “consumer protection is a function of sample volume”\(^{(12)}\). The decision to take a microbiological sample for each unit of raw poultry would require a tremendous laboratory effort. If only a portion of the number of birds processed is examined, there must be some basis for such a sampling procedure. Further, certain administrative problems will require solution. For instance, will the poultry be distributed immediately, to be recalled if the test is positive; or will these be stored till the laboratory analysis is completed? The production flow and storage problems raised by this latter procedure could be enormous.

The list of questions raised by this simple model is great. Such models do help to indicate the kinds of data needed for program analysis. Those variables which may be quantified, should be. For those that may not be quantified, and in the total analysis of the program situation, good judgment in making decisions must still prevail.

The Systems Approach

Fabian\(^{(13)}\) indicates that one of the most immediate and critical tasks of a
systems analysis is to provide a working definition of what is to be accomplished by the proposed program (or system). Objectives for programs are usually stated in very broad terms, i.e. provide a safe food supply or improve a hospital system. The challenge is to define an objective in terms of a specific quantifiable "objective function," so that a comparison of two or more approaches to the solution of the specific objective can be proposed, and the best choice made among them. The goal of "improving a hospital system" can be more specifically stated as the "improvement in the control and utilization of hospital bed occupancy." There are difficulties of defining objectives, and contradictions among multiple objectives.

The next methodological step is model-building. A thorough understanding of the problem and the objectives for attainment should allow a specification of variables and interactions. These models may be in the form of flow diagrams or even verbal or graphic expressions. The more precise models take the form of mathematical expressions. The development of the model with structuring of relationships, and interactions should represent a conceptual analog of the real world system. In any event, the model permits the subsequent utilization of analytical techniques, including econometrics, cost-benefit analysis, simulation and others which are beyond the purview of this discussion. The effective model should point towards the kind of data required and for the search and compilation of data that does not presently exist.

Therefore, the systems approach requires a description of the proposed system, its environment, its functions, its purpose, its interactions, its mechanism of operation. The next step is the application of measures of effectiveness to evaluate the system. Among these, cost-benefit analysis is of particular interest. Klarman(14) describes this type of analysis as entailing a comparison of costs and benefits for a series of programs which may be considered as alternatives or competitors for public funds. The final evaluation is based on choosing the alternative that receives the highest evaluation for implementation. Cost-benefit analysis is analogous to the businessman's calculations on the return from his investments. The critical difference is that some public benefits and costs cannot be evaluated with reliance on market prices, as is the case with business. Benefits are elusive. There are difficulties in predicting and measuring the consequences of a program or policy, including the spill-over effects. Inoculation against a communicable disease benefits the direct recipient, but also those who were not inoculated, because of a decrease in the incidence of the disease.

The cost side is also difficult to measure. For instance, social costs are not easily measurable. Fabian(14) notes that in trying to determine the social cost of urban slums, one would have to measure the cost of crime, fire and health hazards and a variety of moral problems.

Despite the difficulties in this type of analysis, there is an acceptance of the simple fact that economic resources are scarce in relation to the needs, and that all desirable social objectives cannot be pursued simultaneously. Governments, at all levels, must make choices between programs and as to those that will be undertaken and those rejected or postponed. The acceptance of systems analysis in government is highlighted by the initiation by the Federal Government of a P-P-B system throughout the executive branch to be supervised by the Bureau of the Budget. States and larger municipal governments are also adopting these systems.
Planning-Programming-Budgeting systems express the now familiar methodology. The method is intended to set forth the major objectives, to define the programs essential to these goals, to identify resources to satisfy the specific objectives and to systematically analyze the alternatives available. Hitch(15) separates P-P-B techniques into two related and mutually supporting, but distinct phases. The first is "programming" which produces a program or program budget. The program budget is classified by "outputs" which are objective-oriented rather than by "inputs" or traditional classes of expenditures. For instance, the livestock disease control authorities and the public health agency which requires that milk must be from an accredited source are both part of a "program" whose output is tuberculosis eradication in cattle. The resources that are required to satisfy the objective are linked to the "programmed outputs," and the program extends into the future (five years rather than an annual budget) to show the full resource requirements and the financial implications of the programmed outputs.

The second phase in P-P-B methodology is the explicit quantitative analysis (to the extent that is practical), which is designed to increase the value of the objectives achieved minus the value of the resources used by an organization. The "quantitative" feature of this analysis has been stressed repeatedly. The need for quantitation poses difficulties in the analysis of problems in which economic, social and political features dominate. The "systems analyst" is continually striving to broaden the nature and content of the analysis to deal with these critical aspects Novick(16) notes that "...as important as, in many cases more important than quantitative considerations, are problems of a qualitative nature for which we do not have numbers. This does not mean that analysis is not possible just because we cannot quantify. On the contrary, there are many ways of analyzing qualitative problems and it is an essential ingredient of this process that we undertake to do a substantial amount of qualitative analysis in addition to the quantitative work."

One measures those things that are measurable and defines the things that are not readily or possibly measurable. Areas which are not quantifiable at present, may become quantifiable in the future. There is always a requirement for human judgment and values must still be determined and uncertainty dealt with. Enthoven(1) stresses that "good analysis can do a great deal to sharpen the issues, clarify the alternatives available to the decision-makers, and narrow substantially the range of uncertainty, thus, freeing the responsible officials to concentrate their attention on the crucial judgments."

REFERENCES
REPORT OF THE PUBLIC HEALTH AND RADIOLICAL FALLOUT COMMITTEE

R. L. Parker, Atlanta, Georgia; J. F. Stara, Cincinnati, Ohio; I. M. Saturen, Ames, Iowa; W. L. Rottman, South Gate, California; J. C. Jefferies, Hyattsville, Maryland; R. H. Singer, College Station, Texas.

The Public Health and Radiological Fallout Committee Report is composed of the three reports presented to the Committee and two recommendations by the Committee.

REPORTS

The Committee discussed the report on "The Role of Radioisotopes in Veterinary Public Health" presented by Dr. J. F. Stara, National Center for Radiological Health, Public Health Service, which reviewed two major aspects of the role of animal research in radiation biology and bio-effects programs: (a) metabolism and toxicity of radionuclides in various mammalian species and their relationship to human exposure estimates; (b) general principles governing the route of entry and prevailing levels of fallout radionuclides, particularly 131I, 90Sr and 137Cs, in animal products for human consumption.

Reported metabolic data in several animal species were compared with available values in humans for the following radionuclides: Cs (Cesium), Sr (Strontium), Ra (Radium), Zn (Zinc), Po (polonium), Pb (Lead), Pu (Plutonium), I (Iodine), and Ru (Ruthenium). These elements were selected because they are recognized as potential public health hazards, and they represent the various chemical families. Advantages and disadvantages in extrapolation of animal data to man were discussed and summarized.

A general survey of the pattern of uptake and metabolism of 137Cs, 131I, and 90Sr in meat-and-milk-producing animals was presented. Such values give a direct assessment of population exposure since they are part of the human food chain. Also listed are factors which determine the relative biological significance of the different nuclides, and the principal transfer routes into the diet. Radioactive levels in the food chain of the Eskimo population in Alaska (lichen-caribou-man), and reported radionuclides concentrations in human tissues were presented.

Dr. Stara briefly described food additives that reduce body burdens of 90Sr, 137Cs, and 131I by binding them in the G.I. tract thus preventing their absorption process.

The second report, on the new A2/Hong Kong/68 strain of influenza virus, made several interesting points. This virus, which caused extensive outbreaks in the summer of 1968, was first reported by newspapers in southeast Mainland China. It affected Hong Kong and Malaysia and began spreading westward. Several World Health Organization Influenza Centers have studied this strain and compared it with A animal strains. In Prague, inhibition of the Hong Kong strain was shown with RDE treated rat sera prepared against two A/equi-2 strains (Detroit/63 and Miami/63), and A/quail strain, and two A/duck strains. The London laboratory in
tests with post-infection ferret sera showed some HI crossing between the Hong Kong strain and A/equi-2/Miami/63. Neuraminidase inhibition tests, however, indicated that the Hong Kong strain resembles the 1964 and 1967 strains of A2 virus rather than the A/equi-2/Miami/63 strain.

This report suggested that outbreaks of animal influenza should be investigated to identify the etiologic strain and be reported through routine channels so that the activity of influenza virus can be monitored as fully as possible this year.

The third report reviewed the current listeriosis situation in the United States. Listeriosis should be considered a zoonosis; humans have contracted the disease after associating with infected dogs, consuming contaminated milk or infected meat, and handling infected newborn calves. Listeria monocytogenes infection can cause abortion, stillbirth, conjunctivitis, endocarditis, meningoencephalitis, pneumonia, myodermatitis, septicemia, and urethritis in humans. In addition, L. monocytogenes infections have been reported in conjunction with chronic pulmonary disease, neoplastic diseases, alcoholism, diabetes mellitus, and cardiovascular disease. The organism is widespread in nature, and those persons who receive massive exposures or are debilitated prior to exposure may become infected. The case fatality rate for persons with listeriosis in the period 1933 to 1966 was 42%.

In animals the clinical picture includes abortion, meningoencephalitis, and septicemia. The organism has been reported to infect 42 species of mammals and 22 species of birds. It has been isolated from crustaceans, feeds, soil, water and sewage. It had been isolated from feaces of packinghouse workers at a higher rate than from the general population, but since the majority of cases are reported from urban populations, sources of infection other than animal must be considered possible.

The disease is of definite public health importance; however, much of the pathogenesis and epidemiology are unknown, for the organism is difficult to isolate. Four serologic types of L. monocytogenes have been identified, and another species, L. grayi, has recently been reported. The pathological significance of the new species has not yet been defined.

Although the disease is frequently fatal, listeriosis in humans is not a reportable disease in the United States. During the period 1933 to 1966, a total of 731 bacteriologically proven cases were recorded; last year 60 cases were voluntarily reported to the National Communicable Disease Center of the Public Health Service. Through mid-September 1968, 49 cases had been reported to the Center.

USDA reports indicate that cattle are the animal species most often infected with L. monocytogenes, but isolations were made last year from sheep, goats, dogs, cats, chickens, chinchillas, swine, horses, and a heron. Cattle accounted for 819 (87%) of the 941 animal cases.

The National Communicable Disease Center is including listeriosis in its surveillance program and encourages the reporting of human cases. Hopefully, increased voluntary reporting will enable study of the disease to define its epidemiologic characteristics.

RECOMMENDATIONS

The committee recommends that those responsible for radiological monitoring
instruments provide an annual exercise to maintain their efficiency and capability in using these instruments.

The committee recommends a slight change in its name. Recent reports on radiological problems indicate areas of concern having nothing to do with fallout. Toxicology is not presently in the realm of this committee, but recent experiences suggest it may become more and more the concern of this committee. It is, therefore, recommended that the name of the committee be changed to Committee on Public Health, Radiological Exposure, and Toxicology.

This report is respectfully submitted to the Association.
SUMMARY

P.O. Box 70, Ames, Iowa 50010
USDA, ARS, APH, National Animal Disease Laboratory
Joseph R. Sonner, Donald T. Breyman, and Herbert M. Cook

FOR EUTHANASIA OF SWINE

THE USE OF SUCCYNCYLOBENZINE CHLORIDE

PART I
It should kill the animal in essentially 100 per cent of the cases and require no additional action on the part of the exterminator; such as, exsanguination.

It should not jeopardize the disposal of the carcass after death.

The quantity of the drug used should be relatively small to facilitate rapid exposure.

The cost of the drug should be reasonable.

Field studies by Shulz on the use of succinylcholine chloride for swine depopulation in the hog cholera eradication program indicated it had considerable value for this work. However, a previous report by the AMA panel on euthanasia condemned the use of curare form drugs to kill animals.

Succinylcholine was first synthesized in 1906 by Hunt and Taveau. In 1941 Glick synthesized succinylcholine and noted that it was readily hydrolyzed by alkali and an esterase found in horse serum. Bovet et al., in 1951 demonstrated its powerful neuromuscular blocking activity. At about this same time, Costillo and de Beer reported on this skeletal muscle-relaxing effect. Since 1950, succinylcholine chloride has been used extensively as a skeletal muscle relaxing drug in the human as well as the veterinary medical field.

Structurally, succinylcholine consists of two molecules of acetylcholine linked together at the alpha-methyl position and is sometimes referred to as diacetylcholine.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{(CH}_3\text{)}_3 & \quad \text{N} - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C} - \text{CH}_2 - \text{CH}_2 - \text{C} - \text{O} - \text{CH}_2 - \text{CH}_2 - \text{N} \equiv (\text{CH}_3)_3
\end{align*}
\]

Succinylcholine

The action of succinylcholine differs from that of curare in that it produces a persistent depolarization of the motor endplates of skeletal muscle and the surrounding muscle cell instead of inhibiting the depolarizing action of acetylcholine. Also, succinylcholine has an action similar to decamethonium which differs from curare in that the drug first stimulates contractions of skeletal muscle before paralysis takes place. The drug does not affect autonomic nervous transmission or intestinal motility.

Succinylcholine is rapidly broken down in the body by the enzyme pseudocholinesterase. The decay curve of the drug in human serum follows a very steep slope so that after 3 minutes the plasma concentration is virtually undetectable. Cattle have low pseudocholinesterase levels and are 8 to 10 times more susceptible to succinylcholine than are horses and swine.

The purpose of this study was to determine the value of succinylcholine chloride as a euthanatizing drug.

**MATERIALS AND METHODS**

Catheters were surgically implanted in the right femoral artery of 8 female swine weighing approximately 200 lbs. each. One week was allowed for recovery from surgery.
Following recovery from surgery, the swine were placed in a restraining crate and electrodes were attached to record respiratory rate, blood pressure, and heart rate.

A preexposure blood sample was collected and 5 ml. of a 10 per cent solution of succinylcholine chloride were injected into the catheter. The catheter was flushed with 5 ml. of phosphate buffered saline (pH 7.4). Four blood samples were collected as rapidly as possible, the first approximately 30 seconds following exposure and the remainder approximately 10 seconds apart. Two additional samples were collected at approximately 3 minutes and 6 minutes following the injection with succinylcholine chloride.

All blood samples were collected and held in heparinized plastic disposable syringes. Approximately 5 ml. of blood were drawn into the syringe and all air expelled immediately. The syringe was then sealed with a plugged 26-gauge needle to prevent air from entering the syringe.

The oxygen and carbon dioxide were extracted from the blood with a Loenco Model AD-200 blood gas extractor. The gases were then analyzed on a Loenco Model AD-2000 gas chromatograph and recorded on a millivolt recorder.

Since intravenous exposure is tedious and time consuming, other methods of exposure were studied. Exposure routes used were IP, IM, and IT. Seven swine were exposed IM, one IT, and one IP. Each pig weighed approximately 75 lbs. and was given by injection 5 ml. of a 10 per cent solution of succinylcholine chloride. Samples of arterial blood were collected with a 4-inch, 18-gauge needle from the area immediately anterior to the heart. Swine were restrained in dorsal recumbency and the needle remained in the blood vessel until the last sample was collected.

A preexposure blood sample was collected. Approximately 30 seconds following exposure a second sample was collected. The next three samples were collected at approximately 10-second intervals. A 3-minute and a 6-minute post exposure sample were also collected.

RESULTS AND DISCUSSION

All 8 swine exposed IV with 5 ml. of a 10 per cent solution of succinylcholine chloride had respiratory failure within 5 seconds. The mean heart rate of unexposed swine was 128 per minute. Following IV exposure with 5 ml. of succinylcholine chloride, the heart rate was extremely irregular, dropping to approximately 60 per minute. A gradual decrease in heart rate continued for 8 to 14 minutes until heart failure or fibrillation was experienced.

The mean arterial blood oxygen level for unexposed swine was 14.68 volumes per cent. Within 60 seconds following exposure to succinylcholine chloride, the mean arterial blood oxygen level was 4.99 volumes per cent.

From human aviation physiology studies, it was concluded that the critical O2 level of arterial blood for normal man, below which consciousness was not supported, was 60 per cent of saturation. If we assume that a similar condition exists in swine and that normal arterial blood is essentially saturated (95-100 per cent), 60 per cent of saturation for this group of swine would have a mean of 8.80 volumes per cent. The time required to reach this O2 level following IV exposure ranged from 26 to 50 seconds with a mean of 38 seconds (Fig. 1).
Swine exposed IM experienced respiratory failure within 25 seconds post exposure. Respiratory failure was not preceded by signs of apprehension on the part of the pigs.

The mean arterial blood oxygen level for this group of swine prior to exposure was 13.40 volumes per cent. The mean 60 per cent saturation level was 8.04 volumes per cent. From the time of IM injection with 5 ml of succinylcholine chloride until the 60 per cent saturation level was reached was a mean of 43 seconds (Fig. 2).

The assessment of pain in man has been extensively investigated. Kitchell points out that pain is primarily a subjective response; its quantity and intensity are dependent upon the "state of mind" of the subject. Man conceives of pain in animals as he might sense it with his background of real and vicarious experience. However, man can only know about pain in animals and not of pain in animals.
Figure 2. Blood oxygen level of swine exposed IM with 5 ml. of a 10 per cent solution of succinylcholine chloride. Loss of consciousness occurs at or near the 60 per cent saturation level.

Immobilization of the respiratory muscles causing fatal suffocation was cited by the AVMA panel on euthanasia as the basic reason for rejection of curareform drugs for euthanasia. From all outward appearances, this is the consequence of IV or IM exposure with succinylcholine chloride. However, the results presented in this paper suggest that unconsciousness and death result from a rapid loss of blood oxygen rather than from the slower process of suffocation.

Mental anguish on the part of human patients treated with curareform drugs was also cited by the AVMA Panel on Euthanasia as part of the basis for its rejection as an euthanatizing drug. However, in a recent report by Curran et al., the opposite reaction was observed. A mentally retarded patient suffering from tetanus was given large doses of succinylcholine chloride (1 gram per dose) to relax the muscular contractions manifested in the disease. Questioning revealed that he did not mind the profound relaxation and in fact, enjoyed the relief it afforded. In a period of 8 days, the patient received a total of 20 grams of succinylcholine chloride.
Men suffering from loss of blood oxygen in high altitude studies state that it is accompanied by a feeling of euphoria. Whether swine are capable of experiencing a feeling euphoria is subject to question; however, observations have indicated that the experience is not marked with pain.

On the basis of the results obtained in this work, succinylcholine chloride meets the requirements of an ideal drug for euthanasia. It can be administered IM, in small doses (5 ml.), and is relatively inexpensive (20 cents/gram). It kills the animal essentially 100 per cent of the cases and does not require any additional action. It does not interfere with disposal of the carcass since it is rapidly broken down in the body. If proper care is exercised, it poses little hazard to personnel. It is also concluded that exposed animals are rendered unconscious within a short period of time and that this time interval is not marked with pain.

Acknowledgement is given to Drs. R. W. Dougherty and A. H. Sillau for their invaluable assistance in surgical procedures and physiological measurements.

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PART II

FIELD TRIALS USING SUCCINYLCHOLINE CHLORIDE FOR EUTHANASIA OF HOG CHOLERA INFECTED SWINE HERDS

by

N. E. Schulz, D.V.M., B.S.*

The use of firearms as a means to destroy hog cholera infected swine herds has certain disadvantages, which raise questions as to their continued use in disease eradication activities. Disadvantages which must be considered are: the ever present danger of ricochet; accidental firing, persons or animals wandering into the line of fire; contamination of the premises through the discharge of body fluids, after death as well as from gunshot wounds; the unsightly and non-professional manner of execution; and the brutal aspect when marksmanship is not of the highest quality. To overcome these objectionable features, field trials were conducted to determine if drugs might be effective yet practical for the humane euthanasia of swine in hog cholera infected herds.

TRIAL NO. 1

The first of these trials was held in Springfield, Illinois, in December 1966. The objective of this trial was to determine which, if any, of certain selected drugs met the criteria of Dorland's definition of euthanasia, that is, "an easy or painless death."1 Several products were considered; however, many of these had known disadvantages and were therefore eliminated. Other products appeared useful and disadvantages were not so well known. Six of these products were selected for the trial: saturated solutions of (1) sodium nitrite and (2) chloral hydrate, (3) nicotine sulphate, and three proprietary drugs, which contained as their active ingredient (4) pentobarbital sodium, (5) phencyclidine hydrochloride, and (6) succinylcholine chloride. In this trial, all products were injected intraperitoneally, and each was given in dosages considered to be massive and capable of producing death. Two of the drugs, pentobarbital sodium and chloral hydrate, produced only deep anesthesia when administered as described. Three of the products, sodium nitrite, nicotine sulphate and phencyclidine hydrochloride, produced effects which were objectionable including retching and/or dyspnea; and one of these, nicotine sulphate, caused violent convulsions before death in this trial. Succinylcholine chloride produced dramatic results in that death was immediate, and no objectionable features were observed. Five ml. of a preparation containing 100 grams per ml. was utilized.
Conclusions of this very limited trial indicated that one product, succinylcholine chloride had potential for use as an agent for euthanasia, while the other five products failed to meet the criteria by variable degrees.

TRIAL NO. 2

To further evaluate the effectiveness of succinylcholine chloride, the drug was obtained in powder form, and plans were made for its further use under field conditions in a hog cholera infected herd. Such a herd was located in April 1967 in North Carolina, the owner of which gave permission for experimentation. In this trial, three preparations were used. One consisted of a solution of succinylcholine chloride (500 mg./5 cc. tap water), another consisted of 10 cc. of a 50 percent solution of sodium nitrite, and the third was a combination of the two; that is, 500 mg. succinylcholine chloride dissolved in 10 cc. of a 50 percent solution of sodium nitrite.

These drugs were administered by intravenous, intraperitoneal, and intrathoracic routes. The swine, 27 in number weighed 50 to 75 pounds each; and about half of them were visibly ill with hog cholera.

The results of the second trial supported the conclusions of the first. When succinylcholine chloride solution (500 mg./5 cc. H2O) was administered intravenously, instantaneous collapse of the animal was accomplished with heart action ceasing after an interim of two to nine minutes. A combination of the two products, 500 mg. of succinylcholine chloride in 10 cc. of a 50 percent solution of sodium nitrite, injected intravenously also produced collapse instantaneously; however, this mixture produced death sooner than succinylcholine chloride alone in those swine exhibiting severe manifestations of hog cholera. Injections, using the same concentrations, made deep into the sternal musculature using succinylcholine chloride solution alone, or in combination with sodium nitrite, produced death as rapidly as intravenous injections. Further, body wastes were not expelled during the post inoculation interim when any of the above methods were used. Combinations of these two drugs, as well as their use individually, caused objectionable effects when injected into the peritoneal or thoracic cavities. These effects ranged from a mild discomfort to retching, dyspnea or an otherwise general discomfort.

TRIAL NO. 3

A third trial was held in North Carolina to further evaluate the drug combination, succinylcholine chloride and sodium nitrite, under field conditions in a large herd. This trial was conducted on July 13, 1967, in a hog cholera infected herd in which it was necessary to destroy over 2300 pigs varying in weight from 5 to 50 pounds. As these pigs were rather small, it was decided that the drug combination be injected intracardially for ease of administration and to determine the approximate dosage required. This trial demonstrated that intracardial injections were highly satisfactory. As little as 1 cc. (100 mg. succinylcholine chloride in 50 percent sodium nitrite) was ample dosage for the humane destruction of pigs up to 10 pounds, 1½ cc. for pigs weighing between 10 and 25 pounds, and 3
cc. for those ranging in weight from 25 to 50 pounds. Various size needles were used for injections; however, a two-inch, 16 gauge hypodermic needle proved to be the most satisfactory in this trial since intracardial administrations could be made when entering either the right or left side of the pig.

FURTHER FIELD USE

As a result of these trials, succinylcholine chloride solution, 100 mg./cc. aqueous solution, either alone or in combination with a 50 percent solution of sodium nitrite, has been used on numerous occasions in the field for the destruction of hog cholera infected or exposed swine.

SUMMATION

Field trials have shown that a solution of succinylcholine chloride (100 mg. per ml. tap water), or a solution of succinylcholine chloride (5500 mg.) per 10 cc. of a 50 percent solution of sodium nitrite, to be a rapid and humane agent for use in swine euthanasia when injected intracardially, intravenously, or intramuscularly.

COMMENT

As with any agent considered for these purposes, such agents will produce the same reaction whether injection is deliberate or accidental. Therefore, reasonable precautions should be taken to preclude accidental injection. Such measures include the proper restraint of animals for injection, the use of a single dose syringe, and assignment of a safety officer to insure adherence to strict safety practices. With these precautions taken, it is apparent from these trials that succinylcholine chloride can be utilized in lieu of gunfire in destroying swine. Such substitution would prevent unnecessary contamination of premises and the brutal aspects of gunfire as well as eliminate the inherent dangers of gunfire to personnel or property. Use of these drugs is a professional and humane method for the euthanasia of swine.
HOST RESPONSE TO PERSISTENT INFECTION WITH HOG CHOLERA VIRUS

W. L. Mengeling and N. F. Cheville

INTRODUCTION

The existence of swine carriers of hog cholera virus (HCV) was confirmed experimentally by Gibbs in 1933.6 His studies in China followed suggestions of Chinese farmers that chronically ill pigs - survivors of hog cholera (HC) epizootics - were probable carriers of the virus. Similar observations also had been made in the United States.8 By inoculation of HC-susceptible pigs with tissue homogenates, Gibbs identified HCV in button ulcers dissected from the intestines of runt pigs that had had typical signs of HC as long as 92 days previously. As a result Gibbs stated that "Hog cholera virus is prevalent all over east and north central China, and in all probability its continued presence is due largely to the preservation of the virus in chronic carriers during the intervals between epizootics, because the farm animals of China are rather stationary owing to the lack of modern facilities of transportation."

The lability of HCV outside its host7 and the failure to incriminate nonswine hosts as carriers of HCV support Gibbs' hypothesis of the "natural" epizootiology of HC. However, swine management practices common today differ markedly from those common more than 30 years ago. Vaccination with modified live virus, the relatively free movement of pigs made possible by modern transportation, and continuous farrowing have all contributed to a more complex epizootiology.

Numerous sources of HCV are now recognized, but despite extensive investigation of epizootics that occurred in the United States in fiscal year 1968, the source of virus for approximately 15% could not be determined from the evidence available.11 It is likely that swine carriers were involved in some of these epizootics. Moreover, as the national hog cholera eradication program continues and other sources of infection are eliminated or controlled, the importance of swine carriers may increase correspondingly.

The purpose of this report is to summarize past2,10 and recent studies made at the National Animal Disease Laboratory concerning the pathogenesis of chronic HC. Thus far observations have been made of 22 pigs persistently infected with HCV, 3 of which were carriers of virus for 100 or more days.

MATERIALS AND METHODS

Chronic Hog Cholera.—Chronic HC is defined in this report as a lethal clinical form of HC with a duration of illness of 30 or more days.

Virus.—The 331 strain of HCV was used to produce chronic HC. This strain was isolated from an epizootic of HC that occurred in Iowa.9
Pigs.—All 22 pigs that developed chronic HC were specific pathogen-free (SPF), between 2 and 4 months of age when infected with strain 331, and were either hysterectomy-derived or normally farrowed progeny from hysterectomy-derived pigs. Twenty pigs were infected by subcutaneous injection of the virus and 2 pigs were infected by contact with pigs already infected with strain 331.

Collection of Samples.—Blood samples were collected by venipuncture. Other tissues were collected at necropsy from pigs killed during the course of chronic illness. Many of the pigs were killed in terminal illness when their temperature had dropped to subnormal values.

Assay and Analysis Procedures.—Virus, antibody, and serum proteins were identified and quantitated by methods previously reported. Histopathology.—The techniques used have been described.

Observations of Chronic Illness

Clinical Signs.—Based on clinical signs, chronic HC was separated into 3 phases of illness. The 1st or acute phase of illness was characterized by anorexia, depression, elevated temperature and leukopenia. After several weeks the appetite and general appearance of pigs often improved markedly and their temperature decreased, sometimes to normal or slightly above normal values. Leukopenia usually persisted. This general clinical improvement characterized a 2nd phase of illness. Eventually, pigs again became anorectic and depressed and their temperature often was elevated until shortly before death. Leukocytosis was associated with this terminal or 3rd phase of illness. The appearance, temperature, and leukocyte response of pigs during the course of chronic illness are presented (Fig. 1-3).

Viremia and Immune Response.—The amount of HCV detected in the blood of chronically infected pigs at different times during the course of illness varied with different pigs, but a general pattern of viremia was observed. Within approximately 2 weeks after infection, the titer of virus in serum reached a peak and thereafter declined until - with 9 of 12 pigs tested - virus no longer could be detected by isolation in cell culture. The apparent disappearance of virus was transitory, and the titer increased progressively during the remainder of the illness. The above pattern was probably the result of 2 factors; namely, a decrease in the number of cells releasing virus into the blood during the 2nd phase of illness and the amount of serum antibody. Direct staining of tissues with fluorescent antibody indicated that the number of cells producing virus may vary during the course of illness. This aspect will be discussed later. It is probable that most pigs produced antibodies to HCV during the course of chronic illness, however, detection of antibody by serum neutralization is dependent on its being in excess of virus in the blood, as was the case with pig 11746 (Fig. 4). Although such straightforward measurement of antibody was the exception, other findings were considered indicative of the presence of antibody and perhaps other inhibitors of viral infectivity. It was found that in some instances virus could be isolated in cell culture from diluted but not undiluted serum. Furthermore, dilutions of serums often indicated a higher titer of virus than did undiluted serums. Both of these observations, which suggest dissociation of virus and antibody, are exemplified with samples of serum collected from pig 11373 (Fig. 5). On the other hand, when antibody was in great excess of
HOST RESPONSE – HOG CHOLERA VIRUS

virus, as with pig 11746, dilution of serum did not result in isolation of virus in vitro. The difference between titers of virus indicated by in vitro and in vivo results—particularly with serum collected during the 2nd phase of illness—confirmed the presence of inhibitors of viral infectivity in vitro (Table 1).

Gamma Globulin.—An increase in gamma globulin usually accompanied the transient disappearance of HCV from the blood of chronically infected pigs. This finding further implicated the role of immunoglobulins in “masking” of virus present in serum. Moreover, as the disease continued and gamma globulin decreased, virus reappeared in the serum. The decrease in gamma globulins may be attributed to progressive depletion of lymphoid tissues, immune exhaustion, or both. Changes in gamma globulin concentrations during chronic illness are exemplified in Fig. 6.

Localization of Virus in Tissues.—Fluorescent antibody staining of tissues collected from pigs at various times during the course of chronic illness indicated that viral antigen was concentrated primarily in epithelial tissues, e.g., the epithelium of tonsil and ileum, particularly during the 2nd phase of illness. A more general distribution of viral antigen was occasionally seen both early and late in the course of illness. This suggests that associated with the immune response described earlier, lymphoid tissues were refractory to viral replication. Perhaps late in the course of illness, as a result of immune exhaustion, lymphoid tissues became susceptible to the virus. A marked contrast can be seen in the distribution of antigen in a tonsil collected 7 days postinoculation from a pig with acute hog cholera and in a tonsil collected 7 weeks postinoculation from a pig with chronic hog cholera (Fig. 7). The latter pig was in the 2nd phase of illness. Virus could not be isolated in cell cultures from its serum.

Gross Lesions.—Pigs killed early in the course of chronic illness, i.e., 1 to 2 weeks after infection, usually had the classical lesions of hemorrhage indicative of HC. On the other hand, pigs killed late in the course of chronic illness seldom had such lesions. However, a variety of other lesions, some of which were the result of secondary bacterial infection, were observed. In all 3 pigs that lived for 100 or more days, abscesses were found in various tissues. Ulcers of the caecum and colon, infarction of the spleen, and rib lesions were sometimes but not invariably seen. One of the most outstanding lesions was that of a general depletion of lymphoid tissue. Thymic atrophy is shown in Fig. 8.

Microscopic Lesions.—Lymphoid depletion was confirmed by histopathology (Fig. 9). The lesion of perivascular cuffing in the brain that is frequently found in acute HC was slight or absent in brains collected from pigs late in the course of chronic illness.

Secondary Infection.—At least in some cases, secondary bacterial infections were involved in the pathogenesis of chronic HC. The likelihood of secondary infarction is apparently related to the duration of illness. That is, it was found that secondary infections contributed to the death of all 3 pigs that had illnesses of 100 or more days. The most pronounced lesion of each of these pigs was: abscess of the brain, multiple abscesses of the lungs, and multiple abscesses throughout the muscles of the hip and thigh. In contrast, secondary infections were not incriminated in the death of pigs that lived less than 100 days. The reason for this may be explained by the following observations.
To determine the capability of pigs 11746 and 11373 to respond to antigens other than hog cholera virus during the course of illness, \(10^9\) killed *Brucella* organisms were administered subcutaneously in the axillary region. Blood samples were collected at weekly intervals and examined by the tube agglutination test for the presence of *Brucella* agglutinins.

*Brucella* organisms were administered to pig 11746 on the same day as HCV and at 2-week intervals thereafter. The pig responded with the production of agglutinins, and the agglutinin titer fluctuated between 1:20 and 1:80 during the approximately 9-week course of illness. At necropsy, secondary infection was not evident and bacteria were not isolated from any of the tissues examined (blood, liver, lung, spleen, kidney).

*Brucella* organisms were administered to pig 11373 on the 77th day of illness and at 2-week intervals thereafter. The pig did not respond with the production of agglutinins. At necropsy, on the 121st day of illness, multiple abscesses were found throughout the muscles of the hig and thigh. Bacteria, primarily *Proteus* and *Escherichia* spp., were isolated from the abscesses and tissues (blood, liver, lung, spleen, kidney).

Although general conclusions cannot be made from these limited findings, the implication is that during a long course of illness, the immune mechanism may become progressively less effective, thus rendering the pig more susceptible to secondary infection.

**GENERAL COMMENTS**

One of the difficulties encountered in studying the pathogenesis of chronic HC has been that of consistently producing this form of clinical illness. Selection of the strain of virus used in these studies (strain 331) was made from among numerous field isolates and several laboratory strains of HCV and was based on preliminary observations which indicated that this strain of moderate virulence had the greatest potential for producing chronic illness in highly susceptible SPF pigs. Even so, of 69 SPF pigs infected with strain 331 thus far, 33 died of the acute or subacute clinical form of HC and 14 recovered. Recovered pigs were all found immune when their immunity was subsequently challenged with the highly virulent Ames strain of HCV. Thus, only 22 of the 69 pigs developed a chronic clinical form of HC. Furthermore, 6 of the 22 were killed early, *i.e.*, before 30 days of illness, for collection of tissues and consequently a statement that these pigs would have lived for at least 30 days cannot be made with certainty.

As an aside from the primary objectives of these studies, it was established from the above that marked differences exist in the susceptibility of SPF pigs. Such differences are not often manifested with HCV of either very high or low virulence but are readily apparent when pigs are infected with moderately virulent strains such as strain 331.

Based on observations made thus far, most of which are summarized in Table 2, an hypothesis of the pathogenesis of chronic HC is presented. Because of the limited number of observations and the need to bridge numerous gaps in information with conjecture, it is emphasized that the following provides primarily a working hypothesis with which to correlate future observations and on which to base additional experiments.
Early after its introduction into the pig, HCV replicates and spreads relatively freely throughout various tissues. The extent of replication and spread of a moderately virulent strain of HCV such as strain 331 is probably intermediate to what would be found in the same host, infected with other strains of either greater or lesser virulence. Host responses to the presence of the virus include an attempt to clear it from infected tissues. Depending on the success of this response, virus is more or less cleared from tissues other than epithelium and masked in the blood. Detection of homologous antibody, coincident with the apparent disappearance (masking) of virus in serum, is probably a measure of only 1 of several events previously referred to collectively as the immune response. For example, interferon or interferon-like substances may also be important. Such events appear to reach their peak sometime during the interval from the 2nd to the 4th week postinfection. Thereafter, a resurgence of detectable viremia suggests that the immune response becomes progressively less effective.

The following question is therefore presented. Is the apparent impairment of the immune response and antibody production in particular, specific for antigens of HCV, or does it apply equally to other antigens? Attempts have been made to clarify this point experimentally; however, of 28 pigs involved in 2 experiments with this objective, only 1 pig in each experiment developed chronic HC. Results obtained with these pigs (Nos. 11373 and 11746) have already been presented. The progressive increase in detectable viremia which starts relatively early in the course of chronic illness taken together with results obtained from these 2 pigs suggest that a specific impairment of the antibody response to HCV precedes that to other antigens and that general impairment may occur only after an illness of particularly long duration. The latter may be the result of pronounced lymphoid depletion.

The frequent occurrence of acute reactions both early and late in the course of chronic illness allows speculation as to whether the underlying causes are the same in both cases. Although clinical signs are similar, tissue changes are not. For example, hemorrhages are more common and extensive in pigs killed early in the course of illness. In this connection, it is emphasized that after several weeks of persistent infection, pigs develop a tolerance to the "immediate" effects of HCV. Even though blood and other tissues may eventually contain a high titer of virus, a pig may appear almost clinically normal. Titers of the same magnitude in tissues of a pig soon after infection would be associated with marked clinical illness and are characteristic of acute HC. A highly superficial explanation of these observations might be that the molecular basis for the lethal effect of HCV is due to interference with 1 or more metabolic pathways for which alternate, but less commonly used, routes either exist or can be induced. If the major pathway were not entirely curtailed, as might be the case with moderately virulent HCV, perhaps with time an alternate pathway could assume the required function at a sufficient rate to supply the needs of the cell. Thereafter, the "immediate" lethal potential of HCV would no longer pose a problem. Cumulative changes could explain the late (terminal) acute reaction. The above is purely conjectural; however, studies are now in progress to determine changes in certain constituents of the blood which should reflect metabolic alterations characteristic of chronic HC.

Several findings are perhaps of more immediate importance to the eradication program. The general appearance of pigs persistently infected with strain 331, even
during the 2nd phase of illness, was seldom that of entirely healthy pigs. Moreover, pigs that were relatively tolerant to the effects of virus eventually succumbed to the combined effects of virus and secondary invaders. These observations suggest that pigs become neither inapparent nor permanent carriers of HCV. If this is true, the problem of swine reservoirs of HCV is lessened but not eliminated. Chronically ill pigs are, of course, temporary carriers and the absence of classical lesions of HC and the occasional masking of virus by antibody poses a significant problem for their identification. History of previous acute clinical illness of the pig in question or in the herd would bring HC into consideration. Attempts to isolate HCV should be made with both diluted and undiluted serum and if the pig is necropsied, spleen, ileum, and tonsil as well as serum should be examined. Direct examination of tissues with fluorescent antibodies might circumvent the problem of a diagnosis based on isolation of HCV in the presence of homologous antibody. Finally, it is emphasized that a pig persistently infected with HCV also may have a moderate titer of serum antibody.

REFERENCES

### TABLE 1 – ASSAY OF VIRUS IN SERUM OF CHRONICALLY INFECTED PIG 11373

<table>
<thead>
<tr>
<th>Day of illness sample collected</th>
<th>Phase of illness</th>
<th>Titer/ml in vitro</th>
<th>Titer/ml in vivo</th>
<th>Remarks concerning in vivo assay*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2</td>
<td>0-10^2.6**</td>
<td>10^5</td>
<td>Dilutions 10^-2 through 10^-8 were tested. The 10^-3 dilution was administered to 2 pigs and the 10^-4 dilution was administered to 3 pigs. All other dilutions were each given to 1 pig. One pig given the 10^-3 dilution died of subacute hog cholera. Two pigs given the 10^-4 dilution died of chronic hog cholera. All other pigs were either found immune or died following subsequent challenge.</td>
</tr>
<tr>
<td>121</td>
<td>3</td>
<td>10^6.1</td>
<td>10^8</td>
<td>Dilutions 10^-5 through 10^-9 were tested. One pig was used/dilution. Pigs given 10^-5, 10^-6 and 10^-8 dilutions were found immune following subsequent challenge. The pig given the 10^-7 dilution died of chronic hog cholera. The pig given the 10^-9 dilution died following challenge.</td>
</tr>
</tbody>
</table>

*Pigs were given 1 ml. of appropriate dilution by subcutaneous injection.  
**Depending on dilution of serum used for the assay. (See Fig. 5).
<table>
<thead>
<tr>
<th>Observation</th>
<th>I</th>
<th>Phase of illness</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>2-4 weeks</td>
<td>Variable, primary factor in determining length of illness</td>
<td>Usually 2 weeks or less</td>
<td></td>
</tr>
<tr>
<td>Appetite</td>
<td>Poor</td>
<td>Improved, occasionally almost normal</td>
<td>Poor or absent</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Elevated</td>
<td>Reduced, occasionally normal</td>
<td>Often elevated</td>
<td></td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>Leukopenia</td>
<td>Leukopenia</td>
<td>Leukocytosis</td>
<td></td>
</tr>
<tr>
<td>Viremia detectable in vitro</td>
<td>Moderate to high</td>
<td>Absent to moderate</td>
<td>Moderate to very high</td>
<td></td>
</tr>
<tr>
<td>Gamma-globulin level</td>
<td>Normal</td>
<td>Usually hyper-gammaglobulinemia</td>
<td>Hyper-to-hypo-gammaglobulinemia</td>
<td></td>
</tr>
<tr>
<td>Immune response</td>
<td>Yes</td>
<td>Yes early, reduced or absent late</td>
<td>Reduced or absent</td>
<td></td>
</tr>
<tr>
<td>Localization of virus in tissues</td>
<td>General</td>
<td>Epithelial cells</td>
<td>General</td>
<td></td>
</tr>
<tr>
<td>Gross lesions</td>
<td>Classical lesions of hemorrhage</td>
<td>. . .</td>
<td>Lymphoid depletion, button ulcers, infarction of spleen, rib lesions, lesions associated with secondary infections</td>
<td></td>
</tr>
<tr>
<td>Microscopic lesions</td>
<td>Vascular degeneration. Hemorrhage</td>
<td>Glomerulonephritis</td>
<td>Absence of germinal centers, thymic atrophy, extramedullary hematopoiesis, glomerulonephritis, lymphoid depletion, micro-abscesses</td>
<td></td>
</tr>
<tr>
<td>Secondary infection</td>
<td>Unlikely</td>
<td>Unlikely early</td>
<td>Likely</td>
<td></td>
</tr>
</tbody>
</table>

*Clinical signs and, in part, gross lesions agreed with those previously reported.1,3,4,5*
Figure 1. Appearance of pigs during the 2nd phase of chronic illness. Above pig 11373 on the 98th day of illness. Below, pig 11746 on the 42nd day of illness.
Figure 2. Temperature response of pigs 11373 and 11746 during the course of chronic illness. Both pigs were killed on the day their temperature decreased to subnormal.

Figure 3. Leukocyte response of pigs 11746 and 11747. Pig 11746 was persistently infected with hog cholera virus. Pig 11747 was a noninfected, littermate control.
Figure 4. Titration of hog cholera virus and homologous antibody in serum collected during the course of chronic illness of pig 11746. Antibody was in excess during weeks 2 through 8.

Figure 5. "Apparent" differences in the titer of hog cholera virus in serum of pig 11373 based on plaque counts obtained with various dilutions of serum.
Figure 6. Changes in gamma globulin concentrations during the course of chronic illness. Pigs 11373 and 11746 were persistently infected with hog cholera virus. Pig 11747 was a noninfected littermate of pig 11746.

Figure 7. Localization of viral antigen in the tonsil: (left) tonsil collected from a pig with acute hog cholera on the 7th day postinfection; (right) tonsil collected from a pig with chronic hog cholera on the 49th day postinfection. In the tonsil on the right, antigen is localized in the epithelium of the tonsillar crypt.
Figure 8. Moderate thymic atrophy: (left) thymus from pig 11746 persistently infected with hog cholera virus; (middle and right) thymuses from noninfected pig 11747, littermate control, and pig 11753, from another litter but of the same age, i.e., 5½ months at the time of necropsy. Thymic atrophy is frequently more severe than shown here.2
Figure 9. Lymphoid depletion: (left) tonsil collected from pig 11746 at necropsy on the 62nd day of chronic illness; (right) tonsil collected from noninfected littermate (pig 11747) on the same day. Figure 1. Appearance of pigs during the 2nd phase of chronic illness. Above pig 11373 on the 98th day of illness. Below, pig 11746 on the 42nd day of illness.
SPREADING CHARACTERISTICS OF COMMERCIAL HOG CHOLERA MODIFIED LIVE VIRUS VACCINES IN SWINE. II. IN VITRO SERUM NEUTRALIZATION STUDIES

M. R. Zinober and L. O. Mott

Early work in serum neutralization (SN) was done by Craig in 1915, by Healy and Gott in 1916, by Kernkamp and Roepke in 1942, and by Cole et al. in 1951. These workers had the disadvantage of not having an in vitro indicator system and therefore had to obtain the result of their neutralization procedures in the live pig. The principal contribution of these early workers was their demonstration that hog cholera virus (HCV) can be neutralized by antiserum in vitro.

Coggins and Sheffy in 1961 described a SN procedure utilizing a cytopathogenic virus strain previously described by Gillespie et al. Robson et al. and Van Bekkum also have used this procedure for serological studies with HCV.

In 1963, Mengeling et al. described a SN procedure utilizing a fluorescent antibody (FA) technique as the indicator system.

Simonyi utilized the cytopathogenic C24V strain of bovine virus diarrhea (BVD) virus in a SN procedure. This strain of BVD virus had been demonstrated by Darbyshire to have a serological relationship with HCV.

MATERIALS AND METHODS

A report on the spreading characteristics of modified live hog cholera virus (HCV) vaccines included a projected SN phase. One hundred and fifty pigs were placed in contact with vaccinated pigs two days postvaccination with modified live HCV vaccines. Twenty-four contact pigs died of hog cholera and are not included in this report. All surviving contact pigs were bled for serum two times; first, before they were put in contact (P-C) with the vaccinated pigs, then again 28-30 days postcontact (DPC), which was immediately prior to challenge. The day of treatment or contact is considered to be day 0 in every case.

Vaccines.—There were 33 modified live HCV vaccines used simultaneously with antiserum and also without antiserum for swine inoculations. All but one of these vaccines were included in these studies. The single vaccine which is not included is one which transmitted virus in lethal form from vaccinates to all contacts and therefore no pigs were available for these studies. The remaining 32 vaccines consisted of 14, 5 and 13 vaccines of lapine origin, porcine origin and tissue culture origin, respectively.

Contact Pigs.—A total of 126 pigs, consisting of 55, 23 and 48 pigs were exposed to other pigs vaccinated with modified live HCV vaccines of lapine origin, porcine origin and tissue culture origin, respectively.

Tissue Culture.—An established line of pig kidney cells (PK-15) were used. The cells were established on 10.5 x 50 mm. coverslips in short Leighton tubes and incubated at 37°C for 24 hrs., at which time they were used. The cell growth was about 75 percent confluent at that time. The growth medium was Eagle’s minimum
essential medium, with 2 percent normal swine serum.

Virus.—The Ames strain of HCV (AHCV) was used. It was free of variant characteristics and cytopathic effects. The virus was prepared as serum-virus, distributed in screw-cap vials in 2.0 ml. amounts and frozen at -60°C until used. The titer was $10^6$ plaque forming units per milliliter (PFU/ml). For use in the neutralization procedure it was diluted in Earle's balanced salt solution (BSS) containing 10 percent swine serum to give about 100 PFU/0.1 ml.

Serum.—The paired serums were collected P-C and 28-30 DPC and were inactivated at 56°C for 30 minutes.

Neutralization Procedure.—The beta system of SN was used. Twofold dilutions of serum were prepared in Earle's BSS so that each tube had 1 ml. of diluted serum before 1 ml. of the virus was added. All the tests and controls were incubated at 37°C for 1 hour.

Growth medium was removed by aspiration from culture tubes and each of four cultures was inoculated with 0.2 ml. of each dilution of the serum-virus mixture. This volume of inoculum was also used in the controls. The tubes were placed in racks on a mechanical rocker and virus-serum mixtures were adsorbed at 37°C for 2 hours.

After this time, the inoculum was removed from the tubes and, as was demonstrated by Mengeling, in order to prevent the formation of secondary plaques, 2 ml. of antiserum overlay was added to each tube. The antiserum overlay consisted of 0.25 ml. hog cholera antiserum in about 1000 ml. of Earle's BSS containing 10 percent normal swine serum and 0.5 percent lactalbumin hydrolysate. Antiserum was present in a final concentration of about 1:4000. The cultures were then incubated at 37°C for 48 hours prior to processing coverslips for fluorescence microscopy.

From previous studies it had been shown that our SN procedures revealed the presence of some plaque inhibiting factor (PIF) in almost all pigs, including normal pigs. It was therefore decided to determine the PIF level of each pig before placing it in contact with a vaccinate and to use this value in the form of PFU's as a baseline for further calculations. The same dilution of both the P-C and the 28-30 DPC sera was selected for calculation. The dilution which was selected was the lowest one in which there were PFU's on each one of the coverslips. The extent of the increase in PIF after contact was then stated as a percentage of the P-C PFU count and was expressed as percent plaque inhibition (percent PI).

The calculations were made according to the following formula:

$$\text{Percent PI} = \frac{\text{PFU}_{28-30 \text{ DPC}} - \text{PFU}_{\text{PC}}}{\text{PFU}_{\text{PC}}} \times 100$$

where: 
\[ \text{PI} = \text{Plaque inhibition} \]
\[ \text{PFU}_{\text{PC}} = \text{Average number of PFU's of the P-C serum.} \]
\[ \text{PFU}_{28-30 \text{ DPC}} = \text{Average number of PFU's of the serum taken 28-30 DPC.} \]

More than 50 percent PI was taken as evidence of transmission of an antigen with immunizing characteristics and PI of more than 10 percent was taken to indicate probable transmission of such an antigen.
Clinical Reactions.—Clinical reactions were evaluated by consideration of the first day of visible sickness, the number of days of sickness, the severity of the sickness in points and the day of death. The method of determination of the point values is described in an earlier report. The significance of differences was calculated according to the distribution of t with acceptable significance established at $P < 0.05$.

RESULTS AND DISCUSSION

Lapine Origin Vaccines.—On the basis of survival of in vivo virulent virus challenge, 25 of 55 pigs received transmitted virus with immunizing characteristics and 30 did not. By means of SN procedures, 24 of the transmissions (96.0 percent) were confirmed. PI ranged from 50.0 percent to 92.3 percent (average, 66.2 percent) (Table 1).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>In Vivo+</th>
<th>In Vivo+</th>
<th>In Vivo-</th>
<th>In Vivo-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>I</td>
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<td>0</td>
<td>1</td>
<td>3</td>
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</tr>
<tr>
<td>BB</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>CC</td>
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<td>1</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>DD</td>
<td>4</td>
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<td>0</td>
<td>4</td>
</tr>
<tr>
<td>EE</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>FF</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
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<tr>
<td>GG</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Number of pigs | 24 | 1 | 6 | 24 | 55
Number of vaccines | 11 | 1 | 6 | 9 | 14
Percentage of plaque inhibition | 50.0 - 92.3 | 0.0 | 12.0 - 53.7 | 0.0 - 9.8 | 0.0 - 92.3
Average | 66.2 | - | 31.3 | 2.1 | 33.2

*In Vivo + Survival after virus challenge. Transmission of virus with immunizing characteristics.
*In Vivo - No survival after virus challenge. No transmission of virus.
*In Vitro + Serum neutralization plaque inhibition > 50.0 percent.
*In Vitro - Serum neutralization plaque inhibition < 50.0 percent.

Serum neutralization of the 30 pigs which gave no evidence of transmission of viral immunizing characteristics following in vivo challenge revealed that in six of
them there had been an increase in PIF and thus transmission of an antigen. The remaining 24 negative pigs were confirmed as negative by SN.

Of special interest are the six pigs which gave no evidence of transmission of virus with immunizing characteristics in vivo, but which (by SN) revealed the presence of plaque inhibiting capability of 31.3 percent, and therefore probable transmission of HCV with immunizing characteristics. Although these pigs died following challenge with virulent virus, the fact that SN revealed the probable transmission of a virus which induced the production of a PIF suggested the possibility that the clinical reaction to challenge may also have revealed slightly greater resistance than those pigs which did not demonstrate this plaque inhibiting factor. In order to determine this, the clinical records of these six pigs were compared to the clinical records of the 24 pigs which were negative for transmission of immunizing virus both by in vivo and by in vitro procedures, (Table 2).

Table 2 reveals that with the exception of the difference in the occurrence of the first day of sickness this was the case with a very low probability that this could have happened by chance (<0.001). Statistical analysis confirmed that there was actually a resistance factor present in the pigs which had been in contact with the vaccinated pigs. With the exception noted above, these six pigs were sick longer, they had more points, and they died later.

**TABLE 2**

Postchallenge Clinical Reactions of Pigs Following Contact Exposure to Modified Live Hog Cholera Virus Vaccine of Lapine Origin

<table>
<thead>
<tr>
<th>Number of pigs</th>
<th>Transmission of HCV Immunizing Characteristics</th>
<th>Postchallenge Clinical Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vivo</td>
<td>In vitro</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significance: P < 0.05.

The SN test failed to confirm transmission of virus from vaccinates to one contact pig. This pig survived virus challenge indicating that there had actually been transmission of virus with immunizing characteristics.

**Porcine Origin Vaccines.**—On the basis of survival of in vivo virulent virus challenge, 13 of 23 pigs transmitted virus with immunizing characteristics and 10 did not. This transmission was confirmed by SN in every case (Table 3). The range of PI was from 53.3 to 94.3 percent (average, 79.6 percent). Ten of the pigs were negative with a range of PI from 0 - 1.8 percent (average, 0.4 percent). Five of them, however, on SN were found to have PI range from 36.2 percent to 52.1 percent (average, 40.6 percent) indicating probable transmission of HCV with immunizing characteristics (Table 4). Comparison of clinical reactions of these five pigs with those which were negative on both in vivo and in vitro test showed that this was the case with <0.01 probability that this could have happened by chance.
**Tissue Culture Origin Vaccines.**—On the basis of survival of *in vivo* virulent virus challenge, 25 of 48 pigs transmitted virus with immunizing characteristics. This was confirmed in 23 (92.0 percent) of them by SN procedures (Table 5). In these 23, the range of PI was 51.1 - 96.4 percent (average, 80.6 percent).

**TABLE 3**

Transmission of Virus with Immunizing Characteristics from Pigs Vaccinated with Modified Live Hog Cholera Virus Vaccine of Porcine Origin to Contact Pigs. Comparison of Results of Virus Challenge and Serum Neutralization

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>( \text{In Vivo}^+ )</th>
<th>( \text{In Vivo}^* )</th>
<th>( \text{In Vitro}^+ )</th>
<th>( \text{In Vitro}^* )</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>K</td>
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<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6</td>
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<tr>
<td>M</td>
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<td>N</td>
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<td>1</td>
<td>0</td>
<td>4</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>Number of pigs</th>
<th>13</th>
<th>0</th>
<th>5</th>
<th>5</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vaccines</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Percentage of plaque inhibition</th>
<th>Range</th>
<th>(53.3 - 94.3)</th>
<th>(36.2 - 52.1)</th>
<th>(0.0 - 1.8)</th>
<th>(0.0 - 94.3)</th>
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<tbody>
<tr>
<td>Average</td>
<td>79.6</td>
<td>(\text{In Vivo}^*)</td>
<td>40.6</td>
<td>0.4</td>
<td>53.9</td>
</tr>
</tbody>
</table>

*\(\text{In Vivo}^+\)* Survival after virus challenge. Transmission of virus with immunizing characteristics.

*\(\text{In Vivo}^*\)* No survival after virus challenge. No transmission of virus.

*\(\text{In Vitro}^+\)* Serum neutralization plaque inhibition <50.0 percent. Transmission of virus with immunizing characteristics.

*\(\text{In Vitro}^*\)* Serum neutralization plaque inhibition <50.0 percent. No transmission of virus.

**TABLE 4**

Postchallenge Clinical Reactions of Pigs Following Contact Exposure to Modified Live Hog Cholera Virus Vaccine of Porcine Origin

<table>
<thead>
<tr>
<th>Number of pigs</th>
<th>( \text{In Vivo}^+ )</th>
<th>( \text{In Vivo}^* )</th>
<th>( \text{In Vitro}^+ )</th>
<th>( \text{In Vitro}^* )</th>
<th>( \text{First day sick} )</th>
<th>( \text{Day of death} )</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>5.0</td>
<td>11.0</td>
<td>24.4</td>
<td>16.0</td>
<td>3.4</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td>P</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
<td>(&lt;0.01)</td>
</tr>
</tbody>
</table>

Significance: \(P < 0.05\).
Transmission of Virus with Immunizing Characteristics from Pigs Vaccinated with Modified Live Hog Cholera Virus Vaccine of Tissue Culture Origin to Contact Pigs. Comparison of Results of Virus Challenge and Serum Neutralization

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>In Vivo+</th>
<th>In Vito+*</th>
<th>In Vivo-</th>
<th>In Vito-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>P</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
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<td>Q</td>
<td>3</td>
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<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>R</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>S</td>
<td>4</td>
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<td>0</td>
<td>0</td>
<td>4</td>
</tr>
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<td>T</td>
<td>3</td>
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<td>0</td>
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</tr>
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<tr>
<td>AA</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Number of pigs: 23 (In Vivo+), 2 (In Vito+*), 2 (In Vivo-), 21 (In Vito-), 48 (Total)

Number of vaccines: 9 (In Vivo+), 2 (In Vito+*), 2 (In Vivo-), 7 (In Vito-), 13 (Total)

Percentage of plaque inhibition:

<table>
<thead>
<tr>
<th>Range</th>
<th>In Vivo+</th>
<th>In Vito+*</th>
<th>In Vivo-</th>
<th>In Vito-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.1 - 96.4</td>
<td>80.6</td>
<td>46.5</td>
<td></td>
<td></td>
<td>26.5</td>
</tr>
</tbody>
</table>

*In Vivo* + Survival after virus challenge. Transmission of virus with immunizing characteristics.
*In Vito* - No survival after virus challenge. No transmission of virus.
*In Vito* + Serum neutralization plaque inhibition < 50.0 percent. Transmission of virus with immunizing characteristics.
*In Vito* - Serum neutralization plaque inhibition < 50.0 percent. No transmission of virus.

Twenty-three of the 48 pigs were negative by *in vivo* challenge and this was confirmed in 21 of them by SN procedures (Table 6). Two other pigs, however, were negative by *in vivo* challenge but SN indicated that the probable transmission of HCV with immunizing characteristics had occurred. The percentage of PI of the 2 pigs was 44.9 percent and 48.0 percent (average, 46.5 percent). Although examination of postchallenge clinical reactions suggested the actual presence of a PIF, there was an insufficient number of pigs to make a sound statistical evaluation.

The SN test failed to confirm transmission of virus from 2 different vaccines to 2 contact pigs. These pigs survived virus challenge indicating that there had actually been transmission of virus with immunizing characteristics.

Table 7 is a collective presentation of data from all types of vaccine to facilitate comparison.

Evidence of contact transmission of immunizing HCV from pigs vaccinated with modified live HCV vaccine is confirmed by our SN results. Of 63 pigs which
demonstrated transmission of immunizing HCV in vivo, SN confirmed 60 or 95.2 percent which had been exposed to pigs vaccinated with 25 or 33 vaccines. Furthermore, 13 pigs which had been exposed to 11 vaccines were negative for transmission by in vivo challenge and were positive on SN tests. This transmission was further confirmed by an examination of the clinical postchallenge record of these pigs compared to pigs which were negative for transmission both by in vivo and in vitro procedures. In each one of the 13 pigs, although they all died following challenge, the 1st day of sickness was later, they were sick longer, they accrued more points and they died later. With the few exceptions noted in the text, the differences were statistically significant.

**TABLE 6**
Postchallenge Clinical Reactions of Pigs Following Contact Exposure to Modified Live Hog Cholera Virus Vaccine of Tissue Culture Origin

<table>
<thead>
<tr>
<th>Number of pigs</th>
<th>Transmission of HCV immunizing characteristics</th>
<th>Postchallenge Clinical Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vivo</td>
<td>In vitro</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

No statistical comparison made; insufficient number of pigs.

**TABLE 7**
Transmission of HCV-Immunizing Characteristics by Modified Live Hog Cholera Virus Vaccine of All Types

<table>
<thead>
<tr>
<th>Transmission</th>
<th>Number of Vaccines*</th>
<th>Number of Pigs</th>
<th>Percentage of Plaque Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td>In vitro</td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>18</td>
<td>50</td>
</tr>
</tbody>
</table>

*Several vaccines appear in more than one category.

Two vaccines that had been considered negative for causing transmission of immunizing virus were, on the basis of SN procedures, found actually to have been positive for this characteristic. These facts are very important in the present stage of the hog cholera eradication program and will increase in importance as the incidence of the disease declines. The SN procedure described above is a tool which could be used to detect previous exposure to HCV and therefore the existence of foci of subclinical HCV. As the virus becomes scarce, it will become more and more cryptic and arcane and the methods needed to disclose its presence will have to become even more subtle and sophisticated.
SUMMARY AND CONCLUSIONS

1. *In vitro* serum neutralization procedures revealed transmission of hog cholera virus with immunizing characteristics from pigs vaccinated with 27 of 33 modified live HCV vaccines of all 3 types (lapine origin, porcine origin and tissue culture origin) to 73 of 126 pigs by contact exposure as revealed by serum neutralization procedures. This was true regardless of whether the vaccines were administered simultaneously with antiserum or without antiserum. This includes two vaccines which were revealed by serum neutralization procedures to have been transmitted from vaccinates to contacts which were not revealed by *in vivo* procedures.

2. The serum neutralization testing procedure demonstrated transmission of immunizing HCV to 13 additional contact pigs from 11 vaccines of all types as compared with the *in vivo* challenge testing procedure. The *in vitro* test also failed to identify transmission to three pigs which were positive on *in vivo* challenge.

3. Serum neutralization procedures were used to detect previous exposure and the existence of foci of subclinical hog cholera virus. As the incidence of this disease declines serum neutralization procedures can become a valuable aid to detect such low grade foci of infection.

REFERENCES


The authors gratefully acknowledge the technical assistance of V. L. Wiltsey.
STATUS OF STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

M. J. Tillery DVM*
Hyattsville, Maryland

INTRODUCTION

In almost six years of cooperative effort, all States have initiated the hog cholera eradication program and most are operating in advanced phases. Hog cholera incidence has been markedly reduced.

However, some States have been unable to hold to levels achieved earlier. Others appear to be static causing the program to fall behind earlier established goals. Both situations bear on the ability to complete this program by 1972, the date recognized in 1965 by this Association as being "realistic and attainable."

Today, let's review progress through June 1968 and let's examine some of the obstacles which stand in the way of hog cholera eradication by 1972.

Program Status

By July 1, 1968, all States had moved beyond Phase I of this effort. Twelve remained in Phase II and 38 States and Puerto Rico were operating in Phases III or IV. Of the latter, seven were hog cholera free.

*Senior Staff Veterinarian, Swine Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.

U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
The status lost by some States during the year partially offset gains made in
others. Actually, 40 States operated in Phases III or IV at some point. However,
Mississippi and New Jersey retreated to Phase II due to lack of funds to continue
necessary indemnities. Both States have since requested funds to support a return
to Phase III.

Loss of hog cholera free status occurred when Utah and Vermont experienced
a series of hog cholera outbreaks coupled with failure to carry out “free” State
standards in their entirety. With these two States losing status and Washington and
Oregon qualifying as hog cholera free, seven States were hog cholera free at both
the start and close of the year.

More pronounced advancement occurred at lesser phases. For example, 16
States were in Phase IV in July 1968 compared to 13 a year earlier. Twenty-two
States and Puerto Rico were in Phase III compared with 15 last year. Twelve States
were in Phase II, a reduction from the 21 States in this phase last year. No States
remained in Phase I by July 1968.

Several aspects of this advancement should be considered. First, the Western
area in Phase IV has enlarged. Other States reached and maintained Phase IV.
Through these six years of program activity, hog cholera has failed to gain a
foothold in any of these States to force deviation from these standards in order to
eliminate the infection. True, deviations have occurred with a loss in status
resulting; however, these were administrative choices rather than veterinary
necessities.

Probably, the most encouraging advances occurred in the area with heavier
swine populations. By July 1968, all of the North Central and Southeastern States
except two were in Phases III or IV. Indiana, Illinois, and Iowa had periods of at
least 60 days with no hog cholera confirmed.

Program Standards

The ability of the Phase IV States to hold their position and the heavy swine
producing States to depress the incidence of hog cholera again lends evidence to the
practicality of these standards. In the Phase IV States, these rules were challenged
when hog cholera occurred. In seven, incidence was sporadic and eliminated
without dropping below Phase IV. A more severe challenge occurred in North
Dakota when 14 outbreaks occurred over a six-week period. These were eliminated
in accordance with standards and no hog cholera has occurred in the State since.

The standards served those Phase III States with exceptionally high hog cholera
incidence. North Carolina and Missouri had the heaviest incidence during the year.
Even so, there were considerably fewer outbreaks in these States at the close of the
year than at the beginning.

These experiences in Phase III and IV States lend considerable evidence that
these standards are sufficient to eradicate hog cholera. Program officials must guard
against any temptation to apply less action in any situation. Any who elect to
operate a program which does not embrace all actions required at his level must
recognize that he runs a heavy risk of failure. This risk is largely negated by a
program of the intensity demanded by those rules laid down by this Association.
During the year USLSA’s Committee on State-Federal Relations expressed concern that some States might be recognized at a level which was not being carried out. On being surveyed at year’s end, all veterinarians in charge reported that these operations had been reviewed with their cooperating State officials. No deviations from the standards were in operation, although some difficulties were noted in application. Therefore, each State is now at a level of activity which can be carried out.

Static Programs

Some programs appear to have become static. Stationary hog cholera eradication programs often lead to heavier incidences. Therefore, these static programs must be regarded as threats to the gains made not only in those States but others as well.

The main concern is with longstanding Phase II programs. In September 1968, 10 States remain in Phase II. Three of these have occupied Phase II for almost five years. Three of these 10 States did not have any hog cholera in 1968.

It appears that ample time has elapsed for all these States to have completed Phase II with some potentially hog cholera free, had they elected to have operated with reasonable speed. If our aim continues to be hog cholera eradication by 1972, we cannot be tolerant toward these static Phase II programs.

A similar situation has developed in certain Phase III States. Three of the 23 had no outbreaks and two had only one outbreak throughout the year. Therefore, it appears that at least five of these States, have long ago satisfied Phase III-elimination of foci of infection. They should proceed to Phase IV without further delay.

Perhaps the reason for remaining in Phase III overly long is the salvage provisions which must be abandoned in Phase IV. This is risky. Due to information pointing to hog cholera spread from salvaged swine, some Phase III States have abandoned salvage in favor of destroying all swine in infected herds by other means. No State should remain in Phase III merely to retain the opportunity to salvage swine through slaughter as this operation may prove expensive in the long run.

Hog Cholera Incidence

The ultimate measure of success of an eradication program is its effect on disease incidence. In these six years of hog cholera eradication, incidence has fallen at least 80 percent from the estimated 5000 to 6000 outbreaks annually from 1960 to 1962.

In the past year, hog cholera occurred in 379 counties in 34 States, little change from 1967. Hog cholera occurred in five Free States compared to one in 1967.
These "free" States are vulnerable to the introduction of hog cholera. Imported swine, garbage feeding, and inactivated vaccines may reintroduce the disease. Inactivated vaccines have been incriminated in field investigations although experimentation has been inconclusive.

Once having reached hog cholera free status, these States must rigidly adhere to program standards in order to prevent the spread of hog cholera if introduced.

In fiscal year 1968, 849 hog cholera outbreaks occurred, a decrease from the 1960 to 1962 incidence but an increase over 1966 and 1967. To expect that incidence would uneventfully decline over the program's schedule would be unrealistic.
One of the basic eradication activities is to gather sufficient epidemiological information so that future actions have a sound footing of technical data.

One of the areas to examine is reporting systems. In 1968, the confirmation rate fell to 21 percent compared to 70 percent in 1964. This indicates a steady improvement in reporting. This trend must be watched closely, as a direction other than downward is good evidence that reporting systems are failing.
Those in Phase IV must be especially alert to reports of suspicious illnesses ceasing with the elimination of hog cholera. To overlook this program action invites the possibility of hog cholera being introduced and spreading disastrously before detection. Strong information efforts must be maintained so that complacency does not arise in these times of hog cholera freedom.
Laboratory support continues to improve with 94 percent of all outbreaks having been confirmed with laboratory aid in 1968. Now, all swine specimens sent to laboratories should be examined for hog cholera. Several States pursue this course today, and hog cholera is identified from some of these tissues, although the disease was not suspected clinically. These practices probably contributed to increased incidence in 1967 and 1968, as they were seldom followed earlier.

The years 1967 and 1968 have been a transition period from hog cholera control–Phase II–to hog cholera eradication–Phases III and IV. Historically, an upgrading in program status has led to an apparent increase in hog cholera outbreaks.
The reported increase is probably most severe in moving to Phase III as the swine owner may recoup his hog cholera losses through indemnity payments which are initiated here. With most of the transition involving Phase III, these advances have likely contributed more heavily to the increases than earlier movements to Phase I or II.

Some States had an unexpected incidence in 1968. Surely, hog cholera was not anticipated in the hog cholera free States. Of more significance, three States, Georgia, Missouri, and North Carolina, had unexpectedly heavy incidences in the year with 333 confirmed hog cholera outbreaks, 39 percent of the total, although they contain only 11 percent of the swine. Similarities existed in their situations. All three have closely adhered to program standards in the face of adversity. Probably most important, the swine industry as well as cooperating officials in these States have remained firm in their support of those actions found necessary.
Epidemiology

Hog cholera continues to be spread by traditional methods. Movement of swine and area exposure continue to account for 48 percent of the hog cholera. Although vulnerable to actions such as marketing regulations and quarantine procedures, improvement in the administration of such procedures is indicated.

Georgia pioneered in this area in the spring of 1968. With hog cholera commonly spreading through marketing movements, Georgia imposed a statewide quarantine last April. This quarantine, held for 60-days, was instrumental in reducing spread. However, incidence increased partly through market spread after the quarantine was released, and, the quarantine was re-imposed in August 1968. The latter action is less restrictive because it apparently must be held longer to be entirely effective. We should learn much from this effort. When a sudden increase in outbreaks occurs, marketing movements should be watched closely to determine whether action similar to Georgia’s is indicated.

Vaccination continues to account for 21 percent of the outbreaks. Federal and State actions have been taken to limit this problem. These actions have been somewhat effective in that vaccination associated hog cholera declined from the 33 percent in 1967.

In July 1967, Federal regulations were amended to provide for approval of certain vaccines for continued program use. In August, serum alone treatment was provided for swine to be moved into States which would accept them. By July 1968, 35 States would accept these swine.
HOG CHOLERA ERADICATION PROGRAM

States have acted against hog cholera vaccines. By July 1968, seven had restricted use of all vaccines and 23 States had restricted use of modified live virus vaccines. In these 30 States, the restrictions have practically stopped vaccination, with the only use of the vaccines in question being to qualify swine for shipment to States requiring vaccination. An additional six States provide for use of modified live virus vaccines by permit only. Therefore, 36 States have restricted use of hog cholera vaccines ranging from permitted use to virtual prohibition of usage in their programs.

A lag period was expected to occur before these actions had an effect on hog cholera. In the first six months of the year, 471 outbreaks occurred with 27 percent attributed to vaccination. During the last six months, 378 outbreaks occurred with 13 percent attributed to vaccination. Therefore, these actions appear to have become effective about December and hog cholera incidence appears to have again turned downward.

Hog cholera vaccines continued to be incriminated regardless of the propriety of use. Of the 176 vaccine-associated outbreaks, field veterinarians judged the vaccination to have been done properly in 63 instances and to have been done improperly in 113 instances. Improper use involved both lay and veterinary administration.

With use of vaccines continuing to be a major source of hog cholera, these practices should be abandoned. In 1968, 24 percent of the Federal indemnities were spent on vaccine-induced hog cholera. Field veterinarians have incriminated all modified live virus products and most of the inactivated products as causing hog cholera. No more than 25 to 30 percent of the swine population could be vaccinated with the vaccines being marketed. Some individuals—both lay administrators and veterinarians—choose to administer these vaccines contrary to veterinary knowledge as well as State and Federal restrictions. Finally, we know from the field evidence that the vaccines cause hog cholera even when administered properly. With this accumulation of epidemiology and administrative experience, continued vaccine usage in Phase III and IV States appears foolhardy. Even usage in Phase I and II States would appear subject to debate.

Therefore, other States are scheduling restrictions on hog cholera vaccines and proposed amendments to Federal regulations are planned. Under the Federal proposals, modified live virus vaccines and inactivated vaccines could not be shipped interstate into Phase III and IV States after January 1, 1969. Any swine vaccinated after January 1, 1969, in these States could be shipped interstate for feeding or breeding until July 1, 1969, but not thereafter. Phase I and Phase II States would be permitted to import inactivated vaccines if compatible with State requirements. In addition, swine officially vaccinated with inactivated vaccines in Phase I and II States could move interstate beyond July 1, 1969. The reasoning here is that indemnities are not available in these States to help owners recover losses due to hog cholera. Therefore, some means of protection should be afforded them.

Again, the Phase II States are hampering overall progress. These States should advance without further delay as the time may arrive when the needs of the national program will require complete elimination of all hog cholera vaccines. When this time comes, or when all States reach Phase III, no hog cholera vaccines
would remain in field use.

Raw garbage feeding accounted for seven percent of the outbreaks, above the proportion which might be expected with garbage fed to one to two percent of the swine. However, we may be misled here in that this includes hog cholera spread from household garbage usually exempt from State garbage cooking laws. Epidemiologists in at least two States, Georgia and Tennessee, have developed information to incriminate salvage procedures as the basic cause of several outbreaks. Georgia officials conclude that one salvage operation likely was the stimulus which resulted in over 160 outbreaks since January 1968. In many instances, the garbage fed was household table scraps containing pork from certain establishments about the time they had salvaged swine from infected herds. National surveys have indicated that about one percent of the salvaged swine which are passed for food contain virus.

A precaution accompanied the provision for salvage operations in Phase III. This warning was to the effect that salvage operations should cease when they represent a threat to the program. The known risk coupled with the Georgia and Tennessee experience should cause program officials to look upon salvage with suspicion. Some Phase III States have already found salvage through slaughter to be intolerable. Others should watch this operation closely and abandon it as soon as financially able to do so.

Perhaps the time has arrived to eliminate this stigma of garbage feeding together with the disease risks and associated problems. A prohibition would involve close planning with centers which generate garbage so that disposal by other means is orderly and without hardship. Administration of a prohibition might be as expensive as the present inspection system. Such prohibition would eliminate a troublesome source of hog cholera and other diseases. Moreover, the threat of introduction of foreign diseases through permissive garbage feeding would be removed. Therefore, prohibition could be a good investment in disease eradication if successful.

The outbreaks in which no source was established dropped to 16 percent from the 19 percent in 1967. More skill is needed in this area. Otherwise we cannot be entirely sure that methods of spread as yet unknown may exist. However, if such methods do exist, they have not yet interfered with the program.

Goals

The program is lagging behind the industry established goals of 1964. The December 1967 goal of all States at least to Phase III remains to be met. We are almost a year behind schedule here and 10 States must advance to reach this objective. If this goal cannot be met soon, all of us must reassess our ability to eradicate hog cholera by 1972.

The December 1969 goal of all States to Phase IV is but 14 months away. With 34 States yet in Phase II or III, we will be hard pressed to meet this objective. If we cannot do so, there would be serious question as to meeting the 1972 schedule.
Summary

By July 1, 1968, all States were to Phase II and 38 had reached Phases III or IV. Seven States were hog cholera free. Loss of free status occurred when program standards were abandoned to some extent. Considerable evidence has been gathered to support the premise that program standards are effective. Other experiences indicate that lesser procedures are ineffective.

All States reported that they were carrying out the program phases in which they are depicted. Some programs have become static at Phase II or Phase III and are hampering overall progress.

Hog cholera incidence remains at least 80 percent under the level experienced in the early 1960's although increasing in 1967 and 1968. The increase can be attributed to improved reporting, increased diagnostic skill, better laboratory support, transition from Phase II to Phase III, and unexpectedly heavy incidence in three States.

Quarantinable areas accounted for almost one-half of the hog cholera spread, indicating a need for improved operations. Vaccines continue to be incriminated as major sources of hog cholera. State and Federal actions have reduced spread through vaccination. With further action necessary, proposals are planned to eliminate vaccination in all States in Phase III and beyond.
Garbage feeding accounts for a disproportionate amount of hog cholera. Salvage operations have been blamed as the cause of widespread outbreaks. Some have stopped salvage. All should appraise the value of salvage and stop this operation when it adds unnecessary risk to the program. Reassessment of garbage feeding is in order with the feasibility of a prohibition of this practice to be considered.

The program is behind schedule with the 1967 goal yet to be met by 10 States. The 1969 goal will be difficult to meet. If these goals are met, the 1972 goal continues to be realistic and attainable as adopted by this Association in 1965. If not, the 1972 goal is in jeopardy. Those administering the program would likely be called to task by the swine industry inasmuch as failure to meet these goals has not been based on hog cholera incidence but based on administrative choice.
The midpoint of the projected program to eradicate hog cholera was reached in 1968. By this time, all but 10 States with less than 5 percent of the Nation’s swine had reached the final two phases of the eradication program. Your Committee is pleased with the progress made to date. However, we are concerned with those 10 Phase II States still remaining in the control stage of the program. Factors other than hog cholera incidence appear to be limiting advancement of these States.

The Committee received information concerning the use of hog cholera vaccines and related hog cholera outbreaks and feels that this continues to be of vital concern to the eradication program. According to controlled research, modified live virus vaccines are capable of spreading from hog to hog. From field investigation, vaccines accounted for 21 percent of the hog cholera outbreaks occurring in fiscal 1968. Your committee notes that 40 States have applied restrictions to the use or administration of hog cholera vaccines.

The Committee heard reports on proposed Federal regulations which would prohibit interstate shipment of hog cholera vaccines and vaccinated hogs. It was reported that these proposed regulations (1) stop the interstate shipment of all hog cholera vaccines into States in Phases III and IV after Jan. 1, 1969, and (2) stop the interstate shipment of all hog cholera vaccines, except inactivated vaccines, into Phase II States after Jan. 1, 1969. In effect this would eliminate all modified live virus vaccines after Jan. 1, 1969, and inactivated vaccines except in Phase II States after that date. The proposed regulations would also provide that feeder pigs and breeding stock vaccinated after Jan. 1, 1969, could not be shipped across State lines after July 1, 1969, except for these swine vaccinated with inactivated vaccines which were shipped from States in Phase II.

Your Committee supports these proposals. However, we are concerned with the continued use of inactivated vaccines in Phase II States in light of reported problems with these vaccines. Therefore, your Committee recommends that the Animal Health Division and the States involved should take action as soon as possible to eliminate the use of these vaccines.
Also, in regard to the proposed Federal regulations, the Committee recommends that the period for comments on these regulations be limited to 60 days in order to help make it possible to meet the suggested timetable. In view of the fact that a timetable for ending vaccination has been under discussion for more than a year, the Committee felt that the 60-day period is ample for anyone wishing to comment.

Your Committee reaffirms the desirability of providing State and Federal funds to purchase a hog cholera anti-serum supply to be maintained and used in the event of a disastrous epidemic of hog cholera.

Concern was expressed that the purebred breeder is not always properly compensated for the value of swine destroyed in a hog cholera outbreak. Noting that outbreaks in purebred herds constitute only a minor problem and expenditure, nevertheless your Committee recommends an increase in Federal indemnity payments for purebred hogs in line with actual value, and urges the States to take similar action in order to help compensate the purebred breeder properly for any hog cholera losses he may incur.

Recognizing that nearly half of the hog cholera outbreaks in the past fiscal year were attributed to movements and area exposure, both of which can be reduced by proper application of shipping regulations and quarantines, your Committee recommends that the Animal Health Division and the States take action to strengthen this aspect of the program. The Committee points out that these areas are the direct responsibility of regulatory officials.

The Committee recognizes the need for additional trained personnel to be on call in the event of an emergency hog cholera situation in a State. Such an emergency force should be available to assist and supplement regular State-Federal diagnostic teams available within the State. This emergency force would be expected to provide technical assistance in such areas as (1) field diagnostic work, (2) laboratory equipment and personnel, (3) surveillance of suspicious herds, and (4) traceback and information aspects of the program. The Committee believes this plan could help contain potentially explosive situations and thereby avoid disastrous epidemics which might force compromise with program procedures.

Your Committee points to the Phase IV requirement in the standards for the hog cholera eradication program calling for continual surveillance for the presence of hog cholera and notes that the serum neutralization procedure is available for this purpose. Therefore, the Committee recommends that serological surveys be conducted in States in the final phase of the eradication program after vaccination with living vaccines has been discontinued.

The Committee notes that the practicing veterinarian plays a vital role in the hog cholera eradication program and therefore should be kept fully and currently informed on any hog cholera investigations involving his clients. The eradication program should exploit those services available only from the practicing veterinarian.

Finally, recognizing (1) that when vaccination is discontinued the risk of infection through garbage feeding is greater, and (2) that a certain percent of salvaged swine contain hog cholera virus, your Committee recommends that States in Phase III hold salvage to a minimum and exercise the greatest care in any salvage operations conducted. Further, the Committee recommends that salvage in Phase
III States be eliminated at the very earliest moment and in no event later than July 1, 1969. This action would thus parallel the proposed Federal regulations regarding interstate shipment of vaccines, and vaccinated hogs.

Respectfully submitted,

P. B. Doby, Chairman

J. A. Baker

L. D. Mark

W. L. Bendix

D. L. Smith

S. J. Bower

J. B. Taylor

C. H. Mannasmith

M. J. Tillery
REPORT ON THE "SYMPOSIUM ON THE FUTURE OF GARBAGE FEEDING"
Trisponsored by the USLSA Committees on Transmissible Diseases of Swine, Foreign Animal Diseases, and Nationwide Eradication of Hog Cholera

Dr. A. A. Erdmann, Madison, Wisc.; Dr. D. P. Gustafson, Lafayette, Ind.; Dr. Norvan L. Meyer, Hyattsville, Md.; Dr. James H. Steel, Atlanta, Ga.; Dr. W. J. Zimmerman, Ames, Iowa; Dr. M. J. Tillery, Hyattsville, Md.; Mr. Wilbur L. Plager, Lafayette, Ind.; Mr. Rolland Paul, Des Moines, Iowa; Dr. George L. Crenshaw, Davis, Calif.; Dr. E. L. Brower, Trenton, N. J.; Mr. Edward M. Dwyer, Boston, Mass.

This symposium represents the initial effort on the part of these three committees to examine the situation and problems involved in feeding garbage to hogs. It was felt that the symposium provided a valuable forum for the attitudes of all those concerned with garbage feeding.

After considering the information brought forth during the symposium, the three committees make the following recommendations:

1. More information is needed regarding disease problems among garbage-fed swine versus grain-fed animals. Efforts should be made to find out specific information regarding the disease status of garbage-fed herds and their relation to public health.

2. Studies should be conducted on the economic effects of ending garbage feeding.

3. In view of the fact that there appears to be a wide variation in the inspection of garbage-feeding premises and in the application of penalties in enforcing garbage-cooking regulations, State and Federal animal health officials should take the necessary actions to continue to strengthen these areas.

4. Any action to prohibit garbage feeding should be on an individual State basis. At this time, Nationwide action to prohibit garbage feeding is not recommended.

5. These recommendations and a copy of the symposium report should be brought to the attention of the Livestock Sanitary Board or similar governmental body in each State for their information.

The three committees decided to meet again next year to consider the subject of garbage feeding in greater detail.

STATUS OF GARBAGE FEEDING IN WISCONSIN

Dr. A. A. Erdmann

Gentlemen:

Effective July 1, 1968, the Wisconsin Statute became effective, which reads as follows:
Beginning July 1, 1968, it is unlawful for any person to feed public or commercial garbage to swine or to deposit or receive such garbage on any premises where swine are kept, and no swine having fed on such garbage may be sold or removed from the premises.

This law was passed by our legislature in January of 1965 to become effective 2½ years later. I believe this time lag in the effective date was most important in order to allow the industry to prepare or modify their operations to a grain feeding one, and to permit them to write off their investment in equipment, etc. At present, all feeding of commercial garbage to swine in Wisconsin has been discontinued.

This was a relatively non-controversial law when it was passed by our legislature. This proposal was first presented to our swine health advisory council, which is composed of all segments of our swine industry, and they unanimously recommended action in this area. The problem was then discussed with the swine industry and various farm organizations in our state, who all lent their support to this proposed law.

The swine industry was concerned with the disease hazard of feeding commercial garbage, but was equally concerned with the aesthetic effect garbage fed pork would have on the consumer public. During this time, the garbage feeders were kept advised on the pending legislation, and very minor opposition from their point of view was received. When this law was passed, they were notified of this by registered mail, and reminders of the effective date were sent to them every six months. In June of 1968, they were sent a final registered letter again notifying them that the feeding of garbage must cease on July 1st.

TRANSMISSIBLE DISEASE ASPECTS OF GARBAGE FEEDING OTHER THAN TRICHINOSIS AND HOG CHOLERA

D. P. Gustafson, D.V.M., Ph.D.
Purdue University
Lafayette, Indiana

There is an association between the health of animals and the health of man which may be measured by the infectious diseases that either may become affected with, because of the other. This reality has influenced history and is of great importance to all. One of the most important examples dates from Biblical times when some of the earliest records of bubonic plague were made. During the reign of the Emperor Justinian in the sixth century A.D. a pandemic lasting approximately fifty years was estimated to have been responsible for the deaths of more than 100 million people. Recent epizootics noted in the western United States have resulted in some deaths pointing to a potential for future epidemics due to P. pestis.

As world population has increased at an ever increasing rate more and more recognition has been focused on the necessity of coping with public health problems generated by animals. There is general desire to recognize and eliminate
activities in our society which clearly result in the spread of infectious diseases.

Many have been concerned about the part that the practice of feeding garbage to swine has played in the development of reservoirs of infectious diseases. In response, laws or regulations are in effect in all states of the Union which require the cooking of garbage which is to be fed to swine. These have principally been directed to interrupt transmission to swine of disease producing organisms present in garbage prior to cooking. Some have argued that garbage cannot be successfully cooked to effectively achieve this goal. There is no question but that it can be done. Whether it is done is another thing. If we considered that it is done effectively, still there would remain very serious problems; for the basic concern is twofold. There is concern for the health of people who consume the products from the animals fed garbage and the health of those animals fed scraps from those which were fattened on garbage. Neither seem reasonably protected by enforcement of garbage cooking regulations. If garbage feeding premises have an offensive odor about them it is because of bacterial action on the garbage. The myriads of flies on the residue actively, physically make sure of a rather even distribution of the available flora of microorganisms. Since the pens and feeding areas are one and the same in most instances, and are cleaned in the worst operations when the pile-up of glass, metal, and paper waste is in the way, the swine consume the accumulated populations of microorganisms which have been made available to the environment. This hazard then, develops beyond the barrier of garbage cooking but is not totally unique to garbage feeding enterprises as opposed to other procedures of fattening swine. It may be unique in that the decaying substrate is different and is not totally consumed or disposed of prior to the addition of more garbage except in the most carefully operated enterprises.

A typical garbage-feeding operation has a “high load” of hogs on the land continuously. The swine are brought to the property from sources which have assembled them from many other sources. At the time of purchase they weigh from 60-100 pounds and are sold when they weigh 240-250 pounds. When some are sold, more are bought to replace them. An operator will turnover his herd at least two times during a year, and some will produce four times the average number-being-fed. During the first 6 months of 1968 according to records of the Animal Health Division, ARS, USDA, an average of 867,436 swine were being fed garbage on inspected premises. From these figures and a factor of 2.8 (estimated average turnovers) about 2.5 million garbage fed swine will be marketed in the United States and Puerto Rico this year. According to the same records about 42,000 will have been fed raw garbage. These figures are relative only to those premises under inspection. There are about 10,000 enterprises devoted to feeding garbage to swine in the United States. There were some in every state in the nation as of June 1968. Since swine have a dressing percentage of roughly 75% there will be approximately 150 million pounds of pork from garbage fed hogs available for consumption. This is about three-fourths of a pound of carcass pork for each of the 200 million citizens this year. Approximately 3% (2.5 million) of the 85.5 million hogs marketed in the U.S. in 1967 were garbage-fed, and the prospect is about the same for 1968. It is a significant factor in our society and worthy of our concern.

The pattern of spread of infectious diseases in garbage-feeding enterprise environments is lateral. Infected animals brought into the environment share their
infections with susceptible swine already present. Infected carriers and shedders of microorganisms having reached market weight are sent to slaughter and their products are sold to the public. Some of the uncooked scraps from retail cuts are fed to swine on farms and are a source of disease. The same, of course, may be true of pork from farm-fattened swine yet the weight of experience indicates that the chronic infections frequently occur in "open end" operations such as garbage-feeding businesses rather than on farms. It is not difficult to perceive that asymptomatic carriers of disease-producing agents are more prevalent among garbage-fed swine than among farm-raised swine. Swine present on such premises are exposed to the diseases of their fellows, and each succeeding additional group of swine brought in from other collection-and-distribution enterprises, and to those diseases represented in the garbage fed to them which have not been inactivated by cooking as well as to those organisms growing the organic decaying wastes. Among the many bacterial infections that compose the total potential there are some that are of public health importance, others that are more clearly of importance only to other animals including swine and some that are transmissible only to other swine. Those of public health importance include three types of tuberculosis, brucellosis, swine erysipelas, pseudo-tuberculosis, listeriosis, anthrax, tularemia, salmonellosis, and a spirochetal disease - leptospirosis. The fact that most garbage-fed swine are sold when they weigh about 250 pounds puts them into the so-called "hotel trade." Much of the flesh of these swine becomes trimmings used to make sausage, some of which are eaten without being cooked. The problems of Salmonella spp. in fresh and smoked sausage has been studied1, resulting in findings suggesting that a very high percentage of swine carry Salmonella spp. to the abattoir and that pork sausage either fresh or smoked may contain Salmonella of various types. Abattoir problems surely contribute to the contamination of the products, nevertheless the swine entering the holding pens with high enteric elimination of the organisms contribute to the spread of the organisms among those being held for slaughter. Heavily contaminated environments likewise make possible reports such as one presenting that the examination of muscles and of the intramuscular lymphatic nodes of 3494 swine revealed E. coli groups O 111: B4, O 55: B5, and 0 26: B6 to be present among the 64 isolates, all of which are pathogenic for man.6 Similar presentations could be made in each case citing resistances of organisms to processing under varying circumstances so that ultimately they may be found in products on retail counters. The solution of these problems will not be found by the simple expedient of removing garbage-fed swine from the scene, however, a readily recognizable source of many problems would be erased.

Viral diseases transmissible to other swine and other species of animals are kept active for transmission in garbage scraps from the garbage-fed hogs when fed by individual householders to their animals. By pooling diseases from as many sources as the swine in the garbage-fed group represent, they offer the type of reservoir for pseudorabies as has recently been described in California.4 Enteroviruses may also be preserved for other swine in meat scraps. It was found that the salting or pickling of meats for sausage production rendered the virus of Teschen disease more stable. Thus, the virus in CNS tissue used in making Krakow sausage, survived the smoking process and was inactivated during the subsequent scalding only if the time and temperature employed were such as would guarantee that the sausage would remain
above 700°C (1580°F) for at least 30 minutes.³ An even more serious threat of
garbage-feeding to swine was reported in Spain where the greatest proportion of
new outbreaks of African Swine Fever were traced to such sources.⁵ In the United
States, hog cholera continues to appear, partially because of garbage-feeding
practices of householders. The list of potential diseases assembled in garbage-feed-
ing enterprises also include influenza whose relationship to human influenza is
currently under study because of previous conjectures.

Garbage-feeding enterprises seem to be ideally operated 'for the perpetuation of
the disease problems so briefly alluded to here. However, the measure of the
contribution of garbage-fed swine is not clearly defined and judgment must be
provided with objectivity if it is to be lasting and satisfactory to all concerned.

The practice of feeding garbage to swine in the United States is time honored.
Those who have fed garbage to swine began doing so before those who are
concerned with the spread of disease among swine or concerned about the
transmission of infectious diseases to man observed that this widespread practice
could operate to the detriment of the general public. Since hygienists have become
aware of the potential problems inherent in the procedure as practiced, moves have
been made to control the activity. The forces of sanitation have been trying to
make garbage-feeding of swine a less potent force in creating problems for the swine
industry and as disseminators of diseases among humans. If they are successful,
garbage-feeding on a large scale will largely become unprofitable and therefore, will
pass from the scene.

Garbage-feeding FOREIGN ANIMAL DISEASE IMPLICATIONS

By Dr. Norman L. Meyer

The question is often asked: “How long can Foot-and-Mouth Disease virus live
in meat?”

Acids formed in muscle tissue during rigor mortis usually neutralize the virus of
Foot-and-Mouth Disease; however, virus may survive many months if the meat is
chilled or frozen. PH changes in lymph nodes and bone marrow are much less
pronounced, therefore, Foot-and-Mouth Disease virus is even more likely to survive in these tissues. Virus in skins may also survive for long periods of time under normal methods of handling. Since animal products often find their way into garbage, it follows that garbage could contain virus if infected animals are slaughtered. Furthermore, the "chain of infectivity" could be completed if garbage containing virus laden meat scraps is fed to susceptible animals without proper sterilization.

The "chain of infectivity" can be broken in several ways; for instance, by proper cooking. We have not had an outbreak of Foot-and-Mouth Disease in the United States since 1929 because importation of ruminants and swine and products from those animals are restricted. In 1930 Congress passed an Act making it illegal to import ruminants and swine or fresh, frozen or chilled meat from such animals from countries where Foot-and-Mouth Disease or Rinderpest exists. Other by-products of ruminants and swine origin are permitted entry but only under restrictions which remove at least most of the danger of bringing in foreign animal disease virus. Cooked meat, certain partially cooked meats, and certain cured meats are permitted to come into the country, some under restriction for further processing. The most competent Foot-and-Mouth Disease authorities believe these impositions can be made with a reasonable degree of safety. But all processing is subject to human error. Virus undoubtedly reaches this country from time to time, but since 1929 Foot-and-Mouth Disease has not found its way to susceptible animals. At least not in sufficient quantity to cause disease.

Unfortunately, most experts also agree that it is just a matter of time until the virus of Foot-and-Mouth Disease or African Swine Fever, or some similar foreign animal disease is going to get into this country. When it does, if conditions are right to complete the "chain of infectivity", disease will occur.

Garbage feeding could be the necessary line to complete the "chain". For instance, a piece of virus-laden sausage could be smuggled into this country, then thrown into the garbage can because it is spoiled. That same garbage might then be fed to swine without proper treatment. The result could be an outbreak of a foreign animal disease. In addition, if a foreign animal disease occurs in this country, garbage feeding could be responsible for further spread.

Many garbage feeders do a good job of sterilizing their hog feed but others have an occasional problem in complying with cooking requirements. For instance, cooking equipment may break down. With no other source of feed, breakdowns in cooking equipment may result in raw garbage finding its way to the feed trough. We need to decide if garbage feeding is of sufficient economic importance to warrant its continuance in view of the possible hazards.

The 1924 and 1929 Foot-and-Mouth Disease outbreaks in California were caused by feeding raw garbage from foreign ships.

The first outbreak of African Swine Fever in Europe occurred in Portugal in 1957 on a garbage feeder's premise, and this garbage feeder received garbage from the Lisbon airport. Planes arrive in Lisbon from areas in Africa where African Swine Fever exists. It is believed that African Swine Fever virus entered Portugal on meat scraps which were subsequently fed to swine.

In 1952 the Minister of Agriculture and Fisheries in Great Britain appointed a committee to review the policy and arrangements for dealing with Foot-and-Mouth
disease in Great Britain. That committee, known as the Gowers Committee, presented a report in 1954. One chapter in that report is titled, "Causes of Primary Outbreaks in Great Britain and Precautions Against Them". The report sets out the most likely causes of primary outbreaks between April 1, 1938 and 1954 as follows: Of a total of 540 outbreaks, 214 or 40% were listed as origin unknown but possibly contact with swill and 50 outbreaks or 9% were listed as contact with imported meat and bones other than swill. The other outbreaks were introduced in a variety of ways such as birds and live animals.

Starting in 1952 Vesicular Exanthema, a disease of swine clinically indistinguishable from Foot-and-Mouth Disease, swept across the United States infecting 42 states and the District of Columbia. Feeding garbage proved to be the primary source of infection.

It should be pointed out that the outbreaks I have mentioned were associated with feeding raw garbage. It is our opinion that if all the garbage was properly cooked all the time, the danger of spread of foreign animal diseases by this method would be largely removed.

In Summary

1. Garbage feeding has been responsible for spread of foreign animal diseases in the past.
2. While many garbage feeders do an adequate job of cooking which will prevent the spread of disease, a few others feed raw or partially cooked garbage all or part of the time.
3. If the practice of garbage feeding is continued, it will probably be responsible for the spread of foreign animal diseases in the future.

GARBAGE-BORNE DISEASES OF SWINE AND THEIR RELATIONSHIP TO PUBLIC HEALTH

James H. Steel, D.V.M., M.P.H.*
and
Lawrence A. Busch, D.V.M.**

Garbage is the animal and vegetable waste from homes, stores, markets, restaurants, institutions, and other places where food is stored, prepared or served.1 This type of waste material has been fed to swine for centuries. As modern civilization advanced to recent times, the practices of feeding garbage to hogs has not changed substantially, despite the fact that garbage is known to be an important disseminator of disease.

*Immediate Office of the Surgeon General, National Communicable Disease Center, Department of Health, Education and Welfare, Atlanta, Ga.
**Chief, Zoonoses Surveillance Unit, Veterinary Public Health Section, National Communicable Disease Center, Health Services and Mental Health Administration, Department of Health, Education and Welfare, Atlanta, Ga.
Diseases transmitted by garbage are numerous, and affect both man and swine. There are few vehicles that are as responsible for maintaining disease in important domestic food producing animals as is raw, or poorly processed and handled garbage fed to swine. Bacterial and viral pathogens of both human and animal origin in garbage can initiate and promote enteric, systemic, and pulmonary infections in pigs. In addition, detergents, spoiled food, and various foreign objects frequently found in garbage can cause losses in swine droves.

The public health aspects of feeding garbage to swine are of concern to all. The persistence of Trichinella infection in the human population is definitely associated with feeding garbage to swine. Garbage-fed piggeries enhance conditions for the breeding of insects and rodents, for the pollution of ground and surface water supplies, and for odors and unsightliness. These conditions create nuisances and health problems in any community where such installations are found.

Garbage serves as an excellent vehicle for viral, bacterial, and parasitic diseases of swine. Many diseases of public health importance are transmitted in garbage or by feeding of infected meat. Several of these diseases will be discussed briefly from an epizootiologic viewpoint.

**Salmonellosis**

Salmonellosis has been an important and common disease in man and animals for many years. Despite this awareness, the disease appears to be on the increase in man, domestic livestock, and wildlife, especially in the more civilized nations of the world where the levels of basic sanitation are high. The paradox that exists may be due to the practices of concentration and widespread dispersal of animals, the mammoth food production and distribution systems, and the widespread contamination of the environment by poorly treated wastes, including garbage. Such circumstances coupled with the lack of host specificity of the organism have apparently provided the opportunity for the multiplication and spread of Salmonella.

Salmonellosis has many manifestations in animals and man. The most common symptoms in animals are enteritis and diarrhea. Occasionally septicemic forms are observed, especially when the animal's resistance has been lowered by other causes. The first organism of this group to be described was called the hog-cholera bacillus by Salmon and Smith in 1885. Later, the viral agent of hog cholera (also perpetuated in garbage) was identified as the etiologic agent. Today over 1,200 species of Salmonella have been described. Many of these have been isolated from swine.

Transmission in animals is usually by the ingestion of food or water contaminated with secretions or excretions of diseased or carrier animals or birds. The organisms are known to survive in meat, poultry, eggs, and other food products of animal origin. Garbage-fed swine are frequently afflicted with salmonellosis and other enteric diseases because they are exposed to the organisms more often than grain-fed hogs. Poor sanitation maintained in garbage feed lots contributes to the spread of the disease.

Reliable data on the incidence or the economic loss resulting from salmonellosis in domestic livestock is not available, but it surely is substantial. The problem is so broad in scope and depth that all associated groups in public and private
agencies must cooperate if the disease is to be controlled. Presently, the main concern is with the infection in man, but control of salmonellosis in domestic animals is a prerequisite for public health.6

**Tuberculosis**

Swine may be infected with *Mycobacterium tuberculosis* variety *bovis*, *Mycobacterium tuberculosis* variety *hominis*, and *Mycobacterium avium*. The incidence of tuberculosis in swine is related to the opportunity for direct or indirect contact with cattle, humans, and fowl infected with tuberculosis.10

There is no specific program to eradicate swine tuberculosis. Control of the disease in hogs is based on reduction of the condition in cattle, fowl, and man. The most common type of infection in swine is the avian type,10 but the bovine type causes the most serious disease in pigs.9

Lesions of tuberculosis in swine generally are local and confined to the lymph nodes of the throat and abdominal cavity. This is indicative of infection by ingestion.10

Studies have shown that the single most important source of infection for swine may be soil contaminated by feces of tuberculous fowl.10 Modern poultry husbandry practices that recommend disposing of birds over one year of age should decrease the incidence of the disease in the avian species as tuberculosis is a disease of aged birds. This will have a positive effect on swine tuberculosis.

Feeding swine the offal from abattoirs or feeding garbage has caused outbreaks of tuberculosis in piggeries. An outbreak in Denmark from improperly cooked offal from poultry plants was reported in 1959.10

In 1966, an outbreak in Connecticut due to *Mycobacterium bovis* was reported. The evidence incriminated the feeding of improperly cooked offal from cattle reacting to the tuberculin test and which had slight lesions at slaughter. During the interval from September 23, 1964, to June 17, 1965, of the 228 swine in the condemned herd, 151 (66 percent) had lesions of tuberculosis, and of these, 26 had generalized lesions. *Mycobacterium bovis* was isolated. In addition, transmission from animal to animal occurred since some of the infected swine did not have access to the infected offal.11 This is unusual in swine because the average life span of most pigs is not over 8 to 9 months, by which time they are sent to slaughter. Such animals do not live long enough to become active spreaders of the disease. Therefore, swine-to-swine transmission seldom occurs. As a general rule, tuberculosis of swine is a disease contracted from the environment, rather than from other hogs.2

The human form of tuberculosis in swine often has been traced to garbage from hospitals and sanatoria. For this reason, it is dangerous to feed swine garbage from hospitals and institutions. The economic loss that may result from improper disposal of infected garbage with resulting infection in food-producing animals is significant, and appropriate preventative measures are needed to prevent continued losses.10

**Swine Erysipelas**

Swine erysipelas is an acute or chronic infectious disease and is thought to be transmitted by the ingestion of food or water contaminated by infectious excreta
or tissues of diseased or carrier animals. Hagan points out that pork trimmings in raw garbage probably accounts for many sporadic cases. *Erysipelothrix insidiosa* is resistant to smoking and salting; hence pickled and smoked products are capable of causing infections. European authors state that the disease is transmitted by pork scraps in garbage. This has not been commonly observed in the United States.

**Brucellosis**

Swine brucellosis is a generalized infection which is manifested by orchitis in boars, abortion in sows, arthritis, spondylites, and posterior paralysis. In general, the disease in swine has greater clinical resemblance to brucellosis in man than has the disease in cattle. The disease occurs in most swine-raising areas of this country.

Transmission is by digestive, cutaneous, respiratory, and venereal routes. Many cases of human infection with *Brucella suis* have occurred in slaughter house workers. A comprehensive study by Hutchings and associates revealed that *Brucella* organisms were isolated from each of the organs and tissues of swine carcasses which were examined. Of the total of 5,056 cultures examined, 538 (10.6%) were positive for *B. suis* among the 61 infected animals studies. *B. suis* was also isolated from carcasses held at 40°F. for as long as 20 days after slaughter.

In the U.S., brucellosis has been found in farmers associated with infected swine. An outbreak of the disease has been reported in a piggery and attributed to a garbage-borne infection.

**Trichinosis** is a world-wide parasitic disease affecting most mammals. The disease is of primary importance as a public health problem. The principal reservoir and source of infection for man is swine. Swine usually become infected by the ingestion of pork scraps or trimmings that contain viable larvae. They also become infected from eating diseased rodents or other animals. Signs of disease are seldom noticed in swine, although they are known to occur and may be so serious as to cause death. Pig farms where garbage is fed and the premises are infested with rats are important sources of diseased swine.

The garbage-feeding problem is an old one and has long been recognized as the most important link in maintaining the disease in swine. In 1941, the Public Health Service enacted a regulation requiring that all garbage moving in interstate commerce be cooked. After the national enzootic of vesicular exanthema in swine in 1952, all states passed laws requiring the cooking of garbage and the U.S. Department of Agriculture required that no pigs fed raw garbage could move in interstate commerce. These measures all contributed to the reduction of garbage-fed hogs and trichinosis.

*Trichinella spiralis* was formerly widespread in the United States, where one in every six autopsies revealed human infection. Today the incidence, according to autopsy surveys, is less than 4 percent and even lower in persons under the age of 40 where the infection is rare. In 1967 there were only 67 cases reported, which is fewer cases than reported in any previous year. The rate of swine infections varies from less than 0.1% in grain-fed swine to 1% or 2% in those fed raw garbage. All garbage fed to swine is supposed to be cooked, but there are always exceptions. These exceptions can be considered part of the cycle maintaining
trichinosis.

The April 1968 U.S. Department of Agriculture report of the National Status on Control of Garbage Feeding reports 10,043 premises feeding garbage, of which 9,075 were inspected. Of the premises inspected 8,696 (95.8 percent) were cooking the garbage and 379 (4.2 percent) were using raw garbage. The number of swine being fed garbage was 867,841, of which 783,327 were inspected. Of those inspected, 763,785 (97.5 percent) received cooked garbage and 19,542 (2.5 percent) were eating raw garbage. In April 1955, 14,468 premises were feeding garbage; 12,000 (82.9 percent) of these were cooking the garbage. The number of swine being fed garbage was 1,379,307; of these 1,129,595 (82 percent) received cooked garbage, and 249,914 (18 percent) were eating raw garbage.

While the number of swine fed raw garbage has decreased substantially, the most effective control of trichinosis is to forbid the feeding of garbage and offal to swine. Cooking of garbage and offal has proved to be a formidable task, requiring special equipment and care. Where garbage and offal are fed, general sanitation must be excellent, otherwise rats and flies are attracted. Today good sanitation is the exception rather than the rule.

Most animal health authorities would like to eliminate garbage feeding operations since they are a constant threat to the animal health of the nation and are unnecessary in a country with all the feedstuffs needed to raise healthy swine. It is indeed ironical that a nation that spends millions of dollars in exploring outer space still condones the archaic practice of feeding garbage to one of its most important food producing domestic animals. A leading animal health administrator has stated that it costs more to feed hogs garbage, when all the costs involved are tabulated, including the inspectors that perform monthly inspections of the operations. A study in Alabama indicates that it costs that state over $2.00 per hog to inspect garbage feeding premises at the present level. Officials estimate that it would cost $5.00 per hog to inspect these premises satisfactorily. It is their feeling that many of the feeders "realize that they will be inspected only once a month and do not cook when the inspector is not expected." A study in Alabama indicates that it costs that state over $2.00 per hog to inspect garbage feeding premises at the present level. Officials estimate that it would cost $5.00 per hog to inspect these premises satisfactorily. It is their feeling that many of the feeders "realize that they will be inspected only once a month and do not cook when the inspector is not expected."18

The food-animal industries of the United States are valued at more than $50 billion, but a subsidized fringe industry, swine garbage feeding, which produces at the most 2 million market hogs a year with a value of $25.00 per animal or a total market value of $50 million (0.1 percent) can jeopardize the health and wealth of the nation. The incidence of morbidity and mortality is generally higher in garbage-fed hogs, the losses due to condemnation at slaughter are higher, and the costs to all swine producers is higher because of the unpleasant image that garbage-fed hogs perpetuate, and the undesirable product that discourages consumption. Meat inspectors state that garbage-fed hogs can be identified readily due to the offensive odor, abscesses, internal cuts and wounds, hemorrhages, and poor quality of the flesh. Food inspectors concur with the animal health authorities that the market can do without garbage-fed hogs.17

Lastly, there is no need for such operations in the United States. It is about time that State animal and public health authorities become aware of the danger in their midst and take steps to rid the country of the practice of feeding garbage to pigs. Wisconsin was the first state to enact a law prohibiting garbage feeding of commercial pigs, effective July 1, 1968. The midwest should naturally lead in
discouraging garbage feeding as this is the greatest producing area of good meat in the world. If the states do not take action, the nation may face another critical invasion of disease such as vesicular exanthema, except the next time it may come from abroad and endanger the entire animal industry. Foot-and-mouth disease and African swine fever can be garbage-borne diseases, as demonstrated by the 1967 and 1960 epizootics of foot-and-mouth disease in England and the 1958 invasion of Portugal and Spain by African swine fever, which is now established in southern Europe. The costs of trichinosis is a pittance to the meat industry compared with cost of having any foreign epizootic become established in the United States.17

Resistance is to be expected from the remaining garbage feeders and from municipal governments who believe that feeding garbage to swine is economical.

However, the effect of garbage feeding on a municipal refuse collection system is considerable. The garbage must be kept and collected separately, and requires special equipment and time schedules in many situations. In addition, to the local and state government that condones garbage feeding to swine falls the responsibility to see that these facilities are well built, regulated, and properly operated.1 As has been pointed out, this is costly, if done right. Regardless of costs, public acceptance of such practices is the determining factor. The public wants simple and prompt services with little inconvenience.1 Today, when domestic garbage disposals are becoming commonplace, the concept of garbage feeding piggeries is esthetically unbecoming.

The cost of garbage feeding involves the investments in collection, hauling, cooking, labor, veterinary services and supplies, supplemental feeds, and other hidden costs. The cost of cooking garbage in 1955 varied from $1.40 to $2.80 per hundred pounds of hog.1 In 1968, this figure would almost assuredly be two or three times higher due to inflation. When everything is totaled, the cost per hundred weight to raise garbage-fed swine may be equal to or greater than the cost per hundred weight to raise grain-fed hogs which is about $10.00 – $15.00. A 1964 analysis of the New Jersey swine industry showed that, depending on the type of garbage utilized and grain prices, the cost per hundred weight of pork from grain would be comparable and competitive with garbage fed pork.19 Any economic advantage that garbage feeding had can only be less than today's economic squeeze on markets, equipment, and labor.

Feeding garbage to swine involves only the edible waste products produced by society. Other refuse is collected and disposed of by other means. It is evident that garbage can be collected with rubbish without causing health and nuisance problems. Los Angeles County found that collecting garbage separately for hog feeders cost the community and subsidized the swine feeder in the amount of $600,000 a year.1 Numerous methods for refuse disposal, including garbage are available and can be operated economically. These methods include properly planned and operated sanitary landfills, central incineration, on-site incineration, grinding food wastes, and composting.1 There are advantages and disadvantages to each of these methods, but each community must evaluate the methods best suited to its situation.

Summary

There are many diseases of swine and man which can be transmitted by
garbage. They include salmonellosis, brucellosis, swine erysipelas, tuberculosis, and trichinosis. Garbage-feeding of swine is not acceptable to public health, and should be eliminated as soon as possible.

This subject was reviewed some 16 years ago. I am happy to see that it is under serious consideration today. (See Appendix 1)

REFERENCES

Diseases of swine transmitted through garbage are numerous. There are few other vehicles including man and insects that are as responsible for maintaining disease in this important domestic food producing animal as is raw, unprocessed garbage. Garbage is fed to hundreds of thousands of hogs in the United States every year. The losses among droves of thousands of hogs in the United States every year. The losses among droves of thousands of hogs in the United States every year. The losses among droves of thousands of hogs in the United States every year. The losses include disease and death among swine, lower profits for the producers and processors, and poorer quality of meat for the consumer. The public health aspects of feeding garbage to swine are of concern to all. The prevalence of Trichinella infection in the human population must be attributed to the unsanitary practice of feeding raw garbage to swine. The fly and rodent problems created about piggeries is a health problem in any community where such installations are found.

Garbage serves as an excellent vehicle for virus, bacterial, and parasitic diseases of swine. Hog cholera, vesicular exanthema, and foot-and-mouth disease all are reported to be transmitted in garbage or by feeding of infected meat. These diseases will be discussed briefly from an epizootiologic viewpoint.

**Hog Cholera**

Hog cholera is the most important porcine disease in the United States. It is an acute, highly contagious disease characterized by degeneration of the walls of the smaller blood vessels which results in multiple hemorrhages, necrosis, and infarctions of the internal organs. Most swine die within 7 to 10 days from the time the first symptoms appear. The mortality in natural outbreaks is very high. If preventive measures are not taken, it is common to lose nearly all the susceptible animals on the premises. Fortunately, the virus of hog cholera does not affect any other species except swine, both domestic and wild. All experiments to produce disease in other animals have failed. It has never been reported in man.

The transmission of the disease is principally by intimate contact with sick animals or with their secretions and excretions. The virus may also be carried by various methods and agents. One of the most important vehicles of the virus is uncooked pork scraps that are found in garbage. In 1912, an outbreak of hog cholera was reported in Canada, caused by the feeding of infected market pork. In 1917, Birch reported that it was impossible to detect all of the hog carcasses that contain hog cholera virus. In an examination of 21 sugar-cured hams from infected hogs showing no lesions, virus was isolated from 12. the virus was found to persist for 80 days in the meat. Udall states that in the eastern States, probably 85 to 90 per cent of new outbreaks can be traced to virus in pork scraps fed in garbage.
During World War II there were many hog cholera outbreaks in foreign countries that were attributed to garbage from American military bases. Reported outbreaks of the disease occurred in North Africa, England, Australia, and Iceland. Recently it has been reported in France.

The National Committee on Eradication of Hog Cholera of the U. S. Livestock Sanitary Association states, among its recommendations, that "no garbage not sterilized by heating, except that originating on the premises where it is fed, shall be fed to swine. Owners of swine should be advised to avoid feeding uncooked home garbage, because of the danger of spreading hog cholera." The Committee goes on to recommend a public relations program that would disseminate information on the disease to producers, handlers, distributors, processors, and perhaps even to consumers, to mobilize public support for eradication.4

The control and eventual eradication of hog cholera would be one of the outstanding achievements of veterinary medical science. Use of the heat treatment of garbage to be fed to swine will contribute to the success of such a campaign.

Vesicular Exanthema

Vesicular exanthema is an acute infectious disease characterized by vesicles of varying size on the snout, lips, tongue, foot pads, skin between the claws, around the coronary bands, and the dew claws, and also on the teats of nursing sows. It is seen almost exclusively in swine droves that are fed raw garbage. Work by Crawford in 1936-37, indicated that there is slight possibility of indirect exposure, and that the disease probably is spread either in pork trimmings of raw garbage or by direct contact with infected animals.5 The mortality is not great in adult animals, but heavy losses occur among suckling pigs. Swine are the only naturally susceptible animals. Man is not susceptible, even by direct challenge inoculation.

The disease first appeared in 1932 in garbage-fed swine in southern California, and was thought to be foot-and-mouth disease. Seventeen farms had outbreaks that year, and about 18,000 hogs were slaughtered and buried in an attempt to eradicate the disease. In 1933, the disease appeared again and again was handled as though it were foot-and-mouth disease. Concurrent investigations by Traum led to the conclusion that the malady was not foot-and-mouth disease, but a vesicular disease previously undescribed.6

A third outbreak occurred a year later. In 1934, a total of 31 garbage-feeding premises and 95,000 hogs were involved. In spite of stringent quarantine measures, the disease spread to other premises in southern and central California.

In 1935, a few outbreaks were reported. In 1936, it was reported in 14 garbage-feeding establishments involving 19,000 hogs. Between June 1936 and December 1939, no vesicular exanthema episodes were recorded although veterinarians state the disease was present. In 1939-40 there was an epizootic that affected 123 herds. Only 8 herds of these 123 had not been garbage-fed. Of these 8, 4 had contacts with garbage-fed swine.7 Since 1940, there have been outbreaks every year.

Occasionally the disease is seen in stockyards of packing houses in the enzootic areas. When it is recognized in the animal upon ante-mortem examination, veterinary inspectors will not allow the animals to be slaughtered at that time. No doubt, animals that are in the early stages of the disease are not detected, as well as
some animals that are recovering. In any case, it appears that some virus-harboring animals are slaughtered and marketed. Scraps and trimmings of such carcasses and their parts eventually find their way into garbage and are returned to the hog ranches to precipitate new outbreaks. The development of an extensive frozen locker system for the storage of meat makes it possible for infectious agents to remain viable in stored meat for long periods of time.

In the summer of 1952, vesicular exanthema spread eastward. Infected swine were first seen in a hog cholera serum plant at Grand Island, Nebraska. They were said to have originated in a garbage-feeding lot near Cheyenne, Wyoming.

Shortly thereafter, reports of infected animals were received from the Midwest, far west, south, and eastern seacoast. Most of these outbreaks were in garbage-fed hogs, while some were in hogs that had originated on grain-feeding farms and had become infected by contact with diseased animals en route to market. The problem has continued to be of serious concern to all livestock sanitary officials. More than 30 states have had outbreaks. Fortunately, many of these areas have been freed of the disease by the combined efforts of State and Federal livestock sanitary officials.

There is considerable evidence that the disease is maintained primarily on garbage-fed hogs and that no eradication program will succeed until the practice of feeding raw garbage to swine is discontinued. The U. S. Livestock Sanitary Association recommended at its 56th Annual Meeting that feeding of raw garbage to swine be prohibited by State and Federal Laws.

Foot-and-Mouth Disease

Foot-and-mouth disease is an acute, highly contagious disease of clover-footed animals. On rare occasions it has been reported in man as a mild febrile disease. It is the most feared cattle disease of the entire world. The disease is characterized by depression, fever, and vesicular eruptions of the mouth, feet, and teats, and occasionally on the surface of the udder. Lesions may also be seen in the pharynx, larynx, trachea, esophagus and wall of the rumen.

The virus multiplies in the epithelium, which is first invaded after the epithelium is broken down. The virus escapes into the blood and is carried to all organs and tissues.

The mortality in most cattle outbreaks is not high. It usually varies between 1 and 3 per cent, although it may be as high as 50 per cent. The death rate is higher in young animals. The most serious consequences are loss of flesh, cessation of lactation, and long periods during which the affected animals are not productive. Economic losses due to quarantine restrictions are considerable.

Transmission of foot-and-mouth disease virus in infected meat has been known for a long time. England had numerous outbreaks with this source of infection, attributable to shipments of fresh meat from South American countries where the disease is enzootic. In 1942, for example, England had 41 different outbreaks involving 670 premises. On 26 premises where initial outbreaks began, raw garbage had been fed to swine. In 19 instances, hogs were the first species to be affected. A compulsory garbage cooking order was issued late in 1942. The following year, 1943, there were only 6 primary outbreaks, most of which began in swine which presumably had received insufficiently cooked or uncooked garbage. England has continued the cooking of all garbage, and there is no doubt that this order has
reduced the incidence of many communicable diseases of swine, especially of foot-and-mouth disease.

The last two outbreaks of foot-and-mouth disease in the United States occurred in California in 1924 and 1929. The disease also appeared in Texas in 1924. These outbreaks began in swine that were fed garbage from ships. It is presumed that the garbage contained scraps of meat that originated in infected overseas areas. As a result of this experience, ships coming from countries where foot-and-mouth disease exists are not allowed to land garbage in any United States ports. An earlier outbreak, in 1914, was traced to a hog lot in Niles, Michigan. According to the investigators, "The infection may have been introduced in merchandise brought to Niles, Michigan, from South America, which found its way into a hog lot, and in due time caused the development of the disease in hogs which were the first animals found infected." It is quite apparent from the history of foot-and-mouth disease in the United States that hog farms that feed raw garbage are one of the weakest links in our defense against exotic diseases.

**Bacterial Diseases**

Among the more important bacterial diseases transmitted to swine by garbage or animal flesh are salmonellosis, tuberculosis, swine erysipelas and brucellosis. It is well known that pigs develop anthrax after they have fed on carcasses of animals dead of anthrax. Fortunately, anthrax contaminated food is not found in garbage.

**Salmonellosis**

Salmonellosis has many forms of manifestation in animals and man. The most common symptoms of animals are enteritis and diarrhea. Occasionally septicemic forms are observed, especially when the animal's resistance has been lowered by other causes. The first organism of this group to be described was called the hog-cholera bacillus by Salmon and Smith in 1885. Later the viral agent of hog cholera was identified as the etiologic agent. Today nearly 200 species of Salmonella have been described. Many of these have been isolated from swine.

Transmission among animals is usually by the ingestion of food or water contaminated with secretions or excretions of diseased or carrier animals or birds. The organism is known to survive in pork, poultry, eggs and other food products of animal origin. Frequent outbreaks of this disease occur among garbage-fed swine. Poor sanitation maintained in garbage feed lots contributes to the spread of the disease. It has been observed that salmonellosis can be eliminated from the swine if they are moved to clean pens and given a ration containing no raw garbage.

**Tuberculosis**

Swine are susceptible to all three types of tubercle bacilli. The prevalence of avian, bovine and human types depends upon the environment surrounding the swine and the character of their feed. For example, the human type of infection, where it does appear, almost always occurs in swine fed on raw garbage. For this reason, it is dangerous to feed swine uncooked garbage from hospitals and sanatoria. The most common type of tuberculosis in swine is the avian type, because of their exposure to diseased chickens. The bovine form causes the most serious disease in pigs.
Lesions of tuberculosis in swine generally are found in the abdominal cavity of hogs. Infections are contracted most commonly by ingestion; hence, the primary lesions are found in the lymph nodes of the throat and abdominal cavity.

In 1939, Feldman, in a study of 264 garbage-fed hog carcasses, found that 28.4 per cent had tuberculosis. Subsequent studies to determine the types of tubercle bacilli in the diseased carcasses revealed that 74.5 per cent were of the avian type, and 25.5 per cent were of the human type. The offal of diseased chickens would account for the high percentage of avian type infection. The human type bacillus apparently is more frequent in garbage than has been assumed.

Butler and Marsh in another study reported that 30 per cent of hogs fed on garbage from a tuberculosis sanatorium were infected with the human type of disease. It should be remembered that the average life span of most swine is not over 8 to 9 months, by which time they are sent for slaughter. Such animals do not live long enough to become active spreaders of the disease; hence, swine-to-swine transmission probably seldom occurs. Tuberculosis of swine is a disease contracted from the environment, rather than from other hogs. There is no report of its transmission to man from swine.

Swine Erysipelas

Swine 'erysipelas is an acute or chronic infectious disease caused by Erysipelothrix rhusiopathiae suis. It occurs chiefly in swine where the acute form is a septicemia with joint involvement. It occurs chiefly in swine where the acute form is a septicemia with joint involvement. Red patches commonly occur on the skin. Death is sudden usually preceded by respiratory distress. The chronic disease is nearly always characterized by enlargement of the joints. A vegetative endocarditis is seen in many of these cases. The disease also occurs in sheep, turkeys, chickens, geese, pigeons, ducks and wild birds. The causative organism is found on the bodies of fresh- and salt-water fish and in fish products. The infection in man is described as erysipeloid to distinguish it from human erysipelas, a streptococcus infection. The disease is usually a local lesion at the site of injury. The septicemia form in man is very rare.

The infection in swine is thought to be transmitted by the ingestion of food or water contaminated by infectious excreta or tissues of diseased or carrier animals by some authorities. Hagan points out that pork trimmings in raw garbage probably accounts for many sporadic cases. E. rhusiopathiae is resistant to smoking and salting; hence pickled and smoked products are capable of causing infections. European authors state that the disease is transmitted by pork scraps in garbage. This has not been commonly observed in the United States.

Brucellosis

Swine brucellosis is a generalized infection which is manifested by orchitis in boars, arthritis, spondylitis, and posterior paralysis. In general, the disease in swine has greater clinical resemblance in man than has the disease in cattle. The disease in hogs is common in the Midwest, California and some parts of the South.

Transmission is by numerous routes including ingestion, contact, droplet, and venereal. Several reports have appeared in the literature on the isolation of Brucella suis from hog carcasses in packing houses. The most recent and comprehensive
study was that of Hutchings and associates. Their results revealed that Brucella organisms were isolated from each of the organs and tissues of the body which were examined. Of the total of 5,056 cultural examinations, 538 (10.6 per cent) were positive for B. suis among the 61 infected animals studied. B. suis was also isolated from carcasses held at 40°F. for as long as 20 days after slaughter. Recently, Giddens observed an outbreak of swine brucellosis in a piggery, which he attributed to garbage-borne infection.

Parasitic Diseases

Parasitic diseases of swine that affect man are few in number. Those that do cause disease in man are of considerable concern to public health officials. The most important are trichinosis and cysticercosis. Fortunately, porcine cysticercosis in man and swine in the United States is very rare. No human cases have been reported in recent years and few porcine infections. This is not true in other parts of the world. For example, it is estimated that in Mexico there are one million people infected with pork tapeworm. Inasmuch as this is not a garbage-borne disease, it will not be discussed in this paper. The disease is transmitted to hogs by the ingestion of tapeworm eggs or segments passed in the feces of infected human beings.

Trichinosis

Trichinosis, as we have heard, is not an uncommon disease in certain parts of the United States. Outside of the United States the incidence is thought to be lower although a recent paper by Peck points out that trichinosis is quite prevalent in Mexico. By examining microscopically portions of human diaphragm weighing 50 gm. or more, Beck reports an incidence of trichinosis in Mexico City much higher than heretofore reported. He likewise postulates the use of xenodiagnosis as an aid in future survey work.

In swine, natural infection seldom produces symptoms. A heavy artificial infection will induce high fever, diarrhea, stiffness, colic, difficulty in swallowing, dyspnea and edema. The infection is found in many mammals including rats, mice, cats, bears, dogs, walrus, seals, white whales, foxes and man. Otto states that over 25 species have been found naturally or experimentally infected. In addition, with proper preparation, birds and amphibians have been experimentally infected.

Swine and rats are infected by eating scraps of raw pork, or rats or mice containing the encysted parasites. The chief source of infection is pork, and little importance is now attached to rodents as a source of infection. Nearly all swine infections are probably light in nature, inasmuch as there are few records of naturally occurring trichinosis outbreaks. Schwartz has described one such outbreak in Virginia, near Washington, D.C. These light infestations may not be without some benefit in terms of immunity. There is some evidence that an initial infection will confer protection for some time thereafter.

Schwartz has carried on some interesting experimental infections in which he demonstrated that swine were not injured by less than 1 Gm. of infections material. All were made ill by as little as 1-4 Gm., and at this dose, 8 of 14 dies within 7 to 24 days.

It is strange that so few severe natural outbreaks of the disease, with
symptoms, have been reported or observed. It may be that the immunity established by constant exposure to low doses prevents serious episodes of trichinosis in swine.

The first source of porcine infection reported in the United States was in 1888 by Mark in Boston. Between 1883 and 1888, over 3,000 garbage-fed hogs from the vicinity of Boston were examined. The infection rate was between 9 and 16 per cent, with an average of 13 per cent. Concurrently, an examination of western grain-fed hogs revealed only 2 per cent infection.

In 1939, Schwartz reported an infection rate of 4.4 per cent in more than 6,000 garbage-fed hogs, and 0.9 per cent in a similar group of grain-fed hogs. The rate among garbage-fed hogs in an Atlantic seaboard area was over 10 per cent. The number of trichinae in individual diaphragms ranged from 1 to 69,700. According to Otto, in 1940 Kerr reported less than 0.5 per cent infection in California grain-fed hogs, and over 6 percent in raw-garbage-fed pigs, with rates as high as 12 per cent in some lots. No infection was found in hogs fed cooked garbage.

A more recent study by Schwartz indicates that the prevalence of trichinosis in farm raised swine was only 0.63 per cent while it was 11.21 per cent in the garbage-fed hogs by the digestion technique. The microscopic examination did not reveal any infection in farm-raised hogs and 4.81 per cent in garbage-fed swine. The incidence in hogs fed cooked garbage was the lowest with only 0.5 per cent.

It seems evident that most of the porcine infections are associated with the practice of feeding raw garbage. The source of the occasional grain fed swine disease is not at once evident. Kerr reported that one group of grain-fed swine, which he traced, revealed that they had only been finished on grain after having been raised on garbage. He was unable to find any evidence of Trichina larvae in rats or other rodents trapped around the grain feeding pens; but in a study of rodents around garbage feeding ranches, he found about the same rate of infection in the rats as in the hogs feeding there. This would seem to support the earlier statement that the rat is another victim of garbage transmission of the infection.

It is obvious that the control and eventual eradication of trichinosis is dependent upon elimination of feeding of raw garbage to hogs. Uniform State and Federal regulations obviously are needed for the control of garbage feeding. The need of such regulations is so obvious that one wonders why we have allowed 64 years to pass since Mark's report on the situation in Boston. There has been organized resistance to State laws by feeders who believe that cooking will reduce the nutrient value of garbage. In answer to their question, we can point to the success of cooked-garbage feeding in Canada and Great Britain. The piggeries in those countries have been able to raise well-nourished hogs, free of such serious diseases as hog cholera, foot-and-mouth disease and vesicular exanthema. There is resistance to laws for enforcing cooking of garbage from some municipal governments also, who believe that the most economic way to dispose of garbage is by feeding it raw to hogs. Sanitary Engineers have countered this argument by revealing that the costs of garbage and rubbish disposal are between $2 and $3 per capita annually, while the revenue from garbage feeding is only 2 to 3 cents per capita. The comparative costs of these operations should be studied closely to determine the best method of garbage disposal. Atlanta, Georgia, has found that
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incineration is the most efficient and economical method. In the past years, the Atlanta incineration plant has paid for itself through salvage of metal, including tin cans.

Summary

There are many diseases of swine which are transmitted by raw garbage. They include hog cholera, vesicular exanthema, foot-and-mouth disease, salmonellosis, tuberculosis, swine erysipelas, brucellosis, and trichinosis. The transmission by raw garbage of all of these diseases can be prevented either by prohibiting raw-garbage feeding or cooking the garbage. The prevention of these diseases would contribute to improvement of animal health and human health. The control and eventual eradication of trichinosis is dependent upon the prevention of feeding of raw garbage to swine.

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RELATIONSHIP OF GARBAGE FEEDING OF SWINE TO THE TRICHINIASIS PROBLEM IN THE UNITED STATES

W. J. Zimmerman, Ph.D.
Veterinary Medical Research Institute
College of Veterinary Medicine
Iowa State University
Ames, Iowa

Three key components, garbage - swine - pork, comprise the basic trichiniasis cycle which has perplexed the pork industry of the United States for at least a century. If viable trichinae could be eliminated from any of these three facets of the life cycle, trichiniasis would become an extinct disease in the United States and another major milestone would be reached in the never-ending battle against human and swine disease problems. It is recognized that other pathways of infection to man exist beside the garbage - swine - pork cycle, but present knowledge would indicate that these are only of minor significance and in most cases are related to the primary cycle. As with other disease problems, it is clearly more effective, less expensive, and much safer from the public health standpoint to prevent the infection in swine through management practices that it is to attempt to eliminate infections in swine or destroy the parasites in all pork being consumed by the human population. Unfortunately, immunization procedures have not yet been developed for trichiniasis. Detection and subsequent elimination of trichinae infections in swine herds on a national or even local basis is not feasible at this time, since there is currently neither a reliable diagnostic method for field usage nor a drug that is completely effective as a therapeutic agent. Even if both a diagnostic procedure and therapeutic agent were available, economics and the continuing possibility of exposure to infection after field examination would probably rule out use of these procedures. Treatment of pork by heat, refrigeration, or curing under adequate inspection procedures is highly effective but this only eliminates trichinae in a relatively small percentage of the pork consumed. The detection of trichiniasis in swine carcasses at slaughter would make major inroads on the trichiniasis problem by eliminating trichinae in most of the pork consumed in the United States. Although methods such as the pooled sample method would not be applied to home or locally slaughtered swine, the use of packing house diagnostic procedures over a period of relatively few years should eliminate the major source of infection for all swine.

Even without the availability of a vaccine, trichiniasis in swine should be one of the most readily preventable problems that the swine industry faces. Trichiniasis, unlike most bacterial and viral diseases, is nearly always transmitted to swine by ingestion of viable trichinae present in pork scraps and to an undeterminable, but much less extent, from carcasses of infected wildlife. Trichiniasis is not airborne, cannot be carried into a herd by contaminated clothing, feed, or equipment, and is not normally transmitted by exposure to infected animals. Experimentally, it can be transmitted to swine through the feces of infected swine or wildlife or by mechanical transmission by necrophagous insects, but the importance of these modes under actual field conditions is minimal. Other modes of transmission
currently unknown may also exist. The epidemiological investigations being currently carried out in conjunction with a pilot study of the pooled sample method of post slaughter detection of trichiniasis in Iowa swine will give a clearer understanding of the modes of transmission which occur in a state where commercial garbage feeding installations are negligible. It is believed that these studies will show that trichinae are primarily transmitted to swine through meat scraps with garbage being the primary medium for transmission.

The role of garbage feeding in the trichiniasis cycle can best be ascertained by a comparative review of the prevalence of trichiniasis in garbage-fed and farm-raised swine. One of the first to determine the relationship of garbage-feeding to the trichiniasis problem was Mark who conducted studies in the Boston, Massachusetts area. In a preliminary phase, Mark examined 500 midwest swine of unknown origin during 1881 and found trichinae in 19 (2.0 percent). These were probably a mixture of grain-fed and swill-fed swine. Mark, during 1883-1888, examined 3064 swine from the Boston area where the basic feeds consisted of offal, garbage and slops containing table scraps. Trichinae were found in 391 (12.9 percent) of the swine examined. Similarly, 42 (17.9 percent) of 234 garbage-fed swine from Massachusetts state institutions were found to be infected with *T. spiralis*. Thus, even 80 years ago there was ample evidence to incriminate the feeding of raw garbage as a prime source of trichiniasis in swine since the infective rate for garbage-fed swine was 6–8 times that obtained for midwest farm-raised swine. In making these studies, Mark microscopically examined only 1 grain (approximately one-sixteenth gram) of diaphragm from each pig. Trichinoscopic methods normally use 1 gram of diaphragm while artificial digestion studies utilize samples weighing up to 100 grams or more. Hall postulated from this factor that a prevalence of 25 to 50 percent would be a conservative estimate of the actual prevalence of the disease in the garbage-fed swine examined by Mark.

The United States Department of Agriculture began a series of studies during the 1930's to monitor the prevalence of trichiniasis in both garbage-fed and farm-raised swine. These studies, which have been continued by the author in collaboration with the Meat Inspection Division, Consumer and Marketing Service, USDA, utilized the artificial digestion technique. The results are shown in Table 1. Schwartz during the 1930's examined 10,500 diaphragms of swine from garbage feeding centers on both the Atlantic and Pacific seaboards and found 599 (5.7 percent) infected. In contrast, only 126 pigs (0.95 percent) of 13,000 farm-raised swine examined during the same period were found infected. Thus, 50 years after the problem was first pointed out by the studies of Mark, the prevailing rate of infection for garbage-fed swine was still 6 times that for farm-raised swine.

During 1950, Schwartz reactivated studies on garbage-fed swine and found 146 (11.0 percent) of 1325 diaphragms to contain trichinae. Forty-four of the 146 infected diaphragms contained more than 1 trichina per gram with a maximum of 2369. This high prevalence was maintained even in 1954 when 114 (9.45 percent) of 1206 swine which had been fed raw garbage the first part of the period and cooked garbage the latter half were found to be infected. With the advent of garbage cooking during the mid 1950's the infective rate sharply decreased. Schwartz found trichinae in only 2.2 percent of 5723 swine fed cooked garbage during 1954-59. In a comparative study, Schwartz found *T. spiralis* in 0.63
percent of 3031 midwestern swine examined during 1948-52.

Zimmerman and Brandy carried out a national swine study during 1962-65. Prevalences of 0.12 percent and 0.22 percent were obtained for farm-raised butcher and breeder swine, respectively. The prevalence in 5041 garbage-fed swine, however, was 2.6 percent.

The latter figure is somewhat misleading. During 1961, the initial year of the study, prevalences of 6.3 percent and 5.1 percent were obtained from swine from two of the leading garbage feeding states. The prevalences in these states then sharply decreased to levels of 0.4 percent and 0.5 percent during 1963 and were maintained at these levels through 1966.

The apparent downward trend in the prevalence of the disease in garbage-fed swine as indicated by the study of Zimmermann and Brandy, was investigated in a statistically designed national study of 5955 garbage-fed swine during 1964-66 by Jefferies et al. A statistically weighted prevalence of 0.5 percent was obtained. Thus, in a period of 15 years the prevalence in garbage-fed swine decreased from about 10 percent to 0.5 percent, a reduction of 95 percent. The current prevalence of 0.5 percent is still, however, about 5 times that found in non garbage-fed swine.

Not only is the prevalence higher in garbage-fed swine, but the intensity of infection, as indicated by trichina per gram counts, is also generally higher than that obtained from farm-raised swine. Schwartz considered as significant only those infections containing one or more trichina per gram since those infections would be most likely to cause clinical infection in man. In the 1950 study by Schwartz on raw garbage-fed swine, an overall infective rate of 11.0 percent was obtained but only 3.3 percent of the swine had significant infections. During the 1954-59 study when the prevalence was 2.2 percent, approximately one-half the infected swine had significant infections. In two recent studies on garbage-fed swine by Zimmermann and Brandy and by Jefferies et al., 40 percent of the infections contained one or more trichinae per gram. This gave a significant prevalence rate of 0.9 percent in the former study and only 0.2 percent in the latter study. In contrast, Zimmermann and Brandy found that only 12 percent of the infections in farm-raised swine contained more than 1 trichinae per gram; the significant infection rate was therefore, less than 0.02 percent. Thus, while the current overall infective rate for garbage-fed is 5 times greater than for farm-raised swine the significant infection rate is 10 times greater.

The changes in the prevalence of trichiniasis in garbage-fed swine correlates closely with the eradication programs for vesicular exanthema and hog cholera (Fig. 1). With rapid spread of vesicular exanthema through the swine of the U. S. in 1952 and the subsequent passage of garbage cooking regulations, the prevalence in garbage-fed swine was almost immediately reduced from 11.0 percent to 2.2 percent, a decrease of 80 percent. The hog cholera eradication program then reduced the prevalence further to only 0.5 percent in 1964-66. Thus, in a period of less than 15 years the overall prevalence in garbage-fed swine decreased 95 percent. The sharp rise to about 5 percent found in 1961, however, is of major significance. Vesicular exanthema had been eradicated five years previously. Cooking equipment was becoming old, no new major swine disease problems were threatening, and laxity in cooking may have occasionally occurred. Thus, a sharp rise in the prevalence rate occurred. If continual emphasis on proper cooking procedures is not stressed after hog cholera is eradicated, a similar but possibly lesser increase in the
prevalence of trichiniasis in swine may again occur.

The question now arises, with the relatively low prevalence of trichinae now found in garbage-fed swine, as to the relative importance of this group of swine in the perpetuation of trichiniasis. Zimmerman, in 1966, using current prevalence rates and a marketing base of 85,000,000 swine, estimated that about 105,000 trichina-infected swine were being marketed each year in the United States. Ninety-four percent swine were farm-raised while only 6 percent were garbage-fed swine. At first glance this would tend to minimize the role of garbage feeding and place the primary burden of solving the trichiniasis problem on the producers of farm-raised swine. This is not entirely true. If trichiniasis is to be controlled and eventually eradicated, this 6 percent of the infected swine is of major significance for several reasons. First, garbage feeding is concentrated primarily in the coastal metropolitan areas where the pork is marketed. Thus, this infected pork would tend to cycle infections in the same area perpetuating a continual area problem. In contrast, pork from farm-raised swine is dispersed throughout the U.S. The probability of pork scraps from an infected farm-raised swine ending up in garbage fed to swine is much less than that for pork from an infected garbage-fed swine. Since 40 percent of the infections currently found in garbage-fed swine contain more than 1 trichina per gram in contrast to only 12 percent in farm-raised swine, pork scraps from garbage-fed swine would be more likely to initiate infections in swine if fed raw or improperly cooked than would the infected pork generally obtained from farm-raised swine.

Although nearly 100,000 infected swine cannot be attributed to commercial garbage feeding practices, nearly all can be ascribed to garbage feeding per se in the form of table scraps, other household garbage, and, to an unknown but much lesser extent, wildlife carcasses. The feeding of table scraps and other household garbage has declined sharply in the past two decades which the increased emphasis on commercial feeding programs as practiced in the north central swine producing area. However, an undeterminable amount still occurs, especially on smaller farms where both the number of swine and crop production is limited. The feeding of table scraps from the farm home and other sources would bring about a major cost saving on these marginal farms. Some of the swine from these farms would be utilized for home consumption, thus trichiniasis could readily cycle in the herds of these non-registered garbage feeders.

The role of wildlife vectors has not been determined. Zimmermann and Hubbard in 1963 reported finding 15 infected species of wildlife in Iowa. The prevalence rate for fox and mink was over 5 percent. Thus, the feeding of wildlife carcasses to swine, instead of proper disposal by burying, could help perpetuate trichiniasis as well as other diseases. This practice again would be most prevalent on the smaller farms. In discussing the possible role of wildlife in transmitting trichiniasis to swine, the role of rats in garbage feeding installations should be briefly mentioned. Various workers through the years have incriminated rats as an important source of infection for swine. However, the consensus of opinion would not assign little or no significance to rats in the primary trichiniasis cycle. Infection in rats from garbage feeding installations is probably an indicator of poor garbage cooking procedures and therefore examination of rats for trichiniasis could possibly be used as a screening method to evaluate the efficacy of cooking methods.
Studies on the prevalence of *T. spiralis* in Iowa rats has shown that infections are prominent in rats obtained from city and village dumps but uncommon in farm rats. The overall prevalence of 995 dump rats was 6.7 percent while no infections were detected in 324 farm rats. However, since the inherent possibility exists that rats may carry trichiniasis and that swine may occasionally eat rats, rat control programs should be carried out wherever swine are raised.

Since hog cholera eradication is receiving attention at this time, a possible relationship between trichiniasis and hog cholera should also be mentioned. Zimmermann and Schwarte, in limited studies, demonstrated that *T. spiralis* larvae, when isolated by digestion from the diaphragms of hog cholera infected pigs, could mechanically transmit the virus in an active or pathogenic form to susceptible pigs. Injection of the supernatant digestive fluid failed to produce infection. Since the parasites were washed and disinfected before administration to the recipients, the virus was apparently carried within the parasite. The parasite could thus shield the hog cholera virus from certain environmental factors which would normally inactivate the virus.

It is both surprising and disconcerting to anyone reviewing the trichiniasis problem to find that there has never been a specific control program for trichiniasis in the United States, a country noted for its public health oriented control programs. This is especially true in examining the data of the 1930's when 16 percent of human diaphragms obtained at autopsy contained trichinae as did 10 percent of the garbage-fed swine and nearly 1 percent of the farm-raised swine. In the past 3 decades these prevalences have declined to 4 percent in humans, 0.5 percent in garbage-fed swine and 0.1 percent in farm-raised swine. These prevalence rates will probably continue to decrease for a period of time due to the effect of swine feeding programs, the hog cholera eradication program, and the widespread usage of freezer facilities for pork storage. The stigma on pork, however, will continue to exist as long as the inherent threat to human health is present. Therefore, those concerned with the trichiniasis problem should consider formulation of a specific eradication program.

If, or more preferably when, a trichina eradication program is formulated, the garbage feeding problem must be given primary consideration. The best solution would be elimination of garbage feeding such as accomplished in Wisconsin. There will be arguments against this policy stating that garbage disposal would be impossible otherwise in some metropolitan areas and that this would also place undue economic sanctions against the commercial garbage feeders. This may be true to some extent, but the June, 1968 National Status on Control of Garbage Feeding lists only 9483 garbage-fed swine in Illinois, 5867 in Michigan, and 8546 in Ohio. New York state reported only 16,068 swine being fed garbage. The garbage being fed to swine in these highly populated states must represent only a very small portion of the available garbage.

If garbage feeding cannot be eliminated, then increased emphasis must be placed on the strict enforcement of proper cooking procedures and maintenance of equipment. A program for re-evaluation of cooking procedures must also be made. Although the prevalence of trichiniasis in garbage-fed pigs has decreased 95 percent since the advent of cooking, it is still at least 5 times that obtained for farm-raised swine. This would indicate that an increase in efficacy of cooking procedures must
be brought forth if trichiniasis is to be eliminated. Since trichinae are killed by exposure to heat at about $137^\circ$F, the occasional failure to destroy trichinae in garbage would also indicate that other disease organisms may also escape inactivation during the cooking process.

In addition to the problem evolved by commercial feeding of garbage, major attention must also be given to the role of garbage in the perpetuation of trichiniasis in farm-raised swine. An intensive educational program must reach all swine producers, both large and small, citing the hazard of feeding raw or improperly cooked garbage not only in regard to trichiniasis but for other disease organisms also. The danger of feeding wildlife carcasses must also be stressed. If garbage feeding can be eliminated or properly controlled, trichiniasis need no longer be feared. If not, trichiniasis will continue to be a public health problem in the United States.

REFERENCES


TABLE 1

PREVALENCE OF TRICHINIASIS IN GARBAGE-FED SWINE WITH COMPARATIVE STUDIES IN FARM-RAISED SWINE

<table>
<thead>
<tr>
<th>Year</th>
<th>Garbage-fed Swine</th>
<th>Farm-raised Swine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No. +</td>
</tr>
<tr>
<td>1930's</td>
<td>10,500</td>
<td>599</td>
</tr>
<tr>
<td>1948-52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1950</td>
<td>1,325</td>
<td>146</td>
</tr>
<tr>
<td>1954-59</td>
<td>5,723</td>
<td>126</td>
</tr>
<tr>
<td>1961-66</td>
<td>5,041</td>
<td>131</td>
</tr>
<tr>
<td>1964-66</td>
<td>5,955</td>
<td>25</td>
</tr>
</tbody>
</table>

*Butchers
**Breeders
COST OF ENFORCING GARBAGE FEEDING REGULATIONS
AND GARBAGE RELATED HOG CHOLERA OUTBREAKS

M. J. Tillery, DVM*

Each State has laws, rules or regulations which require garbage to be heat-processed before being fed to swine. For the most part, these requirements had their inception as a basic part of the effort to eradicate vesicular exanthema. This program was completed in 1959, and these authorities have been incorporated into the cooperative hog cholera eradication program begun in 1962. Wisconsin prohibited garbage from being fed to hogs in July 1968, and this is the first State to take this action. While there are no Federal requirements as to heat-processing garbage, severe restrictions affect the interstate movement of swine fed raw garbage as well as pork derived from such swine.

Hog cholera eradication standards require that garbage feeding establishments be inspected at least monthly. An exception is that those premises on which garbage derived from school lunch programs, such garbage containing no raw meat scraps, are exempted from the monthly regimen. These feeders may be inspected at the beginning and close of the school term. Such inspection does not relieve these feeders of compliance with the applicable authority as to heat processing the garbage.

Equipment for heating the garbage is also checked. In the case of steam equipment, an inspection is made at three month intervals as to its capability to heat the garbage to boiling for at least 30 minutes. A similar inspection is conducted on direct-fire equipment except that the inspection interval is every six months. It is thought that 10 percent of the premises use steam equipment and 90 percent use direct-fire equipment.

No information is available as to the actual number of herds or the actual number of swine fed garbage throughout a year. Therefore, these estimates are made. March is taken as a representative month in that school lunch garbage is fed during this period as well as that from other sources. During March 1968, there were 9390 known garbage-fed herds containing 850,000 swine. Some feeders discontinue their operation during the year with others taking over the collection and feeding operation in other herds. This turnover results in an estimate of 10,000 herds in which garbage will be fed at one time or another during the year. As to numbers of swine fed garbage, the estimate is 1,700,000 head. Garbage feeders usually purchase heavy feeder pigs (at least 60 pound pigs and often 100 pound pigs or heavier). However, the rate of gain is less than expected from grain-fed operations, therefore, it is estimated that two groups of pigs will be fed on one premises in the year.

The inspection workload is heavier than the minimum required by the standards. This is primarily due to noncompliance with the heat processing requirements. Followup inspections are then conducted in an effort to gain compliance with these restrictions. Noncompliance is common and it is estimated that at least 10 percent of the 10,000 premises must be reinspected at more frequent intervals. Therefore, the number of inspections per year required is derived as follows:

Compliance
10,000 premises x 1.1 inspections (10% reinspection) = 11,000 inspections/month
11,000 x 12 months = 132,000 inspections/month

Temperature inspections
90% direct fire x 10,000 premises x 2 inspections/year = 18,000 inspections/year
10% steam x 10,000 premises x 4 inspections/year = 4,000 inspections/year
10% reinspection of heating equipment = 2,200 inspections/year
Total inspections per year = 156,200

The cost of conducting these inspections is estimated as follows:

Two hours are estimated per inspection (227 workdays or 1816 man-hours/year.
Therefore 908 inspections could be conducted per year by one inspector.
156,200 inspections ÷ 908 inspections per man year = 172 inspectors needed.
Time is also required to locate new feeders and this is estimated to be an additional five percent. Therefore, 180 inspectors are required to inspect the feeders at the required intervals.

In 1965, the proportion of feeders inspected by Animal Health Division personnel was 60 percent and 40 percent were inspected by State inspectors. About
85 percent of the ANH personnel were GS-7; with the remainder being GS-5's. Therefore, personnel costs are computed as follows:

**ANH Cost**

- 92 GS-7 @ $7447 per year
- 16 GS-5 @ $6115 per year
- Total salaries
- Personnel benefits ($782,964 x 9%)
- Total salaries and benefits
- Per Diem (108 inspectors x $600 per year)
- Gas and oil (108 vehicles x $540 per year)
- 108 vehicles (average life of four years, therefore 27 x $1850)
- Total ANH cost

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>92 GS-7 @ $7447 per year</td>
<td>$685,124</td>
</tr>
<tr>
<td>16 GS-5 @ $6115 per year</td>
<td>97,840</td>
</tr>
<tr>
<td>Total salaries</td>
<td>782,964</td>
</tr>
<tr>
<td>Personnel benefits ($782,964 x 9%)</td>
<td>70,467</td>
</tr>
<tr>
<td>Total salaries and benefits</td>
<td>853,431</td>
</tr>
<tr>
<td>Per Diem (108 inspectors x $600 per year)</td>
<td>64,800</td>
</tr>
<tr>
<td>Gas and oil (108 vehicles x $540 per year)</td>
<td>58,320</td>
</tr>
<tr>
<td>108 vehicles (average life of four years, therefore 27 x $1850)</td>
<td>49,950</td>
</tr>
<tr>
<td>Total ANH cost</td>
<td>$1,026,501</td>
</tr>
</tbody>
</table>

**States’ cost**

- It is estimated that State expenses will approximate those listed above. Therefore, the States’ cost would be 40 percent of the total inspection cost which equals $684,334
- Total cost of garbage inspection $1,710,835
- Annual cost per herd (9390) $182
- Annual cost per head $1

These estimates are thought to be conservative in that numbers of garbage feeders may be underestimated. There is little possibility that they are overestimated. In addition, salaries for the inspectors are estimated at the third step of the grade. This may also be an underestimate with little possibility of an overestimate. Therefore, these computations should be regarded as minimal garbage-fed swine inspection costs.

In fiscal year 1968, an estimated $8.4 million were spent by the cooperator's toward hog cholera eradication with $4.6 million spent by ANH and $3.8 million spent by the States. Therefore, garbage inspection expenditures required 20 percent of the funds available, or 23 percent of ANH funds and 18 percent of the State funds which were budgeted for hog cholera eradication.

Despite this heavy annual cost of garbage-fed swine inspection, hog cholera and other diseases occur more frequently in this segment of the swine population than would be expected on a proportionate basis. In fiscal year 1968, seven percent or 61 of the 849 outbreaks were related to feeding raw or improperly processed garbage. Of these 50 herds were depopulated in accordance with Phase III or Phase IV standards. One might be misled here in that some of these outbreaks resulted from feeding household garbage which is usually exempt from heating requirements. Nevertheless, these outbreaks resulted from feeding raw garbage and constitute a sizable program expense for necessary herd depopulation. Therefore, no distinction is made as to household or commercial garbage in computing the depopulation costs of garbage-fed herds which are infected with hog cholera.
Cost of depopulation is calculated as follows:

**Diagnosis**

Two days are estimated per diagnosis. Therefore, one diagnostican can diagnose 114 outbreaks per year. Diagnosticians salaries are calculated at the GS-12 rate. No consideration is given to the cost of negative diagnoses. Therefore, the 61 outbreaks would require 0.5 diagnosticians.

$$0.5 \text{ diagnosticians} \times \$12,986 = \$6,493$$

Personnel benefits (9%) $584

Per Diem @ $600/year x 0.5 diagnosticians $300

Gas and oil @ $540/year x 0.5 diagnosticians $270

0.5 vehicles, average life of four years,

$$0.13 \times \$1,850 = \$241$$

Total diagnostic cost $7,888

Total Federal Cost of Diagnosis (60%) $4,733

Total State Cost for Diagnosis (40%) $3,155

**Epidemiology**

Two weeks are estimated for each investigation by a GS-12 veterinarian. Therefore, a veterinarian investigates 24 outbreaks for source of infection per year. 2.5 veterinarians would be required to establish source of infection in the 61 outbreaks calculated as follows:

$$2.5 \text{ GS-12 veterinarians} \times \$12,986/\text{year} = \$32,465$$

Personnel benefits (9%) $2,922

Total Salary $35,387

Per diem $600/year x 2.5 investigators $1,500

Gas and oil @ $540/year x 2.5 vehicles $1,350

2.5 vehicles, average life of four years,

$$0.5 \times \$1,850 = \$1,110$$

Total epidemiology cost $39,347

Total Federal cost for epidemiology (60%) $23,608

Total State cost for epidemiology (40%) $15,739

**Herd depopulation costs**

Several regulatory people are required to depopulate premises. The expenses are estimated to amount to one week expenditures by a GS-12 veterinarian and one week expended by a GS-7 livestock inspector per depopulation. Therefore, one GS-7 livestock inspector would be required to depopulate the infected herds in Phase III and IV States.
Costs are calculated as follows:

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-12 veterinarian</td>
<td>$12,986</td>
</tr>
<tr>
<td>GS-7 Livestock inspector</td>
<td>7,447</td>
</tr>
<tr>
<td><strong>Total salary</strong></td>
<td><strong>$20,433</strong></td>
</tr>
<tr>
<td>Personnel benefits (9%)</td>
<td>1,839</td>
</tr>
<tr>
<td>Per diem @ $66/year x 2 people</td>
<td>1,200</td>
</tr>
<tr>
<td>Gas and oil @ $540/year x 2 vehicles</td>
<td>1,080</td>
</tr>
<tr>
<td>2 vehicles, average life of four years,</td>
<td></td>
</tr>
<tr>
<td>0.5 x $1,850</td>
<td>925</td>
</tr>
<tr>
<td><strong>Total depopulation cost</strong></td>
<td><strong>$25,477</strong></td>
</tr>
<tr>
<td><strong>Total Federal Cost (60%)</strong></td>
<td><strong>$15,286</strong></td>
</tr>
<tr>
<td><strong>Total State Cost (40%)</strong></td>
<td><strong>$10,191</strong></td>
</tr>
</tbody>
</table>

Indemnity cost

The average herd depopulated in 1968 contained 170 swine.

Average indemnity costs per head were as follows:

**Phase III States:**
- $1795 ANH indemnity per herd
- 1526 State indemnity per herd
- $3320 Total indemnity per herd

**Phase IV States:**
- $2920 ANH indemnity
- 2920 State indemnity
- $5840 Total indemnity

Of the 61 garbage related outbreaks, 11 occurred in Phase II States and these were not depopulated. No estimate is made for costs such as administration of quarantines on these herds. However, 47 of the outbreaks were in Phase III States and 3 were in Phase IV States. Indemnities are calculated as follows:

**Phase III States**
- 1795 ANH indemnity per herd x 47 outbreaks $84,365
- 1526 State indemnity per herd x 47 outbreaks 71,722
- **Total indemnity for garbage related outbreaks** $156,087

**Phase IV States**
- 2920 ANH indemnity per herd x 3 outbreaks $ 8,760
- 2920 State indemnity per herd x 3 outbreaks 8,760
- **Total indemnity for garbage related outbreaks** $17,520

**Total indemnities paid for garbage related outbreaks in Phase III and IV States:**
- By ANH $ 93,125
- By States $80,482*
- **Total paid for garbage related outbreaks** $173,607

*Payment pending in one State
Summary of hog cholera eradication program costs related to garbage feeding.

<table>
<thead>
<tr>
<th>Function</th>
<th>State Cost</th>
<th>Federal Cost</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspection of premises</td>
<td>$684,334</td>
<td>$1,026,501</td>
<td>$1,710,835</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>3,155</td>
<td>4,733</td>
<td>7,888</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>15,739</td>
<td>23,608</td>
<td>39,347</td>
</tr>
<tr>
<td>Depopulation</td>
<td>10,191</td>
<td>15,286</td>
<td>25,477</td>
</tr>
<tr>
<td>Indemnity</td>
<td>80,482</td>
<td>93,125</td>
<td>173,607</td>
</tr>
<tr>
<td><strong>Total Cost</strong></td>
<td><strong>$793,901</strong></td>
<td><strong>$1,163,253</strong></td>
<td><strong>$1,957,154</strong></td>
</tr>
</tbody>
</table>

Percent of total funds utilized in garbage related outbreaks: 21% State, 25% Federal, 23% Total

Garbage fed swine inspection is one of the more costly items in the hog cholera eradication program. The system of inspection probably limits spread of hog cholera through garbage. A system such as this would have to be maintained throughout the program unless garbage feeding were prohibited. While this system likely limits hog cholera spread, the procedure is not perfect inasmuch as 61 outbreaks of hog cholera were blamed on garbage feeding in 1968.

Should the permissive garbage feeding be discontinued, administration of this legislation would likely be as expensive as the present inspection system. However, hog cholera outbreaks related to garbage feeding should drop sharply, and this would represent a program saving. A corresponding decrease in trichinosis incidence as well as other domestic diseases should also decrease in similar fashion, if garbage feeding were prohibited.

Remarks by Wilbur L. Plager

Given at U. S. Livestock Sanitary Association Convention Board Meeting
Held at New Orleans, Louisiana — October 8, 1968

On behalf of the National Swine Record Association I wish to take this opportunity to thank the United States Livestock Sanitary Association for this opportunity to express our views in regard to the feeding of garbage to swine and the many complications involved in it.

First, I would like to point out that the group of purebred swine breeds and breeders I represent have been responsible for every ounce of swine breeding improvement that has taken place during the many years that our associations have been in existence. The Yorkshire breed I represent celebrated the end of its 75th year on April 1st of this year which gives you some idea of the length of time and efforts of our association. Some breed associations are even older in years of service to the swine industry.

Much money, time, and effort have gone into these purebred swine associations during the many years to develop strains or lines of breeding to help the swine industry. Every imaginable type of hog has been developed to produce that which was needed most at a given time. When lard was a needed commodity, the percentage of lard in a hog carcass was greater. Present day demands are for less lard...
and more muscle. Purebred breeders are meeting a challenge and have developed a modern hog for today's needs. He has little resemblance to those early hogs. Today's hog has a much higher percentage of muscle, with a lot less lard. All this has cost a lot of money, time, and effort, thru testing pigs of a litter in swine evaluation testing stations, on the farm testing, plus comparisons in barrow shows and carcass contests, by breeders of purebred hogs. Yes, even many of our top commercial breeders are doing a great deal of showing, cutting, and testing, to find out what combination of breeds and lines are doing the best job.

I was raised on an Iowa farm. I have spent quite a few years now in hog pens on my own farm and others all over the United States plus Mexico, Canada, and Europe. The last 26 years I have had a chance to see hogs raised under every imaginable procedure possible. I have also soon learned that pork products lacked some in prestige in comparison to some of our competition. I saw the need, with others, for a swine industry organization, so was one of many who were responsible for helping organize the National Pork Producers Council and served as its first president. The Swine Industry has needed a national association for a long time to look after its best interests. I have seen many kinds of garbage being fed under many different conditions. I question whether many of these operations could ever help promote the sale of pork. With only about one percent of all the hogs in the United States being fed this way and offering nothing to the industry, is like the tail of the pig wagging the pig as far as our U.S. Swine Industry is concerned. I cannot see any place for garbage feeding other than some products that have not come through a restaurant or a household. I have seen some G.I. rations and candy bars fed on a limited basis in a ration that had been condemned that could have had some salvage value at no expense or harm to the swine industry.

Household garbage has little to offer as hog feed. If all household garbage looks like what I see when I place ours in the trash can at our apartment, this would sure be a true statement. Restaurant garbage certainly has more to offer as feed for hogs but I doubt very much if worth what it costs the swine industry in terms of prestige of our product, especially when only about one percent of the total hog production are fed this way. Fifteen feeders of garbage in the state of Indiana feeding 3,250 hogs should not be permitted or able to create this poor of an image, especially when nearly half of this amount is fed in state institutions.

Legislation for cooking of garbage was a big forward step. Many years ago when Germany started cooking of garbage, they reduced the presence of Trichinosis to less than 1 percent. We are in the process of trying to eliminate Trichinosis. Some will probably point out that there may be less in hogs fed cooked garbage. Dealing with one percent of a ninety million-odd pigs or one million is no comparison. We do not need Trichinosis in hogs fed either way.

Every country who has eliminated cholera, Vesicular Exanthema, or other major swine diseases have had to restrict garbage feeding. I served on the National V. E. Committee some years ago. In my now adopted state of Indiana, three state institutions objected to restrictions on garbage feeding. They were the only three with V. E. outbreaks in the whole state of Indiana. Most all cases of V. E. in the U.S.A. were in garbage feeding herds.

Garbage feeding is not the only thing that has hurt the image of pork. In years past, cattle feeders felt that they must have hogs running behind cattle in feed lots.
They fed these hogs to very heavy weights. The combination of these many ills did not help the image of pork. Fewer hogs are being fed this way today, by far. Marketing hogs at too heavy weights is wrong, if fed behind cattle, garbage or normal procedures. All hurt the swine industry.

Surely garbage can be disposed of in a better way than through hogs. Burying, incinerating, or composting offer some means of disposing of garbage that would let the swine industry free of this unfavorable image handicap. Today, more counties in more states have outlawed the raising of hogs within a county. More will follow this pattern in the future. Feeding of garbage will lead to an ordinance outlawing of hogs being raised in any county far sooner.

Disposal of waste from raising hogs, period, is coming in for more attention today than ever and it will become even a bigger problem to swine producers in the future. I am sure garbage feeding of hogs is nearing its final stage and only a matter of time until it will be outlawed as a method or way of disposing of garbage.

Garbage collectors have had more than their share of controversy and will continue to have enough without feeding it to hogs. A well known national political figure told me, many years ago, that garbage collection was one of the biggest political footballs in municipal government. I am for taking hogs out of this arena and let garbage find another source, or way of disposing of its material. Any way that will not handicap a worthwhile industry such as the Swine Industry will be a better way.

My remarks have not likely been to the liking of those who are feeding garbage but I believe they are in the best interests of Swine Producers in general. I am just as sure that if those who are feeding garbage were to be honest with themselves, that they would agree that the handwriting is on the wall for the elimination of feeding garbage as well as tighter controls on disposal of hog waste will be in certain areas. We can only exist as an industry by facing these changes as they arise or even before they arise. We will eventually be forced into tighter controls. Meeting these problems, possibly before they arise or being forced into them, would be a cheaper and better way out for the good of our Swine Industry.

Statement by Rolland Paul, Executive Vice President
National Pork Producers Council
Presented at
“Symposium on the Future of Garbage Feeding”
USLSA Convention
Jung Hotel, New Orleans, Louisiana
October 8, 1968

The National Pork Producers Council, at their June board meeting, went on record favoring outlawing the feeding of garbage to hogs. The Council notes that approximately only 1 percent of the nation's hogs are fed garbage.

When we realize that the majority of the major pork-producing states feed virtually very little garbage and that only approximately 180 producers of an estimated 240,000 in those states feed garbage at all, the Council feels that the
consumers' image of pork is being hurt by this small percent of producers and production. The Council notes that not only the image but disease problems are greater among garbage fed hogs.

While the Council feels that the outlawing of garbage must be done on a state by state basis, preferably on a 3-5 year plan, we feel that some other recommendations can be developed: (1) support federal regulations to stop interstate shipment of garbage for hog feeding; (2) negotiate with the Defense Department and request that they stop selling garbage for hog feeding; (3) work closely with cities in developing a way of disposing of garbage other than through hogs; (4) secure necessary funds to enforce prohibiting feeding of garbage to hogs through inspection, etc.; (5) encourage 110 percent state inspection of garbage-feeding premises and hogs.

The National Pork Producers Council hopes to furnish a strong voice through the swine industry to enforce the outlawing of garbage feeding on the national level.

CALIFORNIA GARBAGE FEEDING INDUSTRY

G. L. Crenshaw and F. P. Jones

The feeding of waste products to swine in California has shown a rather substantial decline in the past 14 years. As of August, 1968, there were 56,565 hogs being fed garbage compared to 227,936 hogs in June of 1954. The number of ranches feeding garbage has decreased equivalently with 156 establishments feeding garbage in 1968 while there were 602 in 1954.

A number of factors have been responsible for this decline; but, the most significant ones have been the requirements for cooking garbage, a markedly decreased supply of household garbage, increased collection and handling costs, and several years of unfavorable hog prices.

Proper cooking of garbage in California has controlled and eliminated several diseases of economic and public health significance; most importantly vesicular exanthema, hog cholera, and trichinosis.

California has been free of vesicular exanthema for the past five years. We feel confident that if the current methods of cooking and inspection are continued, this record will be maintained.

Hog cholera has been satisfactorily controlled. Currently there are 28 ranches holding permits to vaccinate with modified live virus vaccine. Of the 156 garbage feeders only 14 have vaccination permits. This, we believe, is good evidence that hog cholera is not a problem for our garbage garbage feeders; particularly since in our experience when raw garbage was fed, the modified live viral vaccines were ineffective in controlling the disease, necessitating the use of virulent virus and serum to protect newly-introduced pigs. Furthermore, many of the hog cholera outbreaks in both grain and cooked-garbage-fed swine have been caused by the use of modified live virus vaccine without administering serum simultaneously. The most recent hog cholera outbreak in California was in July, 1967, and was caused by the improper use of modified live vaccine.
Trichinosis surveys conducted in California by the Animal Disease Eradication Division, ARS, U.S. Department of Agriculture, in 1965-66 involving 741 samples collected from 18 premises feeding garbage, yielded no evidence of trichina infestation.

There are some disease problems in garbage-fed hogs not seen as frequently in grain-fed hogs. Perhaps the most important are pseudorabies and nutritional diseases. These diseases are ones that the individual garbage feeder must contend with and do not affect other swine producers. One of the beneficial results from the feeding of garbage that is rarely discussed is the comparative freedom of pigs from intestinal parasites. Evidently garbage has fairly good anthelmintic qualities.

If the concern over the feeding of garbage is esthetic, one must consider dogs raiding garbage cans as a worse problem. Furthermore, the practice of feeding cattle and pigs together is much more repulsive. We must consider that certain species of animals, i.e. dogs, bears, and hogs, are attracted to garbage and readily consume many waste foods.

If hog cholera is spread by the feeding of garbage, it has been done by feeding raw household or improperly cooked garbage. It seems unrealistic to attempt to control a disease such as hog cholera by having states in different phases of the eradication program. Pigs from infected ranches should be slaughtered and not shipped interstate or else the herd should be destroyed.

The feeding of garbage serves the distinct purpose of utilizing and disposing of waste foods. The proper cooking of garbage has been demonstrated in California to effectively control diseases of economic and public health importance. Garbage-fed swine have not been a hazard to the swine industry in our state.

We recommend that the feeding of cooked garbage be allowed to continue with no further restrictions than those currently in effect.

Presented at USLSA Meeting, New Orleans, Louisiana, October 7-11, 1968, by G. L. Crenshaw, D.V.M.

Statement of Dr. E. L. Brower
Director, Division of Animal Health
New Jersey Department of Agriculture
for the
Symposium on the Future of Garbage Feeding
United States Livestock Sanitary Association
New Orleans, Louisiana — October 8, 1968

In 1957, New Jersey enacted a Garbage Feeding Swine Law. In addition to requiring the proper heat treatment of garbage to 212°F. for 30 minutes, the law also requires these farms to meet rigid sanitary regulations. The garbage feeding farms are inspected at least twice a month for sanitation and temperatures are recorded at least once a month. Our force of State and Federal inspectors also conduct periodic weekend checks for cooking compliance. We believe that our farmers as a whole, manage their garbage feeding activities as adequately as this
type of operation permits. Where we find violations, we hold hearings for possible suspensions or revocations of licenses or a monetary settlement in lieu of partial suspension.

At this time, 162 farms are licensed to feed garbage in New Jersey. They contain a total of 87,854 head of swine. This is an average of more than 540 head per herd with some herds as large as 8,000 to 10,000 head. We import more than 60,000 feeder swine each year from other states, principally from the Midwest and South.

The industry in New Jersey annually feeds and markets approximately 150,000 head of slaughter swine with a return of $8,000,000 to $9,000,000.

Another aspect of this business is the contracts the swine farmers have with municipalities for the removal of garbage. The contracts amount to at least $1,500,000 annually, with Philadelphia expending $1,100,000. This represents an average cost per ton to the city of $11.00 for an annual 100,000 tons collected. Were these contracts to be replaced by municipal hauling of garbage with disposal by landfill or incineration, the cost to the municipalities would be approximately $7,500,000.

The municipalities involved state that these garbage feeding swine farmers have the wholehearted support of the municipality and they would oppose any action which might prove a threat to New Jersey's swine feeding industry.

It is estimated that the garbage feeding business in New Jersey has an economic value of $15,000,000 which is not an insignificant portion of the agricultural economy of the State.

We believe that the garbage feeding industry, if properly conducted, is in no way a threat to the health of the swine of New Jersey or a public health menace.

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Report on New England Garbage Feeding
by Edward M. Dwyer, Director
Division of Animal Health
Massachusetts Department of Agriculture

Garbage feeding to swine has been a practice carried on in Massachusetts and the rest of New England since the days of the early settlers. It was a common practice for most farmers to raise one or more hogs during the year, according to the size of the family, and dress them off in the Fall for their own use. It was a very economical way to supply some of the meat for the home.

I am personally familiar with some cases of boys, at an early age, starting out with one or two pigs and expanding their operations over the years into fairly substantial businesses. In some instances they obtained a college education with the money earned from raising hogs.

**ECONOMIC FACTOR:** The garbage feeding industry amounts to eight to ten million dollars a year to the agricultural economy of Massachusetts. There are a number of families gainfully employed in raising garbage fed hogs and have been for generations.

Garbage disposal is a very important financial factor in many of our cities and towns. Incineration is costly and land fill in most cases poses a serious rodent
control problem. It is not good conservation to waste the nutritive value in garbage by burning or using it for land fill. It has been said that many of the starving people of the world today could live in luxury on the waste from American tables.

**NATURAL DECLINE IN THE NUMBER OF FEEDERS:** In 1954 Massachusetts issued 550 permits to feed garbage. In 1968 this number has been reduced to 240, or more than one-half in a fourteen year period. If garbage feeding ever becomes prohibited in Massachusetts, as well as the rest of the New England States, 90% or more of the swine industry will be forced out of business.

**HOG CHOLERA:** The 1967 U.S.D.A. Progress Report states that 5% of the Cholera cases were traced to raw garbage feeding. This is not a large per cent when one looks at some of the other causes, such as intrastate movement 24%; interstate 3%; area exposure 21%; improper vaccination 21%; miscellaneous 8%; and no source 16%.

**CONCLUSION:** We are all quite proud of this country in which we live and consider it one of the most powerful nations in the world. This did not just happen, but came about by hard work, individual initiative, and a free enterprise system. I, for one, value this system and think we should all take a very close look at the direction we are heading at the present time, and refrain from imposing any more laws, rules and regulations than are absolutely necessary. Another item that I think has been overlooked and disregarded too much the last few years is the autonomy of the State.
COMPARISON OF IMMUNOLOGIC PROPERTIES OF A HIGH AND LOW CELL CULTURE PASSAGE OF TRANSMISSIBLE GASTROENTERITIS (TGE) VIRUS FROM A SINGLE ISOLANT


Urbana, Illinois

From the Department of Veterinary Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana, Illinois 61801.

This investigation was a part of the North Central Regional Project, NC-62. The study was jointly supported by funds from the Illinois Agricultural Experiment Station and the Department of Agriculture, State of Illinois. This report is a part of a thesis submitted by the senior author to the University of Illinois in partial fulfillment of the requirements for the Doctor of Philosophy degree.

The observation that high passage cytopathogenic isolants of transmissible gastroenteritis (RGE) virus lose the capacity to produce typical signs of the disease in baby pigs has resulted in controversial opinions concerning the identity of the causative virus.

Most of the scientists now working on TGE believe that loss of pathogenicity for baby pigs by certain TGE isolants is due to their adaptation to cell culture systems.11,13 This theory finds support in the observation that cytopathogenic isolants which are not pathogenic for baby pigs can be used to detect neutralizing antibodies in the serum of animals recovered from TGE.1,2,4 Another group of workers claims that the TGE isolants propagated in cell cultures and described as the causative agent of TGE are only contaminants or viruses merely coincidentally associated with TGE. This theory is based largely on observations that hyper-immune serums produced against cytopathogenic isolants failed to neutralize the infectivity of TGE virus for baby pigs.7,8

In an attempt to resolve this controversy, a comparative investigation of two different viral populations (a high and a low cell culture passage) which had been derived from a single TGE isolant, was undertaken.

MATERIALS AND METHODS

TGE Virus

The thirty-fifth cell culture passage of the Miller* TGE isolant, propagated in primary pig kidney (PK) cell culture in which it caused cytopathic effect (CPE), was used to prepare the stock virus of the low passage (M-35) virus. The fifty percent infectious dose (ID50) of the virus in cell culture was 10^4.5/ml and its ID50 in baby pigs was 10^3.0/ml. The one hundred and eighth cell (PK) culture passage of this isolant was used to prepare the other stock virus (M-108). This latter stock virus had a higher CPE titer (ID50 10^6.5/ml) than the M-35. However, M-108

*Miller tissue culture isolant of the TGE virus received from Dr. E. H. Bohl, Agricultural Experiment Station, Wooster, Ohio.
was not pathogenic for pigs. The TGE virus purified from intestinal contents of infected baby pigs in accordance with the method of Ristic, et al. (designated "Z1") was used to immunize sows by oral exposure. The twelfth cell culture passage of the original Miller RGE isolant was also used to produce antiserums.

**Antiserums**

Antiserums were produced by oral exposure of a sow and pigs and by parenteral injection of rabbits and pigs with TGE virus. The animals were bled approximately 4 weeks after the last exposure. The sera, in 3 or 5 ml aliquots, were stored at -60°C.

The following antiserums were obtained by oral exposure: Serum 1: Sow 847, exposed to intestinal material containing TGE virus; Serum 2: Sow 352, exposed prior to farrowing to a purified preparation of the above intestinal material; Serum 3: Baby pig 128, exposed to the twelfth cell culture passage of the Miller TGE isolate; Serum 5: Pig exposed to the one hundred and eighth (M-108) cell culture passage of the Miller TGE isolant.

The following antiserums were produced by parenteral inoculation: Serum 6: Pig inoculated with the thirty-fifth passage of Miller TGE isolant; Serum 8: Pig inoculated with the one hundred and eighth passage of Miller TGE isolant; Serum 9 and 10: Rabbits inoculated with one hundred and eighth passage of Miller TGE isolant.

**Experimental Animals**

Healthy baby pigs ranging from 3 to 5 days of age were used for infectivity studies. The baby pigs were obtained from herds with no history of TGE and were individually maintained in Horsfall-Bauer isolation units modified by the method of Young and Underdahl.

**Cell Culture Methods**

Primary cell cultures of PK and pig testis (PT) cells were grown in 3, 2 and 1 ounce prescription bottles. Baby pigs from 1 to 4 weeks of age were used as sources of kidneys and testes. Growth medium consisted of Hanks' balanced salt solution (HBSS), 0.5% lactalbumin hydrolysate, 10% heat inactivated calf serum, penicillin (100 units per ml) and streptomycin (100 ug per ml). Maintenance media used during propagation of viruses contained 5% calf serum. A confluent monolayer was usually obtained after 4 to 6 days. Primary PT cell cultures grown on coverslips were used for fluorescent antibodies (FA) studies.

The plaque technique used was that described by Cunningham. Virus titrations in cell cultures were carried out by observing CPE and calculating the titer by the method of Reed and Muench. Viral neutralization tests were conducted in accordance with described methods.

**Fluorescent Antibody Technique**

Conjugates were prepared from Serums 1 and 6. The technique used was similar to that described by La Brec et al. and Schur and Becker.
The procedures for fixing and staining infected cell cultures on coverslips were similar to those described by Konishi and Bankowski.5

Absorption of Anti-TGE Serums with TGE Isolants

The absorption of conjugates with TGE isolants prior to staining infected cells was carried out as follows: The infected coverslip PT monolayers were removed from the tube 48 hours after virus inoculation; 0.3 or 0.5 ml of conjugates were mixed separately with 1.5 or 2.7 ml of infected cell culture fluid from different TGE isolants as well as normal cell culture fluids. The mixtures were incubated at 37°C for 1 hour and then used to stain the infected coverslip monolayers. The final dilution of the conjugate represented two FA units. An FA unit was defined as 1 ml of the highest dilution of the conjugate which stained infected coverslip monolayers.

The absorption of sera with TGE isolants prior to viral neutralization tests were performed as follows: 0.1 ml aliquots of serum were mixed separately with 4.9 ml of fluid from cells infected with TGE virus and also with normal cell culture fluids. The mixtures were incubated at 37°C for 1 hour, following which each mixture was centrifuged at 48,000 x G for 2 hours and then the upper half of the supernatant fluid was pipetted out. The remaining supernatant fluids represented the absorbed sera which were used for viral neutralization tests. The absorbed sera were tested for absence of virus by inoculating 4 bottles of PK cells with each absorbed serum.

RESULTS

Table 1 illustrates the results obtained when coverslip cultures infected with M-35 were stained with two different conjugates previously absorbed with either M-35 or M-108 cell culture fluids. It can be noticed that M-108 did not neutralize all the antibodies in either conjugate used in this experiment. Table 2 summarizes data on serum neutralizations of M-35 TGE virus by anti-TGE serum from a convalescent sow absorbed with either M-35 or M-108 cell culture fluids. When anti-TGE serum was absorbed with M-35 no residual antibody was left which would inhibit ability of the virus to cause CPE in tissue culture cells or the disease in baby pigs. On the other hand, M-108 virus absorbed from the serum only the antibody which inhibits CPE activity of the virus but not pathogenicity in pigs.

Different results were obtained when rabbit anti-M-108 antisera were used in neutralization tests (Table 3). Both CPE and the pathogenicity of M-35 for pigs were neutralized by undiluted rabbit anti-M-108 serum. Normal rabbit serum had no neutralizing effect.

Table 4 summarizes results from experiments conducted on the hypothesis that two viral “serotypes” are required to cause TGE. The M-35 and M-36 virus passages were neutralized with anti-M-108 antisera to form the hypothetical M-35A and

*S. W. 50, rotor and Spinco model L centrifuge, Spinco Division, Beckman Instruments, Palo Alto, California.
M-36A viruses. The M-108 virus was then added to M-35A. In two trials, results were negative, while one trial gave positive results. However, M-36A caused the infection in one case.

Results of pig infectivity studies of the third PK passage of M-35A alone, as well as M-35A in combination with M-108, are presented in Table 5. The results are similar to those described in Table 4. In one trial, the third passage M-35A produced clinical symptoms of TGE and in one trial the third passage M-35A plus M-108 also produced evidence of the disease. These results were inconclusive.

Results of the investigation of a possible dual etiology of TGE represented by a "defective" and a "helper" virus are summarized in Table 6 which presents data on CPE and the pathogenicity of cell cultures for pigs infected with both M-35A and M-108 in different sequences. Cytopathic effect was observed in all but one cell culture. However, none of the cell cultures inoculated with both variants were pathogenic for baby pigs.

It can be seen in Table 7 that the animals exposed orally to TGE isolants tended to develop higher titers in antibodies which neutralized pathogenicity for baby pigs than in antibodies which neutralized CPE. There was no difference between titers of both types of antibodies produced by pigs immunized intramuscularly with cell culture fluids containing M-35 virus. Pig pathogenicity neutralization titers were inversely proportional to cell culture passage, with M-108 by oral exposure giving best results.

DISCUSSION

It can be seen from Tables 1 and 2 that M-108, under the conditions of these experiments, did not remove all the antibodies present in serum from a convalescent animal, while M-35 did. These results suggested a serologic difference between the high (M-108) and low (M-35) cell culture passages of the Miller isolant. However, such a difference does not necessarily imply the existence of two viruses in the low passage.

In Table 3 it can be noticed that antiserum to the M-108 virus did neutralize CPE and pig pathogenicity of the M-35 virus. There was, therefore, an apparent contradiction between results expressed in Tables 1 and 2 and those in Table 3. Several hypotheses can be offered in an effort to provide an explanation for the apparent contradiction of the results.

Hypothesis I: There are two virus "serotypes" and both are required to produce typical signs of TGE in susceptible baby pigs. It may be assumed from results in Tables 1 and 2 that M-35 was a mixture of two serotypes (A + B) and that M-108 was one of them (B). The M-35 (A + B) "combination" was infectious for baby pigs but M-108 (B) was not. However, an anti-M-108 (B) serum neutralized pig infectivity of M-35 (A + B). Therefore, it would be assumed that neither A nor B alone is infectious for pigs but only a mixture (A + B) such as M-35.

In order to prove this hypothesis it would be necessary to demonstrate that the A "serotype" plus the B "serotype" results in a population of viral particles which is infectious for baby pigs. Although in one of three trials, as shown in Table 4 and in one of two trials as shown in Table 5, frank TGE resulted from the mixture of the hypothetical "A" and "B" viruses, both M-35A and M-36A also caused TGE.
Hypothesis II: There are two viruses involved in the etiology of TGE: One is defective virus responsible for pig infectivity and the other acts as a helper during the replication cycle of the defective virus.

It may be assumed from data in Tables 1, 2 and 3 that only the mixture of two viruses, "A" (defective) and "B" (helper) would produce typical signs of TGE in susceptible baby pigs. Failure to isolate "A" virus and demonstrate that the mixture of "A" + "B" was infectious for baby pigs (Tables 4 and 5) could be explained easily. Neutralization of "B" virus of M-35 (A + B) by anti-B serum would not permit replication of "A" virus because it was assumed to be defective; the cell culture fluids would not contain "A" virus and, therefore, the mixture with "B" virus would not be infectious for baby pigs. This hypothesis could also explain why the B virus (cytopathogenic isolant) is so closely associated with TGE outbreaks.

Table 6 presents the results of an experiment designed to provide suitable conditions for replication of a possible defective virus. The mixture of M-35 and anti-M-108 serum was hypothesized to contain the "defective" virus (35A) not neutralized by the antiserum. The cell cultures were inoculated with this mixture and then reinoculated with the hypothetical helper virus B (M-108); different sequences of inoculation were attempted. However, the results did not indicate that a defective virus is required to initiate TGE in pigs.

Hypothesis III: The low passage cell culture isolant (M-35) may contain a greater proportion of inactive viral particles than M-108 which, by competing for antibodies with infectious particles, may remove more antibodies. This hypothesis, although unlikely, could also explain our data. However, the results presented in Table 7 are not readily explained by this hypothesis.

Hypothesis IV: Cytopathogenic isolants nonpathogenic for baby pigs (M-108) are viral particles selected from the original population of TGE virus particles by cell culture passages. Therefore, it can be expected that the serologic characteristics of M-108 would be slightly different from M-35 because of the selective effect of passing viral particles in cell cultures for 73 more times. This hypothesis can explain the results shown in Table 1 and 2 as follows: Serum from convalescent animals will contain antibodies directed against a predominant serotype found in a virulent population of TGE particles. Thus more M-108 cell culture fluids will be required to remove all TGE antibodies from the serum of a convalescent animal than M-35 cell culture fluids.

An explanation of the result shown in Table 3 could be that an M-108 viral population also contains viral particles that are representatives of the original population and parenteral immunization will produce antibodies against these typical viral particles. This would explain why anti-M-108 neutralized the pig pathogenicity of M-35.

Results presented in Table 7 support this last hypothesis. The immune response of animals exposed orally to TGE isolants was found to be higher than that of animals exposed parenterally regardless of the number of the cell culture passage of the TGE isolant used for immunization. In fact, the M-108 virus had the highest neutralizing index against pig pathogenicity. This indicates that the epithelial cells of the intestine of swine "select back" the original TGE viral particles from the viral population used in these experiments.
SUMMARY

Absorption of antiserum to transmissible gastroenteritis (TGE) virus, obtained from a convalescent sow with high and low passage cell culture TGE Miller isolants, revealed a serologic difference. An attempt to isolate the viral particles of the low passage cell culture virus that differed serologically from those of the high passage cell culture virus was made and their role in the etiology of the disease was studied. The results did not support the hypothesis of a requirement of two serologic variants to cause TGE. The hypothesis of a defective virus as the causative agent of the disease was also tested and likewise not supported. The serologic difference between high and low passage cell culture isolants was explained on the basis of population changes due to selective forces of cell culture passages.

TABLE 1

Coverslip Cultures Infected with M-35 Isolant and Stained with Two FA anti-TGE Conjugates Absorbed Previously with either M-35 or M-108 Cell Culture Fluids

<table>
<thead>
<tr>
<th>Conjugate Used</th>
<th>Absorption With Cell Culture Fluids</th>
<th>Infected Coverslip Culture</th>
<th>Noninfected Coverslip Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-M-35, 0.5 ml (FA titer 1/8)</td>
<td>M-108 (1.5ml)</td>
<td>**4/4+</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>M-35 (1.5 ml)</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>NCCF* (1.5 ml)</td>
<td>4/4</td>
<td>0/4</td>
</tr>
<tr>
<td>(Convalescent serum, 0.5 ml (FA titer 1/20)</td>
<td>M-108 (2.7 ml)</td>
<td>4/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>M-35 (2.7 ml)</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>NCCF* (2.7 ml)</td>
<td>4/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* NCCF: Normal cell culture fluids
** Number of coverslips showing fluorescence
* Number of coverslips stained
TABLE 2
Neutralization of Miller-35 TGE virus by an Anti-TGE Serum Absorbed Previously with Either Miller-35 or Miller-108 Cell Culture Fluids

<table>
<thead>
<tr>
<th>Neutralization of M-35 by anti-TGE serum*</th>
<th>Resulting Virus</th>
<th>Observations on Resulting Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CPE**</td>
</tr>
<tr>
<td>I 0.5 ml unabsorbed serum + 0.5 ml of M-35</td>
<td>(I)</td>
<td>0/8</td>
</tr>
<tr>
<td>II 0.5 ml serum absorbed with M-35 + 0.5 ml of M-35</td>
<td>(II)</td>
<td>8/8</td>
</tr>
<tr>
<td>III 0.5 ml serum absorbed with M-108 + 0.5 ml of M-35</td>
<td>(III)</td>
<td>8/8</td>
</tr>
<tr>
<td>IV (a) M-35</td>
<td>4/4</td>
<td>2/2</td>
</tr>
<tr>
<td>(b) M-108</td>
<td>4/4</td>
<td>0/2</td>
</tr>
<tr>
<td>controls (c) serum absorbed with M-35</td>
<td>0/4</td>
<td>0/1</td>
</tr>
<tr>
<td>(d) serum absorbed with M-108</td>
<td>0/4</td>
<td>0/1</td>
</tr>
</tbody>
</table>

* Anti-TGE serum N 1 had a titer of 1/200 against $10^3$TCID50 of M-108. The final dilution used in the test was 1/50.
**The numerator indicates number of positive bottles (CPE) or pigs with TGE signs. The denominator indicates number of bottle cultures or pigs used in experiment.

TABLE 3
Neutralization of Cytopathic Effect in Cell Cultures and Pathogenicity in Pigs of M-35 by Rabbit Anti-M-108 Serum

<table>
<thead>
<tr>
<th>Virus Used</th>
<th>Rabbit Serum N 9</th>
<th>Observations on Resulting Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum Undiluted*</td>
<td>CPE**</td>
</tr>
<tr>
<td>M-35</td>
<td>Control</td>
<td>6/6</td>
</tr>
<tr>
<td>(10^2CPEID50)</td>
<td>Anti-M-108</td>
<td>0/6</td>
</tr>
<tr>
<td>M-108</td>
<td>Control</td>
<td>6/6</td>
</tr>
<tr>
<td>(10^2CPEID50)</td>
<td>Anti-M-108</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* Serum N 9 had an antibody titer of 1/10 against $10^2$CPEID50 of M-108 virus.
**The numerator indicates number of bottle cultures with CPE or pigs with TGE signs. The denominator indicates total number of bottle cultures or pigs used in the experiment.
### TABLE 4
Pathogenicity Tests in Pigs Designed to Investigate the Possibility of a Requirement of Two Viral Serotypes to Cause TGE

<table>
<thead>
<tr>
<th>Virus used</th>
<th>No. Piglets With TGE</th>
<th>No. Piglets Exposed Up to 72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-35</td>
<td>2/2</td>
<td>Vomiting and Diarrhea</td>
</tr>
<tr>
<td>M-35 A*</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>M-108</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>M-35 A + M-108 (0.5 ml)</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>M-35 A + M-108 (0.95 ml)</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>M-36</td>
<td>2/2</td>
<td>Vomiting and Diarrhea</td>
</tr>
<tr>
<td>M-35**</td>
<td>1/2</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>M-35 A + M-108 (0.9 ml)</td>
<td>2/2</td>
<td>Vomiting and Diarrhea</td>
</tr>
</tbody>
</table>

* Virus resulting from the neutralization of M-35 (10^3 CPEID50) by anti-M-108 serum No. 9 (undiluted).

** Virus resulting from the neutralization of M-36 (10^3 CPEID50) by anti-M-108 serum No. 9 (undiluted).

### TABLE 5
Pathogenicity for Baby Pigs of Third Passage of M-35 A Isolants and The Mixture of Third Passage of M-35A and M-108 Isolants

<table>
<thead>
<tr>
<th>Virus used</th>
<th>No. Piglets with TGE</th>
<th>No. Piglets Exposed Up to 72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-108</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>3rd-35A</td>
<td>1/2</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>3rd-36A</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>3rd-35A + M-108</td>
<td>1/2</td>
<td>Vomiting and Diarrhea</td>
</tr>
<tr>
<td>3rd-36A + M-108</td>
<td>0/1</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 6

Pathogenicity and CPE of Mixed Populations of TGE Virus

<table>
<thead>
<tr>
<th>Virus Used Infect The Cell Cultures</th>
<th>Virus Used To Re-infect the Cell Cultures</th>
<th>Bottle Cultures With CPE</th>
<th>Bottle Cultures Infected</th>
<th>Pig With TGE</th>
<th>Pigs Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-35 A*</td>
<td>M-108</td>
<td>3/3</td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>M-108**</td>
<td>M-35 A</td>
<td>3/3</td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>M-108</td>
<td>--</td>
<td>3/3</td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>M-35 A</td>
<td>--</td>
<td>0/3</td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>M-35</td>
<td>--</td>
<td>3/3</td>
<td></td>
<td>1/1</td>
<td></td>
</tr>
</tbody>
</table>

* Hypothetical defective virus present on the mixture of M-35 and anti-M-108 serum.
**Attenuated cytopathogenic isolant assumed to be “the helper.”

### TABLE 7

Neutralization of M-35 (10^2TCID50) by Several Anti-TGE Serums.
Comparison of Titers of CPE Neutralizing Antibodies and Those Neutralizing Pathogenicity for Pigs.

<table>
<thead>
<tr>
<th>Serum Used For Virus Neutralization</th>
<th>Antibody Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 5 Anti-M-108 (obtained by oral exposure)</td>
<td>CPE* 1/20</td>
</tr>
<tr>
<td></td>
<td>PP** 1/80</td>
</tr>
<tr>
<td>No. 8 Anti-M-108 (obtained by parental immunization)</td>
<td>CPE 1/40</td>
</tr>
<tr>
<td></td>
<td>PP 1/40</td>
</tr>
<tr>
<td>No. 3 Anti-M-12 (obtained by oral exposure)</td>
<td>CPE 1/20</td>
</tr>
<tr>
<td></td>
<td>PP 1/40</td>
</tr>
<tr>
<td>No. 2 Anti-ZI-Illinois (obtained by oral exposure)</td>
<td>CPE 1/10</td>
</tr>
<tr>
<td></td>
<td>PP 1/20</td>
</tr>
</tbody>
</table>

* CPE = Cytopathic Effect
**PP = Pig Pathogenicity
REFERENCES


PRESENT PROGRESS ON TRICHINOSIS PROJECT


INTRODUCTION

The Trichinosis Pilot Project, now in operation at the Hormel & Company plant at Fort Dodge, Iowa, came into being through the decision of officials in the Pork Industry and the Agricultural Research Service (ARS) of the United States Department of Agriculture, to "do something about trichinosis," plus the timely development of a practical method for the post-slaughter detection of trichinae in pork in commercial meat packing establishments.

The sequence of events began on or about January 30, 1963, with a discussion between Mr. Carl F. Neumann, general manager of the National Livestock and Meat Board, and Dr. M. R. Clarkson, then associate administrator, ARS, concerning the advisability of instituting some new research on Trichinella spiralis. The appearance of an editorial(1) in February, 1963, containing erroneous information on the incidence of trichinae in swine and incomplete information on means of destroying the encysted larvae in meat, and the occurrence of several outbreaks of human trichinosis that received considerable publicity, led to the decision in ARS to see what could be done to bring about the ultimate eradication of T. spiralis from swine in this country.

The two organizations that are primarily responsible for stimulating the activity that culminated in the initiation of the Trichinosis Pilot Project were the ARS Trichinosis Evaluation Group and the Trichinosis Task Force Study Committee of Livestock Conservation, Incorporated (LCI).

The ARS Trichinosis Evaluation Group, originally named the ARS Tri-Divisional Liaison Committee on Trichinosis, was formed by Dr. Clarkson in July, 1963. It is made up of a representative from each of the three divisions having a major interest in trichinosis, namely, the Animal Health Division, ARS, the Technical Services Division (Meat Inspection), Consumer and Marketing Service, and the Animal Disease and Parasite Research Division, ARS. This group was asked, among other things, to give prompt attention to the development in detail of "what could and should be done about trichinosis."

*Leader, Helminthological Investigations, Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture.
**Veterinary Parasitologist, Technical Services Division, Consumer and Marketing Service, United States Department of Agriculture.
***Veterinary Project Leader, Technical Services Division, Consumer and Marketing Service, United States Department of Agriculture.
****Associate Professor, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa.
*****Staff Veterinarian, Swine Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
The LCI Trichinosis Task Force Study Committee was the result of suggestions made by Dr. Clarkson, at the annual meeting of Livestock Conservation, Incorporated, at Davenport, Iowa, in February, 1964, and by Mr. Neumann, at the National Pork Industry Conference at Purdue University in November, 1965. It was formed early in 1966 by Mr. R. Harvey Dastrup, then executive director of LCI, and held its first meeting in Kansas City, Missouri, at the annual meeting of LCI in February of that year. It is made up of representatives of the National Pork Producer’s Council, American Meat Institute (AMI), American Veterinary Medical Association, Veterinary Medical Research Institute (VMRI), Iowa State University, and the Animal Disease and Parasite Research Division, ARS. At this meeting Mr. J. Russell Ives, Director of Marketing, AMI, chairman of the committee and Dr. W. J. Zimmermann, VMRI, were appointed to serve as a “committee of two” to bring together all available information pertinent to the trichinosis problem before its consideration by the full Task Force for further action.

By April, 1966, the LCI Trichinosis Task Force was convinced that trichinosis in the United States could be eliminated by eradicating trichinae from swine and that the most needed tool for achieving this goal was a system for the rapid and accurate detection of the parasite in these animals.

In November, 1966, the participants in the Swine Health Workshop on Trichinosis Eradication at the National Pork Industry Conference in Waterloo, Iowa, unanimously adopted a resolution that the Conference, “support and encourage the USDA and Veterinary Medical Research Institute, Iowa State University, to move into a program of increased research and eradication of trichinosis.”

Mr. Ives submitted to the LCI meeting in Des Moines, Iowa, in February 1967, recommendations that LCI actively support any trichinosis program initiated by the U. S. Department of Agriculture.

At this same meeting, Dr. Zimmermann discussed informally with pork industry representatives his recently developed pooled sample technique for the post-slaughter detection of trichinae in commercial meat packing establishments. This procedure\(^2\) is carried out by pooling 5-gram samples of diaphragm muscle from 20 hogs, grinding them in a meat chopper, and digesting the meat in an incubator in artificial digestive juice at 100° to 110°F. Briefly, when the digestion is complete, the upper two-thirds of the supernatant fluid are siphoned off, the residue concentrated in a small flat bottomed watch glass, and examined microscopically for trichinae. If trichinae are found, the affected group is retained. Diaphragm samples are obtained from each hog in the infected lot and individually subjected to the same procedure. When the trichinous hog is located, the intensity of the infection is determined and the carcass set aside for processing to destroy the parasites.

Dr. Zimmermann suggested that this procedure might be used to examine hogs in a commercial slaughtering plant because the carcasses are usually refrigerated from 12 to 24 hours before they are cut up and the different cuts subjected to further processing before being marketed. Dr. Zimmermann also suggested that this procedure might be used in a program looking toward the ultimate eradication of \(T. spiralis\) from swine in the United States. This suggestion was enthusiastically received by members of the pork industry.
A few days after the LCI meeting, the ARS Trichinosis Evaluation Group was asked to review Dr. Zimmermann's procedure. The consensus was that the technique had merit, but that its sensitivity should be determined.

In July, 1967, Dr. Zimmermann reported that the limit of reliability of the test could be assumed to be 1 larva/2.5 grams of meat. Since 1 trichina/gram of diaphragm had been previously determined to be the lowest concentration that the method would be required to detect consistently, the procedure was considered ready for a field trial.

A preliminary test was carried out in August, 1967, at the Hormel plant at Fort Dodge, Iowa. Although no trichinae were found in the 2,730 hogs examined during this period, the parasite was detected in all 4 "blind" control samples to which trichinae had been added.

This trial was most important in that it provided evidence that the method could be readily adapted to the high-speed slaughter procedures utilized in this country. The trial demonstrated that (1) every hog slaughtered during a normal work-day at a packing plant running at the rate of 460 hogs per hour could be examined for trichinae with little interference to the normal work flow, (2) the results were available before the next day's cutting operations, and (3) the number of samples in each pooled lot could be increased to 25 without decreasing the sensitivity of the test.

In October, 1967, the Technical Services Division (MI), C&MS, informed the ARS Trichinosis Evaluation Group that the divisions responsible for federal meat inspection would accept the procedure in principle, pending more definite information on its administration, control, manning, and financing, for use in federally inspected establishments as an alternative to official methods outlined in Part 318 of the Regulations for the acceptance of pork in so-called "restricted products."

At the end of October, 1967, the Trichinosis Evaluation Group recommended to ARS that the technique be tested as a pilot project for 1 year under conditions which would permit a computation of costs and potential savings to be made as well as allow for making refinements of the procedures.

In November, the Animal Health Division, ARS, reported that it was ready to trace trichinous hogs to the farm of origin. At the end of November, 1967, ARS asked the Trichinosis Evaluation Group to determine the course of action to be taken in reference to a 12-month pilot project to evaluate the technique.

From this point, plans for the inauguration of the Trichinosis Pilot Project developed rapidly. In January 1968, Mr. Ives (AMI) informed the Trichinosis Evaluation Group that Hormel and Company, Austin, Minnesota, would consider housing the project in one of its plants. Cudahy Laboratories, Omaha, Nebraska, offered to supply pepsin for a year's operation and Wilson Laboratories agreed to supply half of the required quantity of pepsin. Mr. Keith Myers, president of LCI, reported that the National Livestock and Meat Board and the National Pork Producer's Council would lend financial support. The Animal Disease and Parasite Research Division, ARS, and the Animal Health Division, ARS, agreed to provide funds for labor and equipment, and the Consumer Protection Program, Meat Inspection, C&MS, agreed to administer and carry out the procedures within the packing house and to furnish the salary of one laboratory technician.
Early in March, a Memorandum of Understanding covering the proposed cooperative trichinosis pilot project was prepared for approval by officials of Livestock Conservation, Incorporated, ARS, and C&MS. This document was approved and signed by all parties by the end of May, 1967, and by June, LCI had completed all contracts with its collaborators.

In June, Dr. F. N. Hughes, C&MS, was transferred to the project as Project Leader. Equipment for the laboratory, ordered in April, began to arrive in Fort Dodge the latter part of June. Dr. Zinter, C&MS, spent 8 weeks at the Hormel plant beginning June 22, overseeing the installation of equipment, hiring of personnel and supervising the project during the shake-down period and first 4 weeks of operation.

THE TRICHINOSIS PILOT PROJECT

The Trichinosis Pilot Project began full-scale operation on Monday, July 24, 1968.

THE DAY-TIME OPERATION

The number of hogs to be slaughtered during the day is ascertained and reported to the project leader.

Sufficient digestive fluid is then prepared to digest the number of diaphragm samples that will be collected.

As the carcasses move along the rail, lot numbers are marked on the hocks of each hog, and diaphragm samples are collected in appropriately numbered containers.

The first samples arrive in the laboratory about 8 a.m. Each lot of samples is ground in a clean meat chopper and put in an appropriately numbered stainless steel beaker. Digestive fluid is added and the beakers and their contents are placed in the incubator where the digestive process takes place under constant agitation for 10 hours.

This procedure is repeated about 10 times during a normal working day at intervals of 45 to 60 minutes until all the samples have been put in the incubators.

THE NIGHT-TIME OPERATION

The night crew reports for duty 1 hour after the stirring apparatus for the first series of samples put in the incubator has been stopped.

These workers siphon off about two-thirds of the fluid in the beakers, pour the remainder of the contents through 60-mesh screens into appropriately numbered 10-inch baermann funnels, rinse the beakers, and fill the funnels to the top of the screen proper with warm water. An hour later, they fill a smaller 5-inch funnel from each 10-inch funnel. After 1 hour, the samples for microscopic examination are collected in small flat-bottomed watch glasses. This examination requires from 1 to 3 minutes. The reader records the results of each examination, including those of control lots to which known numbers of trichinae have been added.

If trichinae are found, the number of the infected lot is posted on the laboratory door so that the project leader can put retained tags on all the carcasses in that lot.
The lot is then held for reexamination. Portions of diaphragm muscle weighing from 45 to 50 grams are removed from each hog, given an identifying number and processed as was the original pooled sample.

If the trichinous hog is located, the tattoo is noted, and the retained tags are removed from the other hogs in the lot. The trichinous hog is then processed to destroy the parasites. The name of the owner or dealer, whichever is indicated by the tattoo, is then sent to the Animal Health Division's Office in Des Moines, Iowa, at which time the herd owner is contacted and a study is made to determine the mode of transmission, with a view to preventing further occurrences.

RESULTS

As of the end of the tenth week (September 27, 1968), 157,399 hogs had been examined for trichinae (Table 1). Eighteen harbored the parasites. Ten hogs had infections greater than 1 larva/gram; 8 had less than 1 larva/gram. Infections in the first group ranged from 1.3 to 152.2 larva/gram of diaphragm muscle (Avg. 32.4). Those in the second group ranged from less than 0.01 to 0.45 larvae/gram (Avg. 0.115).

Fourteen of 141,165 hogs in the 180- to 300-pound class, 2 of 7,123 hogs in the 300- to 400-pound class and 2 of 9,111 hogs weighing 400 pounds or more were trichinous. The overall incidence of trichinae infection in the 157,399 hogs was 0.0114%.

As far as the authors are aware, all of the swine examined thus far originated on Iowa farms. Trichinous hogs were recovered from 11 premises. During the 10-week period, two of these farms where infection was detected made a second shipment. Trichinous hogs were found in both of these shipments. The only condition these farms had in common was a rather heavy rat infestation. Investigations of the remaining farms have not been completed. In 5 instances, the trichinous hog could not be located because the infections were too light to be picked up in the 45- to 50-gram samples examined from the individual hogs.

COST OF OPERATION

The per-head operating cost of the project through September 20 (wages and pepsin) and through September 27 (remainder of the items) and the per-head investment in laboratory equipment (prorated over a 10-year period) are recorded in Table 2.

These results indicate that the examination of swine for trichinae in a plant slaughtering 3,000 to 4,000 hogs per day can be accomplished for about 10.286 cents/head. If the average dressed weight is assumed to be 140 pounds, the cost/pound is about 0.73 cents.

PROBLEMS

The most important problem concerned identification of the samples throughout the procedure. While this still presents difficulties, greater care by project personnel will remedy the situation.

Personnel problems occurred at times, but were resolved.

It was discovered that an immersion heater used to maintain the digestion fluid
at 110°F, until it could be used inactivated the pepsin that came into direct contact with it. This problem was solved by preparing the solution with water at 120°F. The large volume of solution in the vat was sufficient to keep the temperature above 100°F. between preparation and use.

The presence of an excess of fat in the samples produced a milky fluid that interfered with the microscopic examination for trichinae. This problem was solved by collecting the meat samples after the leaf fat had been removed from the carcass.

At one point, the trichinae added to the control samples could not be recovered. This was corrected by using recently infected rat meat containing encysted trichinae. No further difficulty has been experienced.

FUTURE PROSPECTS

It is anticipated that the project will run for the full year as planned. An increase in efficiency has permitted the work to be done with one less employee. It is hoped that other refinements may be made that will result in further economies.

Officials of the Processed Food Products Division, C&MS, have discussed the possible use of the pooled sample technique in its surveillance of hams subjected to the dry-cure process in the South.

It appears that the technique may form the basis for a national trichinosis eradication program.

SUMMARY

A brief historical account is given of the development of the Trichinosis Pilot Project, which is the result of the cooperative effort of the pork industry and the United States Department of Agriculture. The actual application of the pooled sample digestion technique for the post-slaughter detection of trichiniasis in swine in commercial meat packing establishments, developed by Dr. W. J. Zimmernann, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa, is described. During the first 10-week period of the operation of the project at the Fort Dodge plant of Hormel and Company, 157,399 Iowa hogs were examined for trichinae. Eighteen (0.011%) were infected. Ten hogs had infections from 1.3 to 152.2 larvae/gram of diaphragm muscle (Avg. 32.4). Eight had infections ranging from less than 0.02 to 0.45 larvae/gram (Avg. 0.15).

Thirteen infected hogs came from 11 farms; 5 with very light infections could not be identified. Two farms contributed trichinous hogs on two separate occasions during the 10-week period. The cost of the examination/head was calculated to be 10.286 cents, or approximately 0.73 cents/pound of dressed weight. Possible applications of the results and future prospects for the technique are briefly discussed.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the very fine cooperation between the Animal Health Division, Animal Disease and Parasite Research Division, both of ARS, the Consumer Protection Program, Consumer and Marketing Service, United States Department of Agriculture, Livestock Conservation, Incorporated, and its
collaborators, Hormel and Company, National Livestock and Meat Board, National Pork Producer's Council, Cudahy Laboratories and Wilson Laboratories, without which this project would not have been possible.

REFERENCES

TABLE 1

Post-slaughter detection of trichinous pork in a commercial plant by the pooled sample digestion technique

<table>
<thead>
<tr>
<th>Week</th>
<th>Calendar Days</th>
<th>No. Hogs Examined</th>
<th>No. Trichinous Hogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/22 - 7/26</td>
<td>13,360</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7/29 - 8/2</td>
<td>15,730</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>8/5 - 8/9</td>
<td>14,495</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>8/12 - 8/16</td>
<td>15,453</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>8/19 - 8/23</td>
<td>13,819</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>8/19 - 8/30</td>
<td>15,778</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>9/1 - 9/7</td>
<td>16,122</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>9/9 - 9/13</td>
<td>16,097</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>9/16 - 9/20</td>
<td>19,484</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>9/23 - 9/27</td>
<td>17,061</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>157,399</td>
<td>18</td>
</tr>
</tbody>
</table>

TABLE 2

Per-head operating and equipment cost

<table>
<thead>
<tr>
<th>Item</th>
<th>Cents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wages</td>
<td>7,569</td>
</tr>
<tr>
<td>Pepsin</td>
<td>1.585</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>1.019</td>
</tr>
<tr>
<td>Loss of meat sampled</td>
<td>0.350</td>
</tr>
<tr>
<td>Miscellaneous (paper towels, soap)</td>
<td>0.080</td>
</tr>
<tr>
<td>Depreciation, rent, utilities</td>
<td>0.160</td>
</tr>
<tr>
<td>Operating cost</td>
<td>9.943</td>
</tr>
<tr>
<td>Laboratory equipment (10-year depreciation)</td>
<td>0.343</td>
</tr>
<tr>
<td>Total cost/head</td>
<td>10.286</td>
</tr>
</tbody>
</table>

The passing of Dr. J. D. Ray, a distinguished member of this Committee, is deeply felt by his colleagues and friends. His cheerful disposition and dedication to the livestock industry will keep him in our hearts.

The subcommittee on Transmissible Gastroenteritis, composed of three members of this Committee and three members of the Conference of Laboratory Diagnosticians have submitted their report which is appended to this report of the Committee on Transmissible Diseases of Swine. The report of the subcommittee was approved, however, reservation was expressed concerning the language used to indicate that the etiological agent of TGE has been clearly established.

The pilot project to evaluate the pooled-sample method of diagnosis of trichinosis began July 24, 1968, at the Hormel Packing Company plant in Fort Dodge, Iowa. It is being supported by the USDA, the Cudahy Packing Company, the Wilson Packing Company, American Meat Institute, and the National Pork Producers Council. A separate report of the results of the first 10 weeks activity was presented in the general sessions of the Association as a project report from this Committee. This Committee was pleased by the favorable results thus far achieved and commends the progressive spirit of the three cooperating agencies of the USDA and the cooperative attitude of industry in the effort to effect practical eradication of trichinae from U. S. swine.

Discussion of the report brought out a recognition of the desirability of the planned investigation of sources of trichinae infections in wildlife and the relationship of these infections to those in swine. Plans for further research activity include studies of means of diagnosis of trichinae infections in living animals.

Pseudorabies infections in swine continue to increase in numbers in Indiana and have been recognized in 1968 in California, Illinois and Georgia. The disease may cause deaths among all ages of swine as it now occurs in the United States. The characteristically brief duration of the presence of the disease in a herd of swine often has resulted in a lack of recognition of the disease. Because of the relatively broad spectrum of species susceptible to the disease, its impact is often felt by
TRANSMISSIBLE DISEASES OF SWINE

those who own cattle, sheep, dogs and cats in addition to swine. The disease spreads rapidly among swine. Some become inapparent carriers and assist in the perpetuation of the disease.

In a letter to this Committee, the Hog Cholera Advisory Council to the Wisconsin State Department of Agriculture indicated interest in the criteria used to establish which diseases of swine lend themselves to practical eradication programs. In the exchanges that developed it was decided that a subcommittee to be appointed by the chairman would review the question and provide a report at the 1969 Annual Meeting.

Recently initiated efforts of the ARS to establish a computerized animal disease data processing program is commended. The program should eventually provide for accurate appraisal of the relative importance of various swine diseases, and of the effectiveness of regulatory approaches. Currently, reliance for priorities of the importance of diseases is placed on opinions of knowledgeable people. A recent poll of 2200 swine producers, conducted by the National Pork Producers Council, indicated that conditions in order of importance to them were (1) Mastitis, metritis and agalactia, (2) TGE, (3) scours of unknown cause, (4) virus pneumonia, (5) atrophic rhinitis and others such as mycoplasma infections, and erysipelatous arthritis all of about the same concern value.

In the past year, field evidence worthy of reporting suggests that atrophic rhinitis may be increasing in prevalence again as antibiotic resistance strains of agents causing the disease develop. Another disease, "bloody dysentery" or vibronic dysentery has been observed to be obviously more prevalent this year than in the recent past and with similar reason being suggested. Surveys are to be conducted with respect to influenza in swine in the winter of 1968-69 inasmuch as an epidemic of Asian flu in man is anticipated. Certainly, there is need to apply modern research tools to the long suggested role of swine as a source of new strains of influenza virus to which man may be susceptible.

The Committee participated jointly with the Committee on Nationwide Eradication of Hog Cholera and the Committee on Foreign Animal Diseases in a Symposium on the Future of Garbage Feeding. A report of the Symposium from the three Committees was submitted to the Association for approval.
REPORT OF THE SUB-COMMITTEE* ON TRANSMISSIBLE GASTROENTERITIS OF SWINE


Although the Sub-Committee’s original objective was to establish criteria for the diagnosis of transmissible gastroenteritis (TGE) of swine, the Committee on Transmissible Diseases of Swine requested that its objectives be broadened so as “to evaluate progress that is being made in the study of TGE”. Consequently, the Sub-Committee is briefly reviewing, and expressing some opinions on, those topics which are thought timely and pertinent to the parent organization. The literature review encompasses, primarily, those reports made during the past year.

1. ETIOLOGY

There have been several additional reports on the characterization on TGE virus which, in addition to those previously made, further indicate the virus to be: ribonucleic acid (RNA)-containing (1,7,8,11), ether labile, stable or nearly stable at a pH of 3, spherical or pleomorphic in shape (1,11), somewhat variable in size but generally in the range of 70 to 110 nm in diameter (1,9,11), Electron microscopic examinations, as made by negative staining procedures (1,11), and thin sections of infected cells (9,11), have indicate its similarities to certain other viruses, including some of the oncogenic viruses, the avian infectious bronchitis virus and, especially, the human virus strain 229E.

All of the TGE viral isolates so far studied have been immunologically similar (5).

There was one report (8), published during the past year, which cast doubt on the role of the above-described virus as being the etiological agent of TGE. However, the authors did not characterize the agent or agents which they believe to be the cause of TGE.

2. DIAGNOSIS

The available information on diagnostic methods supports the statements made in the 1966 and 1967 reports of this Sub-Committee. Mentioned were villous atrophy in the jejunum and ileum, neutralization test in cell culture, viral isolation in cell cultures or pigs, and fluorescent microscopy. An additional diagnostic sign, which was not referred to in our previous reports but which can be useful, is the absence of grossly-detectable chyle in the mesenteric lymphatics of the small intestines (4). However, this observation would be valid only if there is milk in the stomach. There have been no

*This is a sub-committee of the Committee on Transmissible Diseases of Swine. Three members are from this Committee and three from the Conference of Veterinary Laboratory Diagnosticians.
additional reports on the use of the bentonite agglutination (BA) test for detecting viral antigen. In contrast the value and application of the neutralization test for diagnosis, titrating antibody, and comparing the antigenic similarities of isolates have been confirmed and elaborated upon during the past year (5,10).

3. **PATHOGENESIS**

   The pathogenesis of TGE has been further clarified during the past year by reports on the ultrastructural changes in the jejunal cells of infected pigs (9), on the altered enzymatic activity of the epithelial cells of the small intestine (6,9), and on changes of the fluid and electrolyte balance (2). All of these findings tend to support the idea, as proposed by others, that the clinical signs and pathological changes probably result from the destruction or altered function of the epithelial cells of the small intestine.

4. **VACCINATION**

   The TGE inactivated vaccine as produced by Diamond Laboratories, Inc., was not relicensed by the Division of Veterinary Biologics, A.R.S., U.S.D.A., because of doubtful or inadequate efficacy. As a result, the vaccine was no longer commercially available after October 1967.

   Modified live virus TGE vaccines are being studied and tested by commercial companies. However, others have had difficulties with this type of vaccine, in not providing adequate immunity to the suckling pigs of the vaccinated sows.

   It has come to the attention of this Sub-committee that there is an increased interest in the use of live virulent TGE virus for “vaccinating” pregnant swine. This “planned infection” procedure is accomplished by the oral infection of swine at least three weeks prior to expected farrowing, and is designed to provide passive immunity to the suckling progeny of the immunized sows during the critical period of the first few weeks of life. Although known to be effective, in providing immunity, this procedure has certain inherent dangers, such as: (a) Infection will be established from which the disease might be unintentionally disseminated to other herds and, in so doing, cause considerable harm. (b) Pathogens other than the TGE virus might be transmitted unknowingly. (c) Persistent or enzootic TGE could be established, especially in those herds on a frequent or continuous farrowing program.

5. **RESEARCH ON TGE SHOULD INCLUDE STUDIES ON THE:**
   
   (A) Mechanisms of active and passive immunity.
   
   (B) Development of standardized methods for evaluating immunizing agents, and especially methods for challenging piglets to determine their passive immunity.
   
   (C) Role of the various types of immunoglobulins in colostrum and milk as related to passive immunity.
   
   (D) Various clinical manifestations of the disease, especially in herds containing immune sows.
   
   (E) Viral transmission, including the role of non-porcine species.
(F) Use of antiviral agents for protecting newborn pigs.
(G) Prevalence of TGE as determined by serological surveys.

REFERENCES

FOOT-AND-MOUTH DISEASE VIREMIA

G. E. Cottral and H. L. Bachrach


SUMMARY

Viremia was found as early as 4 and as long as 120 hours post inoculation (HPI) in cattle inoculated in the tongue epithelium with foot-and-mouth disease virus (FMDV) at the dosage level of $10^4$ to $10^5$ mouse LD$_{50}$ units. At a higher dosage level ($10^{10}$), viremia occurred within 2 HPI. At a lower dosage level ($10^4$) there was an eclipse phase of 8 to 10 HPI. Likewise, viremia was delayed for 24 to 48 HPI when virus exposure was by intramuscular inoculation or by aerosol. When cattle were inoculated with a mixture of 6 FMDV types, one of the virus types predominated in the gross lesions, but was not necessarily the virus type found in the blood.

With cattle inoculated in the tongue epithelium, the combined data gave a peak of the viremia curve of $10^{4.4} +$ mouse LD$_{50}$/ml at 40 to 42 HPI. In some cattle the peak of viremia occurred at 24 HPI and in others at 72 HPI. The mean titer was $10^{0.0}$ or above for 12 to 78 or 20 to 72 HPI, depending on the virus strains used. Viremia was found in all cattle tested between 8 and 66 HPI, while at 120 HPI only 8% had viremia.

When animals were inoculated with the RNA of purified FMDV A-119, viremia was found in steers, cats and rats, but not in monkeys, frogs, dogs or horses. The whole virus produced viremia in all these hosts except dogs and horses. Only the steers had clinical signs and lesions of FMD infection.

Viremia and footpad lesions were produced in muskrats by inoculation of guinea pig adapted FMD viruses. Viremia without clinical signs or lesions of FMD was produced in turtles and snakes.

INTRODUCTION

Prior to the establishment of their etiology, many infectious diseases were known to be transmissible by the inoculation of blood from affected animals. Perhaps, the first demonstration of this was Eilert's work with anthrax in 1836.1 The terms, virus and viremia, were used with broader connotations before true viruses were discovered. The current concept of viremia began about 1897 when Loeffler and Frosch,2 for the first time for any animal virus infection, established the etiology of foot-and-mouth disease (FMD). They were aware of FMD viremia. Some of the subsequent studies on FMD viremia have been reviewed by Mohlmann3 and by Dhennin et al.4

Viremia in FMD is important in regard to the importation of dried blood, which is used mainly in the manufacture of adhesives for the plywood industry. FMDV could survive some of the dilution and drying processes used to prepare dried blood. Information regarding the inactivation of FMDV in blood is given in a recent report.5

Viremia is also a useful adjunct in the study of modified live virus FMD vaccines.6 When the susceptibility of various wild animals or unusual hosts to FMD
is being investigated, the presence of viremia may be the principal means for
determining that virus replication has taken place, especially when clinical signs and
lesions are not found. An understanding of the limits of viremia may be important
in determining when infected animals should be slaughtered during an outbreak of
FMD. It also would be fundamental in a study of potential arthropod vectors.

This report is concerned with viremia produced by the inoculation of FMDV
and the RNA of FMDV into cattle and certain other domestic and wild species of
animals.

MATERIALS AND METHODS

Experimental animals. The methods for management and feeding experimental
animals in isolated rooms has been described previously. For viremia studies 78
grade Hereford steers were used. Virus detection tests and titrations utilized 48
others. The cattle were 14 to 24 months old and they averaged about 700 lbs. The
4 cats were female tabbies about one year old. The 4 rats were adult male albinos of
the Wistar strain. The 6 dogs were young adult males of mixed breeding, averaging
about 25 lbs. The 4 horses were grade mares of the Hunter class about 10 to 12
years old. The 8 monkeys were young adults of both sexes: 5 Rhesus, Macaca
mulatta; 2 Formosan, Macaca cyclops, 1 Capuchin, Cebus capuchinus. The
European brown fallow deer, Dama dama, was an adult male. The 7 Louisiana
frogs, Rana grillo, were about 35 cm. in length. The 2 adult female snapping turtles,
Chelydra serpentina, were captured on Plum Island and had a carapace about 30 cm.
in length. The two garter snakes, Thamnophis sirtalis, were adult males about 25
cm. in length and were captured on Plum Island. The raccoon, Procyon lotor, was a
young adult male.

The 6 muskrats, Ondatra zibethica, were caught in live traps on Plum Island.
They were young adults of both sexes. Previous experience had shown that
muskrats became ill and died when provided a swimming tank of regular laboratory
chlorinated tap water. Consequently, they were provided fresh swamp water from
their natural habitat which was changed weekly. The muskrats were fed twigs from
swamp plants, lawn clippings, apples and cereal-type canned dog food. The normal
rectal temperature of the muskrats was 98.6F.

Routes of inoculation. The muskrats and raccoon were inoculated in the
footpad of their hind feet, the turtles and snakes intramuscularly, and the other
species intramuscularly or in the tongue epithelium as mentioned in results.
Anesthesia was used for tongue inoculations.

One steer was exposed to an aerosol of FMDV A-119 generated by a
DeVilbiss glass nebulizer for one minute. A Millipore filter located near the
steer’s head sampled the air for 3 minutes and the filter material was then removed,
ground in a mortar and washed with 3.0 ml of tryptose phosphate broth. After
centrifugation of the suspension of filter material at 800 x g, ten-fold dilutions of
the supernatant fluid were titrated for virus in suckling mice.

*The monkeys and the deer were obtained from the National Zoological Park, Washington,
D. C.
Brugh.
Collection of blood samples. Whole blood was collected in vials containing heparin at a concentration of 1.0 mg/5.0 ml of blood. The animals were bled from the jugular or femoral veins appropriate to the species, except the rats, muskrats and the turtles, which were bled from the tail, and the frogs and snakes, which were destroyed to obtain heart blood. For titrations, 10-fold dilutions of blood were made in tryptose phosphate broth of pH 7.4. Viremia detection tests and titrations were made in cattle, suckling mice and bovine kidney cell cultures, using techniques previously reported.8,9,10

Viruses. The history of the virus strains used has been previously given.8,9 The major portion of this study was done with FMDV strain A-119. The other strains used were: O-M11, SAT-1/2 RV-11, SAT-2/1 RHO-1, SAT-3/1 RV-7, Asia-1/1 PAK-1, CANEFA: A-1, O-2, C-3 and O-9, and the guinea-pig-adapted strains, A-GB and C-GC. Virus suspensions were made from FMD infected bovine tongue epithelium and guinea pig footpads by the method previously reported.10

Preparation of purified virus and infectious RNA. The method for preparing purified FMDV A-119 and C-CANEFA-3 was previously described by Bachrach et al.11 Likewise, the preparation of infectious RNA of FMDV A-119 was described by Bachrach.12

Diagnostic tests. The constant serum-variable virus dilutions neutralization test was used. The test technique, neutralization index (NI) interpretation and challenge of immunity were previously described by Cottral et al.8 The technique for collection of esophageal-pharyngeal fluid and methods for assay were previously reported.13

RESULTS

Viremia in cattle. A total of 264 virus titrations in mice were made to detect viremia in 23 cattle inoculated in the tongue epithelium with 10^4 to 10^5 mouse LD50 units of FMDV A-119. Blood samples were taken from 1 to 144 HPI and viremia was detected as early as 4 HPI and as late as 120 HPI. With some cattle viremia was not found until after 6 HPI and with some it ended by 90 HPI. Thus, of the 264 samples 69 were negative. This data was used to construct a viremia curve which was fitted by a cubic polynomial (Y=A+BX+CX^2+DX^3). In order to include the negative titrations, the data was converted to antilogs for computations and then back to logs. The 195 positive titrations and the viremia curve from the computer print-out are given in Fig. 1.

Similarly viremia titrations were made with 42 other cattle inoculated with 5 other FMD viruses with the number of cattle for each as follows: 10, A-1; 6, O-2; 14, C-3; 6, O-9; and 6, O-M11. A total of 174 titrations were made between 12 and 144 HPI and viremia was detected for as long as 120 HPI. There were 44 samples that were negative. The data obtained from the 5 viruses were combined and the computer print-out of the curve is given in Fig. 2.

With FMDV A-119 (Fig. 1), the peak of the viremia curve of 10^4.45 ± 0.07 was reached at 42 HPI and the mean titer was 10^3.0 mouse LD50/ml or above from 20 to 72 HPI. The combined data from 5 other viruses (Fig. 2) gave a peak of the viremia curve of 10^4.49 ± 0.1 at 40 HPI and the mean titer was 10^3.0 or more from 12 to 78 HPI. Thus, Fig. 2 depicts a broader curve than Fig. 1. The highest virus titer observed was 10^5.8 mouse LD50/ml. There were considerable differences
between the titers obtained with individual cattle, resulting in a great spread in the titer range at any given time.

The data from the 438 viremia titrations were combined to construct a frequency distribution curve to reflect the percent of the samples that were positive in relation to time (Fig. 3). At 4, 5 and 6 HPI 25, 50 and 83% were positive, respectively. Between 8 and 66 HPI all cattle had viremia. At 72, 90, 96, 108 and 120 HPI, there were 98, 67, 32, 20 and 8% positive, respectively. Clinical signs and lesions of FMD within 24 HPI were evident in all cattle.

To simulate a natural infection, 2 steers were inoculated intramuscularly with the low dosage of about $10^1$ PFU of FMDV C-3. For comparison, 2 other steers were inoculated with $10^6$ and $10^{10}$ PFU of the same virus strain. The high dosage of $10^{10}$ PFU in a small volume (1.0 ml) was obtained by use of purified, concentrated virus. Viremia data obtained with these steers is given in Table 1. Viremia was not detected in the two steers inoculated with $10^1$ PFU until 10 and 14 HPI and persisted as long as 105 and 120 HPI, respectively. At a dosage of $10^6$ PFU, viremia was detected at 5 HPI and for as long as 105 HPI. With the high dosage of $10^{10}$, viremia was present as early as 2 HPI and persisted for as long as 96 HPI. The $10^1$PFU virus dosage resulted in the appearance of tongue lesions, fever and foot lesions, for one steer at 22, 30 and 48 HPI, respectively. The lesions and signs did not appear until 48 HPI in the other steer. Virus dosages of $10^6$ and $10^{10}$ PFU resulted in the steers having tongue lesions at 12 and 8 HPI, fever at 14 and 20 HPI and foot lesions at 30 and 48 HPI, respectively. Rumen lesions were not detected, but 3 out of the 4 steers had evidence of heart lesions; one steer inoculated with a low virus dosage did not. At 21 days after inoculation, serums from the steers had neutralization indexes of 5.8, 5.9, 6.3, and 5.9, respectively, as per virus dosage as listed left to right in Table 1.

A steer was exposed to an aerosol of FMDV A-119 for one minute. A Millipore filter located near the steer's head sampled the air for 3 minutes and the virus recovered from the filter material had a titer of about $10^2$ mouse LD$_{50}$/ml. The steer did not have clinical signs or lesions until about 72 hours after exposure, at which time, tongue and foot lesions were evident. Viremia with titers of 4.5, 3.0, and 2.2, respectively, was found at 48, 72, and 96 hours, but not at 24 or 120 hours after exposure.

Cattle also were inoculated with the Asian and 3 African types of FMDV for comparison with 3 South American strains of types A, O and C. The daily viremia titers with these viruses from 24 to 120 HPI are given in Table 2. The virus dosage of each of these 7 viruses was approximately $10^5$ bovine ID$_{50}$ units. The highest titers were found at 24 HPI with 3 of the virus strains, at 48 with 3 others and at 72 HPI with one virus strain. The results in Table 2 probably reflect individual cattle variations in response more than they do virus strain differences.

Multiple FMD infection. Two steers were inoculated intramuscularly with a mixture of 6 types of FMDV, Table 3. The dosage of each type of virus was adjusted in accordance with its ability to produce gross lesions when inoculated via the intramuscular route as determined in a previous study. The relative amounts of bovine ID$_{50}$ units in the mixture for types O, C, SAT-1, SAT-2, SAT-3 and Asia-1 were 0.16, 0.39, 0.05, 0.02, 0.31 and 0.08, respectively. The total virus dosage was approximately $10^6.5$ bovine ID$_{50}$ units in a volume of 5.0 ml. The 2
FOOT-AND-MOUTH DISEASE VIREMIA

steers did not have viremia, clinical signs or lesions at one DPI, but at 2 DPI, viremia, fever, salivation and lesions of the tongue and feet were found. Material from each lesion of the mouth and feet was harvested when full development was reached on the 2nd or 3rd DPI.

Steer 1. (Table 3) had 6 individual oral lesions (5 on the tongue) and lesions of all 4 feet. The virus type isolated from all 10 lesion sites was SAT-1. At 110 DPI, SAT-1 virus was isolated from the esophageal-pharyngeal fluid of this steer. SAT-1 FMDV also was isolated from the blood of steer 1, at 2 and 3 DPI, with the highest titer at 2 DPI. However, at 4, 6 and 7 DPI, type SAT-2 was isolated from the blood. Viremia was not detected at 5 DPI. Serum samples taken at 7, 14 and 21 DPI had the highest neutralization indexes with SAT-1 virus (Table 4). At 14 and 21 DPI, the serum NI also were at a high level with SAT-2 virus.

Steer 2. (Table 3) had only 2 individual tongue lesions and lesions on all 4 feet. The virus isolated from these 6 lesions was type C. Virus was not isolated from the esophageal-pharyngeal fluid from this steer at 110 DPI. The viremia was of type SAT-2 throughout its' course from 2 to 6 DPI. The highest titers were found at 3 and 4 DPI. Serum samples taken at 7, 14 and 21 DPI had consistently high NI with type C virus and at 14 and 21 DPI higher NI with SAT-2 virus (Table 4). Thus, the virus types isolated from these two steers is supported by the serological findings. Attempts to find other virus types in the lesions, blood or harvested isolates, by growing the virus in tissue culture in the presence of type-specific antiserum for the type initially isolated, were negative. The virus titers of samples of the lesions in the 2 steers varied from 10^4.44 to 10^8.1 PFU/ml.

Viremia in other hosts. Since viremia usually is an indication that virus replication has taken place in a host, viremia was utilized to determine if the FMD susceptibility of certain unusual hosts would be altered when viral RNA is inoculated as compared to whole virus. Purified FMDV A-119 and the RNA extracted from it both were used to inoculate 2 each of the following animals for each preparation: cattle, cats, rats, monkeys, frogs, dogs and horses. The dosage of RNA or whole virus for each species is given in Table 5. Both the tongue epithelium and intramuscular routes were used. Viremia was found at 24 HPI only in steers, cats and rats following inoculation of RNA. The whole virus produced viremia in these same species and also in monkeys and frogs. Dogs and horses were refractory to both inoculums. Only the steers developed signs and lesions of FMD. As a control, a sample of FMDV A-119 RNA with a titer of 2.4 x 10^3 PFU/ml was treated with crystalline pancreatic RNase at 37 C for 5 minutes. This material was inoculated into a steer, using doses of 20 ml intramuscularly and 2.0 ml in the tongue epithelium. The steer did not develop FMD and at 14 DPI it was fully susceptible (within 24 HPI) to a challenge inoculation of FMDV A-119. Bovine kidney cell cultures also inoculated with a portion of the treated RNA were negative, whereas untreated RNA infected cell cultures.

Steers, dogs, monkeys and frogs were inoculated intramuscularly with FMDV A-119 harvested from bovine kidney cell cultures (not purified). The high and low virus dosage for each species is given in Table 6. With a low virus dosage, a steer had viremia from 24 to 96 HPI. Viremia was not detected in a dog or in a Formosan monkey. Two monkeys (Rhesus and Capuchin) had viremia at 72 HPI. However, blood from the Rhesus monkey at 48 HPI and from the Capuchin monkey at 72
HPI apparently contained sufficient inactive or modified virus to immunize the steers that were inoculated with it. These steers did not have clinical signs or lesions of FMD, but they were resistant to challenge with A-119 and had serum NI of 5.4 and 3.8, respectively.

A monkey (Formosan) that received a high virus dosage had viremia at 4, 24, and 72 HPI. A high dose of FMDV given to a dog resulted in viremia at 4 HPI, but virus was not detected thereafter. Three frogs were used and viremia was detected at 4, 24 and 72 HPI. One frog was destroyed at each bleeding time. A steer inoculated with a high virus dosage also had viremia at 4, 24 and 72 HPI. Only the steers had clinical signs and lesions of FMD. Serum taken at 10 DPI from these various hosts, except the frogs, had NI of 3.3 or more in those hosts that had shown a viremia. The dog with the high virus dose that had viremia only at 4 HPI apparently was not infected and did not develop antibodies.

Guinea-pig-adapted strains of FMDV, A-GB and C-GC, were used to inoculate turtles, snakes and muskrats. Both turtles had viremia at 48 and 72 HPI and the titers given are the averages of the 2 readings (Table 7). One snake was destroyed at 24 HPI and the other at 48 HPI to obtain samples, and viremia was found in both instances.

The muskrats were inoculated in the footpads and viremia was detected at 24, 48, 72 and 144 HPI. The virus titers given in Table 7, are the average of two or more readings, except that only one muskrat was tested at 144 HPI. Within 48 hours, 3 of the muskrats had gross footpad and tiny tongue lesions, one with A-GB and 2 with C-GC viruses. Histological examinations of the lesions verified that they were typical FMD lesions similar to those found in guinea pigs and cattle. The NI of 10 DPI serums from these muskrats were 3.4, 4.0 and 4.2, respectively. Four of the muskrats had a fever from 1 to 3 days. An adult male raccoon also inoculated in the footpads with about 10^6.7 mouse LD_{50} units of FMDV C-GC failed to develop viremia or other signs of infection.

A European brown fallow deer was inoculated in the tongue epithelium with 10^5 bovine ID_{50} units of type SAT-1 FMDV. Within 24 HPI the deer had clinical signs and tongue lesions of FMDV. Foot lesions were found at 48 HPI. Viremia was detected at 24, 48 and 72 HPI with titers of 2.8, 4.1 and 3.2 mouse LD_{50}/ml, respectively. At 22 DPI, the serum NI was 6.6 with SAT-1 virus.

**DISCUSSION**

When susceptible cattle are inoculated in the tongue epithelium with FMDV, viremia may be found within 2 hours or may be delayed for 14 or more hours, depending upon the virus dose, virulence and host influence. Thus, the length of time viremia precedes clinical signs and lesions may vary from about 8 - 40 hours. If the virus dose is high, the initial viremia may be predominantly composed of the inoculated virus particles. When lower virus doses are given, the host replicated virus particles probably are the main constituents of the viremia. Dhennin et al.,^4^ using a virus dose of about 10^7 found viremia in guinea pigs from 45 sec. to 72 HPI, but were unable to find viremia in cattle at 45 sec. and 2 HPI. In the insusceptible or less susceptible hosts, such as adult horses and dogs, a virus dose of about 10^4 PFU or more may vanish in the tissues without producing a viremia. For these reasons the virus dosage of 10^4 to 10^5 mouse LD_{50} units were considered adequate dosage
10^5 mouse LD_{50} units were considered adequate dosage levels for the cattle viremia studies.

At the virus dosage levels used, some cattle responded by having an early, very rapid rise in viremia titer. In others, the rise was more gradual to a delayed peak viremia titer at 72 HPI. This may have been caused by the release of virus from the slower developing secondary lesions. None of the cattle studied exactly followed the viremia curves constructed from the data. However, when all of the data was considered, the peak of the viremia was between 40 to 42 HPI. The range in virus titers at any given time is as important as the constructed curve. The variations in viremia titers are probably more influenced by variations in host response than by virus strain differences. If tissue cultures had been used instead of mice for virus assays, the titers with most viruses probably would have been slightly higher. With the frequency distribution curve, the percentage of cattle that may be expected to have viremia at any given time after inoculation can be determined. The ascending portion of the curves (Figs. 1, 2 and 3) were probably influenced by virus dosage, whereas the descending portion may more nearly reflect what happens in natural infection.

In this study, the shortest duration found for viremia in cattle was 3 days and the longest was 5 days. Regardless of the virus dosage or the route of inoculation used, viremia persisted in cattle for no longer than 5 days. Thus, when cattle were inoculated intramuscularly or by aerosol exposure, the starting time for viremia was delayed one or more days, but the duration still did not exceed 5 days in this study and in a previous report. Likewise, the data presented by Seibold, et al. on the intramuscular inoculation of modified FMDV A-119 showed viremia patterns that were erratic, but they did not exceed 5 days. This is not to say that viremia for 6 or more days does not occur. Waldmann and his co-workers, using acetone to coprecipitate virus and albumin from blood plasma, found virus one or more times from 7 to 58 DPI in 13 cattle or 2.6% of the population studied. The cattle were being used for the production of hyperimmune serum, and many were inoculated with virus several times.

It is generally found that FMD antibodies are present at a significant level in the blood of cattle by 6 DPI. Presumably, the decline in virus titer in the blood is at least partially related to the rise in antibody level. However, 4 separate attempts at this laboratory to demonstrate virus in whole blood, serum or plasma after the usual time of termination of viremia were negative.* Homogenization with trichlorotrifluoroethane followed by centrifugation was the technique used in these attempts to disrupt the antigen-antibody union. Sodium dodecylsulfate treatment alone and with the above technique was also tried without success.

There is no evidence for a true separate secondary viremia in FMD as does occur in some virus diseases. However, if more than one FMD virus is present, as was the case in the data presented in Table 3, the period of viremia may be extended by one of the other viruses. From this data, it also can be seen that in multiple FMDV infections, cattle may have viremia of a different virus type than that found in the gross lesions. Also in multiple virus infection certain FMD viruses may be prevented from replicating in the usual sites of predilection for gross lesions and blocked from participating in the viremia by the more adaptable viruses, by the
host influence or by chance. These lost viruses apparently find other body cells where they are replicated in sufficient numbers to stimulate some antibody production. Perhaps, some of the virus neutralization test results were due to cross reactions. Two of the viruses, SAT-1 and SAT-2, were given at a much lower unit level than the other 4 viruses used to infect these 2 steers. Despite this, these viruses replicated at a higher level. Cunha et al.\textsuperscript{19} also found a tendency for certain viruses to predominate when mixtures of FMD viruses were given.

Seibold, et al.,\textsuperscript{6} found that virus recovered in cell cultures from the early viremia of steers subclinically infected with a modified FMDV had more intradermallingual pathogenicity than virus recovered at termination of viremia. Others have reported that the virus obtained from primary FMD lesions was more virulent than virus recovered later from the secondary lesions.\textsuperscript{1}

When the RNA of FMDV is inoculated into an animal it is immediately in danger of being inactivated by the enzyme RNase. The RNA inoculated in the steers, cats and rats via the tongue epithelial and intramuscular routes apparently was able to get into host cells before inactivation occurred. In the monkeys and frogs the RNA did not survive. In dogs and horses neither the whole virus nor the RNA survived. It was hoped that the use of RNA might extend the host range beyond that of the whole virus, but this was not the case. When viremia occurs after RNA inoculation there can be little doubt that the virus found in the blood is host replicated virus, since the blood contains considerable RNase. Mussgay\textsuperscript{20} used the intracerebral route of inoculation for mice in his RNA study because of the lower RNase content of brain tissue.

Others have shown that some young dogs are susceptible to FMD.\textsuperscript{21} However, in this study, dogs were not susceptible and viremia was only found at 4 HPI with a dog that received a high dose of virus. No significant antibody response was detected in the dogs. In species that are susceptible to FMD, antibody production occurs with relatively small doses of inactivated FMDV, even without the use of an adjuvant.\textsuperscript{22}

Rhesus, Capuchin and Formosan monkeys should be considered somewhat susceptible to FMD, mainly because the viremia studies indicate that virus replication took place. The replicated virus appears to be modified since in two instances cattle that were inoculated with monkey blood apparently were immunized without clinical evidence of infection. Thus, tissue cultures of monkey cells may prove useful as a method for making modified live FMDV vaccine. Occasionally people have become infected with FMD and lesions have been found. There are less than 50 authenticated cases of FMD in man.\textsuperscript{23} Therefore, there is additional evidence that FMDV replicates in primates.

Muskrats are susceptible to guinea-pig-adapted strains of FMDV and some have lesions in addition to viremia. The viremia in snakes may have been due to residual virus from the inoculum. However, in frogs and turtles the results indicate that virus replication did occur and that viremia persisted for at least 72 HPI. Serological studies were not done with these species.

The deer had a viremia pattern similar to that found for cattle. The SAT-1 strain used was originally isolated from a kudu in S. Rhodesia. This may have made it a better strain to use for the deer than other strains of bovine origin.
Other studies with various species of animals have shown the following results on the duration of FMD viremia in hours: cattle – 18-103, swine – 29-50, 24-72, 12-96; sheep – 12-66, 24-72, goats – 16-92; guinea pigs – 12-72, 14-54, 13-168, 45 sec – 72; mice – 1-60, 6-366.

After contact exposure, sheep had FMD viremia from the 4th to the 7th day. Also with contact exposure, cattle had FMD viremia from 1-6 days before vesicles were observed.

Sutmoller et al. found that FMD viremia in cattle was prevented by the administration of type-specific antiserum intravenously about 3 hours prior to inoculation of virus, but the appearance of vesicular lesions was not always completely suppressed. In vaccinated cattle, both viremia and vesicular lesions were prevented.

In a study of viremia caused by oral vaccination with poliovirus, Melnick et al. summarized the general pattern as follows: free virus (i.e. readily isolated) found for 3-5 days and antibody-bound virus found for 4-6 days. Viremia studies with live virus FMD vaccines were reported by Asso et al.

Cottral et al. reported on the relationship of viremia to the occurrence of FMDV in the semen of bulls. Viremia was found from 2 to 92 HPI and virus was in semen from 12 HPI to 10 DPI.

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Fig. 1. Curve of viremia titers from 23 cattle inoculated with foot-and-mouth disease virus A-119. Titers are $\log_{10}$ mouse LD$_{50}$/ml.
Fig. 2. Curve of combined viremia titers from 42 cattle inoculated with foot-and-mouth disease viruses (5 strains of Types A, O and C). Titers are log_{10} mouse LD_{50}/ml.
Fig. 3. Frequency distribution curve of 438 viremia titrations from 65 cattle inoculated with foot-and-mouth disease viruses (6 strains).
**TABLE 1**

Viremia Titers of Four Steers Comparing High and Low Dosages of Foot-and-Mouth Disease Virus C-CANEFA-3 Inoculated into Tongue Epithelium.

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</tbody>
</table>

*Virus dosage and viremia titers for the 4 steers are in log10 total PFU and by log10 PFU/ml, respectively. N=negative. The virus used to obtain 10^10 dosage was grown in BHK cell cultures and then purified. The other preparations were of bovine tongue tissue origin and were not chemically treated.

**TABLE 2**

Viremia Titers of Seven Steers Each Inoculated in Tongue Epithelium with a Different Type of Foot-and-Mouth Disease Virus.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Viremia Titers Hours After Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>A-CANEFA-1</td>
<td>2.8</td>
</tr>
<tr>
<td>O-CANEFA-2</td>
<td>2.9</td>
</tr>
<tr>
<td>C-CANEFA-3</td>
<td>4.9</td>
</tr>
<tr>
<td>SAT-1/2</td>
<td>4.4</td>
</tr>
<tr>
<td>SAT-2/1</td>
<td>3.5</td>
</tr>
<tr>
<td>SAT-3/1</td>
<td>2.7</td>
</tr>
<tr>
<td>Asia-1/1</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*The virus dosage varied from about 10^3 to 10^4. The viremia titers are in log10 mouse LD50/ml.
### Foot-and-Mouth Disease Viremia

**TABLE 3**

Foot-and-Mouth Disease Viruses Isolated from Gross Lesions, Pharynx and Blood of Two Steers Inoculated Intramuscularly with a Mixture of Virus Types O, C, SAT-1, SAT-2, SAT-3, and Asia-1.

<table>
<thead>
<tr>
<th>Virus types found in gross lesions and OP-fluid*</th>
<th>Viremia titers and virus type for DPI**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth   Feet   OP-FL</td>
<td>1</td>
</tr>
<tr>
<td>Steer 1. SAT-1 SAT-1 SAT-1</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>SAT-1 SAT-1 SAT-2</td>
</tr>
<tr>
<td>Steer 2. C C N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>SAT-2 SAT-2 SAT-2</td>
</tr>
</tbody>
</table>

*OP-oesophageal-pharyngeal. The mouth and foot lesions were harvested on 3rd and 4th days after inoculation and their virus titers varied from $10^{4.4}$ to $10^{8.1}$ PFU/mL. The total number of lesions found and typed was 10 for Steer 1. and 6 for Steer 2. **Viremia titers are in log_{10} PFU/mL. DPI-Days post inoculation. N=negative. Virus types were determined by neutralization tests. The total virus dosage given to each steer was about $10^{6.5}$ bovine ID50 units.

### TABLE 4

Neutralization Indexes of Serums of Two Steers Inoculated Simultaneously with Six Types of Foot-and-Mouth Disease Virus.

<table>
<thead>
<tr>
<th>Host</th>
<th>Serum DPI*</th>
<th>A</th>
<th>O</th>
<th>C</th>
<th>SAT-1</th>
<th>SAT-2</th>
<th>SAT-3</th>
<th>Asia-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steer 1.</td>
<td>7</td>
<td>3.1</td>
<td>3.8</td>
<td>4.5</td>
<td>5.5</td>
<td>2.6</td>
<td>4.7</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.8</td>
<td>3.3</td>
<td>5.1</td>
<td>7.1</td>
<td>6.6</td>
<td>4.5</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>3.3</td>
<td>3.7</td>
<td>5.8</td>
<td>6.4</td>
<td>6.6</td>
<td>4.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Steer 2.</td>
<td>7</td>
<td>1.9</td>
<td>3.9</td>
<td>4.9</td>
<td>4.1</td>
<td>3.2</td>
<td>3.9</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.7</td>
<td>3.3</td>
<td>4.9</td>
<td>3.8</td>
<td>6.3</td>
<td>4.0</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2.6</td>
<td>2.9</td>
<td>4.8</td>
<td>3.7</td>
<td>5.6</td>
<td>3.8</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*DPI= Days post inoculation serum harvested. A NI of 3.0 or more is considered significant. **Type A virus was not inoculated into steers. The total virus dosage was about $10^{6.5}$ bovine ID50 units.
TABLE 5

Viremia in Various Animal Hosts 25 Hours After Inoculation of Foot-and-Mouth Disease Virus or Its RNA (A-119)

<table>
<thead>
<tr>
<th>Hosts*</th>
<th>Total dose of Virus or RNA</th>
<th>Virus</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steers</td>
<td>4.8</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Cats</td>
<td>4.1</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Rats</td>
<td>3.7</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Monkeys</td>
<td>3.9</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>Frogs</td>
<td>3.4</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Dogs</td>
<td>4.2</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Horses</td>
<td>4.8</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

*Two animals were used for each kind of inoculum. The routes of inoculation were tongue epithelium and intramuscular. The virus or RNA total dose is given in log_{10} PFU.

**P=positive, N=negative. Steers, bovine kidney cell cultures and mice were used for the detection of viremia and all three gave identical results.

TABLE 6

Foot-and-Mouth Disease Viremia (A-119)

In Steers, Dogs, Monkeys and Frogs

<table>
<thead>
<tr>
<th>Host</th>
<th>Virus Dose</th>
<th>4</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>Clinical FMD</th>
<th>Host Serum NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steer</td>
<td>4.8*</td>
<td>3.5*</td>
<td>5.5</td>
<td>5.6</td>
<td>3.8</td>
<td>P</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>3.8</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>N</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>3.8</td>
<td>0/1</td>
<td>0/1(1)</td>
<td>3.8</td>
<td>0/1</td>
<td>N</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Capuchin monkey</td>
<td>3.8</td>
<td>0/1</td>
<td>0/1</td>
<td>1/2(1)</td>
<td>0/1</td>
<td>N</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Formasan monkey</td>
<td>3.8</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>N</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Formosan monkey</td>
<td>7.0</td>
<td>1/1</td>
<td>3/4</td>
<td>1/1</td>
<td></td>
<td>N</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>7.4</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
<td></td>
<td>N</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Frogs (3)</td>
<td>6.3</td>
<td>1/1</td>
<td>3/6</td>
<td>1/2</td>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steer</td>
<td>8.5</td>
<td>1/1</td>
<td>3/6</td>
<td>5.4</td>
<td></td>
<td>P</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

*Virus dosages and viremia titers are in log_{10} bovine ID_{50} units and per ml., respectively. The route of inoculation was intramuscular. Fraction=number of test steers positive/number used. N=negative, P=positive. NI=neutralization index of host's serum 10 days after inoculation. (1)= 2 test steers that did not have clinical signs or lesions of FMD, but had 14 DPI serum NI of 5.4 and 3.8, respectively, and were resistant to challenge (IM) with A-119 virus.
FOOT-AND-MOUTH DISEASE VIREMIA

TABLE 7
Foot-and-Mouth Disease Viremia in Turtles, Snakes and Muskrats*

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Virus Strain</th>
<th>Virus Dose</th>
<th>Virus Titers</th>
<th>Hours After Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turtles (2)</td>
<td>A-GB</td>
<td>6.7</td>
<td>N</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>144</td>
</tr>
<tr>
<td>Snakes (2)</td>
<td>C-GC</td>
<td>6.4</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Muskrats (3)</td>
<td>A-GB</td>
<td>6.7</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Muskrats (3)</td>
<td>C-GC</td>
<td>6.7</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
</tr>
</tbody>
</table>

*Viruses dosages and viremia titers are in log10 mouse ID50 units and per ml., respectively. The route of inoculation for turtles and snakes was intramuscular and for muskrats the footpads. N=negative. Three of the muskrats had gross lesions of their footpads and tongues within 48 hours and the neutralization indexes of the sera were 3.4 to 4.2 at 10 days after inoculation.
SHEEP AND GOATS AS FOOT-AND-MOUTH DISEASE CARRIERS

By

John W. McVicar and Paul Sutmoller

Foot-and-mouth disease (FMD) outbreaks in the United States have been eradicated by slaughtering all infected and exposed animals of susceptible species followed by appropriate disinfection procedures. An early diagnosis of the disease minimizes the impact of the eradication campaign on the livestock industry. A more dangerous situation develops when the disease is difficult to recognize clinically. Then the outbreak may continue to spread unrecognized and infected animals which escape detection may become carriers of the virus. FMD in sheep and goats often produces an obscure clinical picture and Burrows in 1966, reported that sheep could become carriers. It appeared necessary to study the disease in these species further and to obtain information on the carrier state in goats as well as in sheep. Most reports of the experimental disease in sheep and goats involve animals infected by inoculation of the virus into the tongue, oral mucosa, or epithelium of the foot1,2,5,6,8 although some include a few animals infected by contact.5,8 In this study, sheep and goats were exposed to FMD virus by intranasal instillation, contact, and by exposure to clinically diseased steers to produce a more natural course of the disease.

MATERIALS AND METHODS

Sheep and goats: One to 3-year-old sheep and goats of mixed breeding were used. In some experiments 1 to 6 week-old kids were included with their mothers. All animals were housed in isolation rooms previously described.3,7

Viruses: Four virus strains were used. Virus Type A, strain 4691 (A-4691) was isolated from carrier cattle in Brazil.9 The other 3 strains had been isolated in field outbreaks in South America and were designated A-CANEFA*-1 (A-1), O-CANEFA-2 (0-2), and C-Tierra del Fuego (C-TdF).

Virus exposure: Each group of sheep and goats was exposed to the virus by 1 of 3 different methods.

Group I was exposed by intranasal instillation of 0.2 ml. of virus suspension containing approximately 10,000 plaque forming units (PFU). Care was taken not to injure the nasal mucosa.

Group II was exposed by contact with sheep and goats of Group I. They were admitted to a room housing the Group I animals approximately 5 hours following the intranasal inoculation.

Group III was exposed to a steer infected by intradermalungual (IDL) inoculation of virus suspension. Exposure was continued for 6 days from the day of inoculation.

*Comisión Asesora Nacional para la Eradicación de Fiebre Aftosa.
The following scheme shows the number of animals in each group:

<table>
<thead>
<tr>
<th>Group I: Inoculated with</th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^4$ PFU intranasally</td>
<td>A-1 0-2 C-TdF A-4691 Totals</td>
</tr>
<tr>
<td>Sheep</td>
<td>4 4 4 13 25</td>
</tr>
<tr>
<td>Goats</td>
<td>3 4 4 4 15</td>
</tr>
</tbody>
</table>

Group II:
Contact exposure to Group I

| Sheep | 2 2 2 0 6 |
| Goats | 2 2 2 0 6 |
| Kids  | 0 4 0 0 4 |

Group III:
Contact exposure to IDL infected steer

| Sheep | 6 6 6 0 18 |
| Goats | 6 6 6 0 18 |
| Kids  | 4 5 6 0 15 |

Totals: 27 33 30 17 107

Animals were examined daily for clinical signs and heparinized blood samples were taken for viremia studies for 14 days following exposure (DPE). Epithelial tissue was collected from lesions as they occurred and assayed for virus.

Antiserum treatment: Approximately one-half of Group I was given type specific convalescent antiserum prior to virus exposure. Serum was given intravenously from 20 to 60 minutes before intranasal instillation of the virus. Dosage varied from 30 to 100 ml. depending upon the size of the animals.

Collection and treatment of samples: The instrument used for the collection of oesophageal-pharyngeal (O-P) samples (Fig. 1) was designed by Dr. R. Burrows.* It was passed through the mouth and pharynx into the oesophagus and then was moved up and down in the upper oesophagus and oropharynx rather vigorously. Mucus, epithelial cells, often bits of feed or regurgitated rumen contents, and saliva were trapped in the central groove of the instrument. This material was rinsed from the instrument by agitation in a vial containing 5 ml. of chilled tissue culture fluid (Hanks’ balanced salt solution with 0.5 percent lactalbumin hydrolysate) containing antibiotics (2,000 units of sodium penicillin G, 2 mg. of dihydrostreptomycin, and 25 units of nystatin** per ml.) and 2 percent normal bovine serum. Routinely, the instrument was passed and rinsed 3 times. The resultant mixture of the material collected and the tissue culture fluid was referred to as the oesophageal-pharyngeal (O-P) sample. All samples were kept in an ice water bath and processed as soon as possible. In no instance did the time interval between collection and inoculation of tissue culture exceed 3 hours.

*The Animal Virus Research Institute, Pirbright, Surrey, England.

**Mycostatin, E. R. Squibb and Sons, 745 Fifth Ave., New York, N. Y.
A portion of each sample was treated with trichlorotrifluoroethane (TTE) as follows: 2½ ml. of sample and 2 ml. of TTE were placed in the Micro Chamber of the Micro-Homogenizer attachment of an Omnimixer.*** After immersion of the chamber in an ice water bath, the mixture was emulsified at 30,000 rpm for 2 minutes. The emulsion was centrifuged at 40°C for 30 minutes at 800 x g and the aqueous phase used for virus isolation. Both treated and untreated portions of each sample were inoculated onto primary bovine and swine kidney cell monolayers in 4-oz. prescription bottles for plaque assay. Approximately 0.5 ml. of the treated material was inoculated into 15 ml. of tissue culture fluid on a primary bovine and primary swine kidney cell culture in 4-oz. prescription bottles. These monolayers were examined daily for cytopathic effect for 3 days.

Circulating antibody: The serum protection test was performed using unweaned mice according to the method described by Cunha et al.4 with results reported as the mouse protection index.

RESULTS

In this report the term carrier refers to those sheep and goats from which virus was isolated at least 28 days following exposure. An animal was considered to be infected if virus could be detected in lesions, blood, or an O-P sample or if a significant postexposure rise in mouse protection index was demonstrated. In no instance was severe clinical disease observed. Some animals appeared slightly depressed during the height of the febrile reaction. If oral lesions did occur they tended to be superficial, healed rapidly and usually did not interfere with the normal intake of feed. Foot lesions were rarely observed. Some appeared late in the course of the disease and often only a single foot was affected.

The number of animals in Groups I and II, infected and carriers, are presented in Table 1. The intranasal instillation of 10,000 PFU of FMD virus is an efficient method of infecting both sheep and goats. Over 90 percent of the animals inoculated in this manner became infected. Administration of type specific antiserum prior to inoculation did not prevent infection or the establishment of the carrier state. Ten of the 16 sheep and goats in Group II became infected. Thus, of 56 animals exposed to FMD virus, 47 became infected, and of these infected animals, 40 became carriers. It appears that the sheep and the goats had essentially the same infection and carrier rates.

The infected steers used to expose the Group III animals went through a severe course of FMD and were removed after a 6-day contact period. The O-P samples and serum were obtained from the sheep and goats at 14 DPE. The results of this type of exposure in producing infection is presented in Table 2. Forty-five of the 51 sheep and goats exposed to virus in this manner became infected. Again there was no appreciable difference between the total number of sheep and goats which became infected.

Nine sheep in Group I, inoculated with A-4691 virus, were maintained beyond 28 DPE and sampled periodically to determine the duration of the carrier state. One sheep died after 3.5 months of causes not related to foot-and-mouth disease. The number of sheep that continued to be carriers are presented in Table 3. Three out of 8 animals were still positive after 9 months. At this sampling, 18 month-old

***Ivan Sorvall, Inc., 100 Pearl St., Norwalk, Conn.
Hereford steers were inoculated, by the IDL route, with the untreated O-P sample from 2 of the positive sheep. Each steer received 2 ml. of the sample from a single sheep and both steers developed severe generalized foot-and-mouth disease.

**DISCUSSION**

The technique used for processing the O-P samples was essentially that described by Sutmoller and Cottral for treatment of O-P fluid obtained from cattle. The 5 ml. of tissue culture fluid used to rinse the instrument produced a more dilute sample than those from cattle. It was necessary to add 2 percent normal bovine serum to the tissue culture fluid to ensure thorough emulsification when samples were treated with TTE. The Micro Chamber available with the Micro-Homogenizer attachment for the Omnimixer was very useful and allowed the treatment of only 2 ml. of the sample.

In this study, no attempt was made to demonstrate virus strain differences in relation to infection or carrier rates. The four strains used readily infected both sheep and goats and a high percentage of these animals became virus carriers.

It is interesting that the virus present in the untreated O-P sample from sheep, nine months following infection, was fully virulent for cattle. The long stay in the sheep did not render the virus incapable of producing generalized disease when inoculated into cattle. The 3 sheep, still virus carriers after 9 months, do not represent an endpoint and we will continue to sample this group.

Foot-and-mouth disease manifests itself much the same in sheep and goats. Under our experimental conditions, severe clinical signs were not observed, however, the clinical course of the disease in the field might be more severe. In the laboratory, finely chopped hay was fed and the small size of the isolation rooms restricted the movement of the animals. If these sheep and goats had been required to graze for a considerable portion of the day they might possibly have shown more pronounced signs of lameness or oral discomfort. It is also possible that another virus strain might produce more severe clinical signs. The fact remains, that under our conditions, it was a rare occurrence for an animal to show clinical signs that would be detected by any but the most observant shepherd or herdsman. If a closer examination revealed only a minor lip lesion or a single sore foot, it is only a matter for conjecture as to how long it would be before the possibility of an FMD outbreak might be reported to regulatory officials. The disease might spread unnoticed leaving in its wake sheep and goats that were still virus carriers.

**SUMMARY**

Four strains of foot-and-mouth disease virus readily infected both sheep and goats exposed intranasally or by contact. A high percentage of these animals became virus carriers.

Virus isolated from sheep 9 months following infection proved to be fully virulent for cattle.
ACKNOWLEDGMENTS

The writers gratefully acknowledge the excellent technical assistance of Messrs. D. Zaveski, W. Parrish, and H. Mazzaferro, Jr., and the clerical work of Mrs. J. R. Faller.

<table>
<thead>
<tr>
<th>TABLE 1. SHEEP AND GOAT CARRIERS OF FMDV AT 28 DPI</th>
</tr>
</thead>
<tbody>
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FOOT-AND-MOUTH DISEASE VIREMIA

TABLE 3. DURATION OF CARRIER STATE OF SHEEP INOCULATED INTRANASALLY WITH A-4691 VIRUS

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*One sheep died of causes other than FMD.

REFERENCES


Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Greenport, Long Island, New York 11944
Instruments used for collection of oesophageal-pharyngeal (O-P) fluid from sheep and goats. The smaller instrument is used for lambs and kids.
IMMUNOGENICITY OF NANOGRAM TO MILLIGRAM QUANTITIES OF INACTIVATED FOOT-AND-MOUTH DISEASE VIRUS. II. COMPARATIVE RESPONSE OF GUINEA PIGS AND STEERS

D. O. Morgan, P. D. McKercher and H. L. Bachrach
Plum Island Animal Disease Laboratory
Animal Disease and Parasite Research Division
Agricultural Research Service, U. S. Department of Agriculture
Greenport, New York 11944

Purified inactivated foot-and-mouth disease virus (FMDV) has been shown to be an effective immunizing agent in several species of animals.foot-and-mouth disease virus can be effectively inactivated by N-acetylthielenimine* (AEI) with no apparent deleterious effect on its immunogenicity. The inactivation of concentrated preparations can be estimated with a greater degree of confidence, that is, innocuity of a large number of vaccine doses can be tested with a small volume of material. However, the efficient utilization of a concentrated vaccine preparation is dependent upon diluting to its optimum concentration. Achieving the optimum dilution of a vaccine requires a knowledge of the dose-response of the species in which it is to be used. For comparative purposes, the neutralizing antibody responses to varying doses of purified AEI-inactivated FMDV, type A, strain 119 (FMDV, A-119) were determined in guinea pigs and steers, and the immunity of the steers was challenged.

MATERIALS AND METHODS

**Virus:** Foot-and-mouth disease virus, type A, strain 119 was passaged once in suckling mice, 150 times in primary calf kidney cultures, and once in baby hamster kidney cells (BHK-21, clone 13 of MacPherson and Stoker). The virus was purified, as previously described in detail, by methanol precipitation, extraction with organic liquids, one cycle of centrifugation into CsCl and one cycle of centrifugation through an organic solvent mixture into CsCl. The purified virus at a concentration of 4.59 mg/ml based on its extinction coefficient \( \varepsilon_{190} = 76 \) was stored in, and subsequently diluted with 0.2 M NaCl containing 0.05 M sodium phosphate, pH 7.5.

**Virus Inactivation:** Thirty ml of virus (2.62 mg/ml) was inactivated with 0.05 percent (v/v) AEI at 37 ± 0.5°C for 48 hr. A control consisting of virus without AEI was included. Aliquots were taken at appropriate intervals (Fig. 1) throughout the inactivation process, and the AEI reaction was stopped in these aliquots by the addition of 1/10 volume of 20 percent (w/v) sodium thiosulfate. Infectivity of these aliquots was assayed in suckling mice and by plaque assay (Fig. 1).  

*1-acetylaziridine (AEI), 99% pure by gas-liquid chromatographic analysis, was donated by the Dow Chemical Company, Freeport, Texas.
addition, innocuity of the virus preparation after 48 hr of AEI treatment was tested by inoculation of 2 ml (0.65 mg/ml, 0.1 ml/site) intradermalingually in each of six steers.

Fig. 1. Inactivation of FMDV, A-119 with 0.05 percent AEI, 37°C. Log$_{10}$ of PFU/ml vs. hr of AEI-treatment. □ — control preparation containing no AEI; ■ — AEI-treated preparation; — — extrapolated portion of curve.

Measurements of Physical Integrity of the AEI-Treated Virus:

a. Complement fixation (CF). Samples of the material taken prior to, during and after inactivation were tested by the CF test to determine whether or not treatment with AEI degraded the 140 S virus particle. Significant virus degradation would be indicated by a change in the CF titer.

b. Absorbance-temperature (A-T) measurements. As previously described, A-T profiles of purified FMDV would also show the degree of intactness of the virus. Consequently, A-T profiles were determined for each aliquot taken during the inactivation process.
Antigen Dosage in Guinea Pigs: Five different amounts of inactivated virus ranging from 10 nanograms (ng) to $65.5 \times 10^{4}$ ng (Fig. 2) obtained by serial 16-fold dilutions were each tested with and without oil adjuvant in twenty, 400-500 gm, female guinea pigs of the Duncan Hartley strain. The guinea pigs were given injections subcutaneously (1 cm lateral to the spines of the vertebrae in the mid-dorsothoracic region) with either 1 ml of an inactivated virus preparation alone or emulsified with 1 ml of oil adjuvant. At 6, 28, 56 and 84 days postinoculation (DPI), five guinea pigs from each group were exsanguinated by cardiac puncture and individual sera prepared and held at -10°C.

Fig. 2. Means of guinea pig responses, expressed in mouse log$_{10}$ PD$_{50}$ values, at 6, 28, 56 and 84 DPI to varying quantities of FMDV, A-119 inactivated with 0.05 percent AEI at 37°C for 48 hr. ■ indicates response with oil adjuvant; □ indicates response without adjuvant.

Serum/Virus Neutralization Determinations: A constant amount of low-passage tissue culture FMDV, A-119 was mixed with varying dilutions of individual guinea pig serum and incubated at 37°C for 1 hr. Each mixture was then injected into 5- to 9-day-old unweaned Rockefeller H strain mice to detect unneutralized virus. Neutralizing capacities of sera were computed as mouse protective dose$_{50}$ (PD$_{50}$) values by the method of Karber as described by Finney.
Antigen Dosage in Steers: Aliquots of three of the preparations of inactivated virus (i.e., 655,360 ng, 40,960 ng, 160 ng) used to vaccinate guinea pigs were also tested in Hereford steers (Fig. 3). Nine steers were given injections subcutaneously midway between the base of the ear and the point of the shoulder with 1 ml of the inactivated virus preparation emulsified with 1 ml of oil adjuvant. Blood samples were obtained prior to vaccination, 7 DPI, and at 14-day intervals thereafter; individual sera were prepared and held at -10°C.

Challenge of Immunity of Vaccinated Steers: The immunity of the vaccinated animals was challenged 120 DPI by contact exposure to experimentally-infected animals. Two of five unvaccinated control steers placed in contact with the immunized animals were inoculated subcutaneously in the tongue with 10,000 mouse lethal dose50 (MLD50) of FMDV, A-119 guinea pig vesicular fluid. The inoculated controls developed typical foot-and-mouth disease (FMD) lesions within 48 hr, whereas the uninoculated controls did not develop lesions for an additional 48 hr. The steers were examined for signs of FMD daily for 14 days after exposure.

RESULTS

Virus Inactivation: The kinetics of inactivation of purified FMDV, A-119 (2.62 mg/ml) during its treatment with AEI (0.05 percent) (v/v) for 48 hr at 37°C, which
was determined by measuring infectivity by plaque assay, indicate a first order reaction (Fig. 1). Titration of the AEI-treated material in suckling mice closely paralleled the plaque assay results. In addition, none of the six steers given injections of virus treated with AEI for 48 hr developed any lesions of FMD. Altogether 20 percent of the total volume of the AEI-virus mixture after 10 through 48 hr of incubation failed to initiate signs of infection in the three sensitive hosts used.

The control sample without AEI, agitated and held at $37^\circ C$ for a similar period, dropped appreciably in PFU/ml but not as rapidly as AEI-treated preparations (Fig. 1).

**Physical Integrity of the Virus:** Neither A-T profiles nor CF results indicated appreciable change in virus intactness during the inactivation process in either the AEI-treated or control sample.

**Neutralizing Antibody Response in Guinea Pigs:** Mouse PD$_{50}$ values obtained from sera of guinea pigs, which had been inoculated with the two largest doses of inactivated virus, i.e., 655,360 ng and 40,960 ng, without adjuvant were of the same magnitude at all bleeding periods (Fig. 2). While oil adjuvant appeared to exert little influence on the responses to these large doses at 6 DPI, it markedly enhanced the responses at 28, 56 and 84 DPI (Fig. 2).

Response to the intermediate dose (2,560 ng) without oil adjuvant increased slightly between 6 and 28 DPI and decreased thereafter. With oil adjuvant, response to 2,560 ng of inactivated virus, while appreciably higher than response to this dose without oil adjuvant at 28 DPI, did not reach a maximum until 56 DPI and maintained that response through 84 DPI. At 84 DPI, the 2,560-ng dose response with oil adjuvant was approximately 1000 times larger than the response without oil adjuvant (Fig. 2).

With oil adjuvant the response to the 160-ng dose was similar at all bleeding periods (6, 28, 56 and 84 DPI), whereas without oil adjuvant response to this dose was not detectable after 6 DPI.

Mean PD$_{50}$ values to 10 ng of inactivated virus were appreciable without adjuvant at 6 and 28 DPI and with adjuvant at 6 DPI. This dose elicited no measurable response with adjuvant at 28 DPI and later times with or without adjuvant.

**Neutralizing Antibody Response in Steers:** The mean PD$_{50}$ values of 7-DPI sera obtained from cattle inoculated with the two larger quantities of inactivated virus tested (655,360 ng and 40,960 ng) were similar (Fig. 3) and individual responses within the two groups overlapped. The response at 7 DPI to the 160-ng dose was more than 10-fold less than responses to the two larger doses. Except at 21 days, mean PD$_{50}$ values increased with the antigen dosage. Responses were maximal from 49 through 91 DPI. Only the response to the lowest dose showed an increase post challenge. In a few instances, individual responses to the largest and smallest doses overlapped each other; overlaps in responses with the intermediate dose were more frequent.

**Challenge of Immunity of Vaccinated Steers:** One of nine steers vaccinated with purified, AEI-inactivated FMDV, A-119 developed clinical signs of FMD during the 14-day exposure to experimentally-infected steers. It had been vaccinated with 655,360 ng of inactivated virus emulsified in oil adjuvant 120 days
previous to exposure. This steer developed only mild foot lesions; by contrast, the control animals developed fever and severe generalized lesions.

Post-mortem examination of all steers confirmed the preceding results; namely, all controls and the one vaccinated steer showed evidence of the occurrence of FMD.

**DISCUSSION**

Assuming that the inactivation of FMDV by AEI, which occurred without appreciable degradation, remained first order beyond 6 hr, 10^5.4 PFU/ml would still be present at 48 hr. It is reasonable to assume, therefore, that the chance occurrence of an infectious unit of virus in the largest dose of vaccine (655,360 ng) used in this investigation was infinitesimal. In addition, one can expect no more than 2½ percent (p < .05) infective samples in a virus solution from which 120 samples have been removed and shown to be negative in steers (i.e., 20 inoculations on the tongue of each of six steers).

The neutralizing antibody responses in guinea pigs to varying doses of inactivated FMDV with and without oil adjuvant (Fig. 2) are in good agreement with those of an earlier study in which 4-fold increments of antigen were used. Oil adjuvant is ineffective in enhancing the production of neutralizing antibody in guinea pigs at 6 DPI regardless of the antigen dose. It appears, therefore, that oil adjuvant has little or no effect on the production of early neutralizing antibody of the 19 S class. In contrast, oil adjuvant markedly increases the production of late neutralizing antibody to 160 ng and larger antigen doses by 28 DPI, and the increase is accentuated both at 56 and 84 DPI. Moreover, no significant antibody was produced after 6 DPI in response to the 160-ng dose without adjuvant. The neutralizing antibody which would be present in guinea pigs at 28 DPI and later is known to be of the 7 S class. In agreement with an earlier result, the influence of oil adjuvant is most striking on the intermediate dose (2,560 ng) of inactivated virus, and without oil the response to this dose decreases after 28 DPI; by 48 DPI, the response with adjuvant is 10,000-fold larger than without adjuvant. By contrast, increases in response mediated by the oil adjuvant to the two largest doses (i.e., 40,960 and 655,360 ng) at 84 DPI were only about 35 fold. Thus, both the amount of antigen and time of testing are important factors in assessing the effect of the oil adjuvant. As anticipated, the response of individual guinea pigs to the two smallest doses (i.e., 10 and 160 ng) was erratic, perhaps, due to variations in the minimum amount of antigen required to stimulate antibody production.

The neutralizing antibody in guinea pigs and steers can be compared at three antigen concentrations or as a function of time. The PD50 values of the earliest sera taken after the injection of 160 ng, 40,960 ng and 655,360 ng of inactivated virus in oil adjuvant increased with increasing dosage, but the dose-responses in guinea pigs (Fig. 2, 6 DPI) and steers (Fig. 3, 7 DPI) were nearly identical. However, the response in guinea pigs at 28 DPI to the two largest doses was approximately 25-fold larger than that in steers at 21 or 35 DPI; conversely, the response to the 160-ng dose was about 64-fold larger in steers than in guinea pigs. By 84 DPI, the response in guinea pigs to the two larger doses was only six times that in steers, whereas the response of steers to the 160-ng dose had increased to 125 times that of guinea pigs. In view of the limited number of steers in this experiment, the
results were confirmed in a second group of nine steers given injections of the same lot of inactivated virus in oil adjuvant. Thus, steers were much more responsive than guinea pigs to the smallest dose at 28 DPI and later times, but their response to the larger doses did not match that of guinea pigs.

Prior to challenge at 120 days, the PD50 values of the sera of vaccinated steers had declined slightly from the maximum. After challenge by contact infection, there was little increase in PD50 values except in those steers which had been vaccinated with the smallest dose of inactivated virus. The absence of marked secondary responses was possibly due to the fact that antigen was still being released from deposits of emulsified vaccine and/or the fact that the PD50 value at 120 days was still quite high at the time of challenge.

The reason why one of the steers, which had been vaccinated with the largest dose of inactivated virus, developed foot lesions on challenge is obscure. Susceptibility of animals such as this one having $10^{4.5}$ PD50/ml of circulating antibody is not easily explained.

On post-mortem examination of the steers, the local reaction produced by the vaccine appeared to involve the skin and was easily removed with the skin. The underlying musculature was involved only when it inadvertently had received a portion of the vaccine. Of the nine steers given injections of a constant volume of vaccine, three had no visible reactions, three had small reactions (35 X 35 mm) and three had slightly larger reactions (75 X 25 mm). There appeared to be no correlation of the size of the reaction with either the immune response shown by the animals or the antigen dose. Microscopically, lesions were identified as foreign-body granulomas characterized by the presence of oil vacuoles.

**SUMMARY**

The immunogenicity of a purified preparation of foot-and-mouth disease virus, type A, strain 119 (30 ml, 2.62 mg/ml) inactivated with 0.05 percent N-acetylenimin (AEI) at 37°C was compared in steers and guinea pigs. Kinetics of its inactivation indicated a first order reaction resulting in an extrapolated value of $10^{5.4}$ PFU/ml at 48 hr. Complement-fixation and absorbance-temperature measurements indicated little or no virus degradation during the AEI treatment.

Five different amounts of inactivated virus in 16-fold increments from 10 nanograms (ng) to $65.5 \times 10^4$ ng were tested with and without oil adjuvant in guinea pigs. Response to vaccination was assessed at various intervals by serum/virus neutralization tests and calculated as mouse protective dose50 (PD50) values. Oil adjuvant had little influence on the guinea pig response at 6 days postinoculation (DPI) to any antigen dose. At later testing periods (i.e., 28, 56 and 84 DPI), response to the three largest doses was enhanced by oil adjuvant. Without adjuvant the response to the 160-ng dose of antigen was significant only at 6 DPI; with adjuvant the response to this dose was significant at all testing periods.

Aliquots of three of the preparations (655,360 ng, 40,960 ng, 160 ng) used in guinea pigs were also tested with oil adjuvant in steers. The PD50 values at 6 DPI increased with increasing dosage, and the dose-response of steers and guinea pigs was nearly identical. At 28 through 84 DPI, guinea pig responses to the two larger...
doses were much higher than those of steers; however, the response of steers to the smallest dose was many-fold larger than that of guinea pigs. All three steers vaccinated with 160 ng of inactivated foot-and-mouth disease virus, type A, strain 119 with oil adjuvant withstood challenge exposure at 120 days postvaccination.

ACKNOWLEDGMENTS

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REFERENCES


VESICULAR DISEASE COMMITTEE

1968 Report


The vesicular disease committee reports as published in the proceedings of the United States Livestock Sanitary Association provide a continuing summary on the status of vesicular diseases in the United States and in other parts of the world and on vesicular disease research.

INCIDENCE OF VESICULAR DISEASES

FOOT-AND-MOUTH DISEASE — In most countries of Europe there was a marked decrease in the incidence of foot-and-mouth disease (FMD) during 1967 and the first half of 1968. Extensive vaccination programs and the cyclic nature of the disease have no doubt contributed to the presently favorable position. Foot-and-mouth disease virus (FMDV) type O was the predominating type in Europe during this period. However, type A was also identified in Denmark, Poland and Italy, and type C occurred in France, Spain, Portugal, Hungary, and Poland. Details of the extensive epizootic in Great Britain during the winter of 1967-68 have been presented and will appear in a separate report in the proceedings.

West Germany reported the most dramatically improved FMD situation with a decrease in number of premises affected from almost 5,000 in 1966 to 3,332 in 1967 and only 10 during the first half of 1968.

France continued to maintain a very favorable position with 17 outbreaks in 1967 and 28 outbreaks during the first seven months of 1968.

The incidence of FMD has also been remarkably low in Belgium, the Netherlands, Denmark, Switzerland, Poland, East Germany, and Hungary. Austria, Rumania, Czechoslovakia, and Yugoslavia have not reported outbreaks since 1966, Albania since 1961, and Bulgaria reported no cases in 1967.

The position in Italy has substantially improved from 1,569 outbreaks in 1966, to only 216 in 1967. During the first six months of 1968 only 14 outbreaks were reported. Spain experienced an epizootic during 1967 involving 25 provinces and during the first half of 1968 has reported 108 outbreaks. In the European part of the USSR there appears to be some improvement with 1,449 outbreaks reported in 1967.

Greece experienced several limited outbreaks during 1967 but no outbreaks have been reported during the first half of 1968. Turkey reported 2,068 outbreaks in 1967 of which virus type O was identified from 1,141 outbreaks. Sub-type A22 was identified in only 8 of the outbreaks.

In Asia, FMD continues to occur enzootically and seasonally. India reported
3,438 outbreaks in 1967 as compared to 3,738 in 1966. Thailand reported 101 outbreaks in 1967 of which 20 were caused by virus type Asia 1. Virus types O and A were also identified. Virus type O and A were identified in Vietnam during 1967. Information is not available concerning the incidence of FMD in Continental China.

Virus types identified in Africa during 1967 were, Upper Volta-SAT2; Ghana - type A; Nigeria - type A; Sudan - type A; Kenya - types O, A, C and SAT2; Uganda - type O and A; Tanzania - type O; Malawi - type A; Rhodesia - type SAT2; Mosambique - type SAT 1; South African Republic - type SAT 2; and Angola - type A.

In South America FMD continues to occur in sporadic and enzootic form with little notable change. Virus types O, A and C were reported during 1967 in all the enzootic countries except Venezuela and Ecuador. Brazil reported 205 outbreaks in 1966 and 116 outbreaks in 1967. Argentina reported only 327 outbreaks during the first quarter of 1967. However, an epizootic in the early spring in the province of Buenos Aires spread rapidly reaching a peak of 1,292 outbreaks in September. A total of 4,634 outbreaks were reported for the year. Columbia reported 232 outbreaks in 1967 with the highest incidence in June, July, and August. Virus type C was identified for the first time in Columbia during August of 1967. FMD has not been reported in Curacao since 1961, Guadelupe since 1964 and French Guiana since 1960.

The Inter-American Bank has established a policy of financial support to assist countries in South America that have or plan nationwide programs for FMD control and eradication. Loans have already been extended to Chile and Paraguay for $2.3 million and $2.8 million respectively. Loan requests totaling approximately $30 million are in advanced stages of preparation by Argentina, Brazil, Peru and Uruguay. Bolivia and Ecuador may also request loans.

Argentina, Brazil, Chile, Paraguay and Uruguay have established a regional Technical Commission of Animal Health to unify their efforts and policies with respect to livestock diseases. Members have agreed to export only livestock that has been vaccinated or revaccinated during a 15 day period before shipment, to establish standards for vaccine production and control, and to exchange technical information and research findings.

At present FMD causes livestock losses estimated at $400 million a year in South America - not counting lost sales by meat exporting countries where the disease is in evidence.

VESICULAR STOMATITIS

UNITED STATES- Through September of 1968 vesicular stomatitis (VS) has been diagnosed on nine premises in LaSalle and Catahoula Parishes of Louisiana. A yearling hog at Larto, Louisiana had suspicious lesions and neutralizing antibodies to New Jersey VS. This was the same herd in Catahoula Parish in which New Jersey VS was diagnosed during 1967. These antibodies may be from last year's infection.

In mid-May the first VS cases occurred in two horses penned at the headquarters of the Saline Game Management Area (SGMA) in LaSalle Parish approximately 10 miles west of the 1967 VS outbreak. In mid-June the yearling hog was observed in Catahoula Parish. On June 20, several horses were reported affected on two premises approximately two miles from the SGMA headquarters. On July 18 bovine tongue epithelium was collected from a herd at Jena, about 15
miles north of the outbreak area. This tissue was positive for New Jersey type VS on the complement fixation test. The owner first observed the disease a week previously and 10 of his 140 cattle had been affected by the time of the first investigation. Lesions were observed on tongues, dental pads, and teats. Positive swine and bovine serum samples were obtained from three other herds in the Jena area; these herds had been affected about the first of July.

Epidemiological interest following the diagnosis of VS in swine last year in the Larto Lake area caused the Louisiana Wildlife and Fisheries Commission which operates the Saline Game Management Area (SGMA) and Louisiana livestock sanitary officials to collect serums from the deer in the area during the fall kill of 1967.

Eight of ten white-tailed deer serums (ages 1½-3 years) contained neutralizing antibodies for New Jersey VS virus. None of 101 white-tailed deer serums collected at the Delta National Wildlife Refuge near the mouth of the Mississippi River or of 82 deer serums from Dr. Frank Hayes, Southeastern Cooperative Wildlife Disease Study (serums from Georgia, Alabama, Florida, North Carolina, South Carolina, Virginia, and Virgin Islands), contained VS antibodies to either New Jersey or Indiana type of VS virus.

During August, an attempt was made to detect arthropod transmission of VS virus. Thirty litters of one day old Swiss Webster white mice were exposed (1 to 2 litters per night), for one night as sentinel animals in the SGMA and then held in isolation for two to three weeks. The mice were suspended in roofed cages during exposure. Sick or dead mice obtained from three litters on the second, fourth, and eighth day were stored frozen for later attempts to isolate virus. Sick or dead mice from two other litters on the 15th and 16th day were also frozen for virus isolation trials.

A preliminary survey of VS in wildlife was started with the collection and testing of the following species in Louisiana, Texas, and Georgia:

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>New Jersey Type</th>
<th>Indiana Type</th>
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<tbody>
<tr>
<td>Louisiana (SGMA)</td>
<td>Sigmodon hispidus (cotton rat)</td>
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<tr>
<td>Collected</td>
<td>Sigmodon hispidus (juvenile)</td>
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<td>0/1</td>
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<tr>
<td>May 27 &amp; 28</td>
<td>Reithrodontomys sp. (harvest mice)</td>
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<td></td>
<td>Peromyscus sp.</td>
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<td>0/6</td>
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<tr>
<td>Bled Sept.</td>
<td>Syrniun varium (barred owl)</td>
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<tr>
<td></td>
<td>Sylvilagus aquaticus (swamp rabbit)</td>
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<td>0/2</td>
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<tr>
<td>Texas (Webb Co.)</td>
<td>Sigmodon hispidus</td>
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<tr>
<td>Collected</td>
<td>Neotoma micropus</td>
<td>0/4</td>
<td>0/4</td>
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<tr>
<td>June 6 &amp; 7</td>
<td>(Southern Plains Wood Rat)</td>
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**VESICULAR DISEASE**

*Reithrodontomys sp.*

(Hidalgo Co.)

*Sigmodon hispidus*

Collected

June

*Neotoma micropus*

Unidentified Mice

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*Number positive/number tested in 1:20 serum dilution.

Virus isolation efforts were negative from pools of heart, liver, and spleen and of kidney and bladder from each of the two Louisiana suspects.

The New Jersey VS antibodies in the deer and juvenile cotton rat collected in the Saline Game Management Area may have epizootiological importance. The number of rodents tested is too small to be more than a preliminary inquiry, but positive wildlife serums were in correlation with the area of known active infection. **MEXICO** - During calendar year 1967 veterinarians of the Joint Mexico-U. S. Commission for the Prevention of Foot-and-Mouth Disease carried out 58 field and laboratory investigations for vesicular disease in Mexico. Twenty-nine were caused by New Jersey VS, 6 Indiana, 5 were double fixation and 1 revealed animals with both New Jersey and Indiana infection in the same herd. All of these except 1 case of Indiana and 1 of New Jersey occurred in 9 States in the southern half of Mexico. Through September of 1968 there have been 42 investigations. New Jersey type VS had been diagnosed on 19 premises, Indiana VS on 9; double fixation occurred with 7 cases and the remaining were negative. The pattern of cases this year is about the same as for last year with most of the cases occurring in the southern half of Mexico. Cases occurring in the northern states were 1 of the Indiana type in Chihuahua and 1 double fixation in Coahuila. The decreased incidence of vesicular stomatitis in Mexico during the last two years is parallel to that observed in the United States.

**RESEARCH ON VESICULAR DISEASES**

**VESICULAR EXANTHEMA OF SWINE** - Researchers at the University of California have continued to conduct basic research on the virus of vesicular exanthema of swine (VES). These workers have examined the changes in tissue cultures of pig kidney cells infected with VES virus type A-48 by electron microscopy eight hours after infection or at the time of maximal virus production. Progressive nuclear degeneration and accumulation of dense aggregates were found in the cytoplasm of the infected cells. Large crystalline aggregates of virus were seen in cytoplasmic foci 14 hours after infection. The particles in the crystals were approximately 27 millimicrons in diameter. Virus particles were not observed in the nucleus of infected cells during any stage of the infection.

Morphogenesis of a type of VES virus (VESV) was studied in tissue culture propagated kidney cells. Virus particles first appeared free in the cytoplasm of infected cells 4 hours after infection. At 7 hours after infection, virus particles were seen associated with cytoplasmic cisternae. Crystalline arrays of virus particles were observed in the cytoplasm of infected cells as early as 8 hours after infection.
crystalline arrays of virus particles which are demonstrated in the cytoplasm of cells infected with VESV were morphologically in accordance with those shown for other small RNA viruses of the picorna virus group.2

The virus of VES was shown to propagate in cultures of swine kidney cells and produce cytopathic effects many years ago. Some antigenic types of the virus have been shown to form plaques on monolayers of adult swine kidney cell line under agar. Zee et al3 using methyl cellulose overlay medium have described an accurate and reproducible plaque assay method for several of the immunologic types of VESV. The concentrations of methyl cellulose had an influence on the size of plaques formed by the virus showing an inverse relationship between concentration of methyl cellulose in the overlay medium and plaque size. During the course of these studies, the workers also observed that the absorption of VESV to monolayers of pig kidney cells at 37°C was found to reach a maximum within 60 minutes. It is now evident that all immunologic types of VESV may be expected to produce plaques in appropriate tissue culture systems.

VESICULAR STOMATITIS - While vesicular stomatitis (VS) has not reached epizootic proportions in the U. S. for the last two years, it continues to exist widely in Mexico, Central and South America. The epizootiology of VS has been examined during the last year by at least two investigators. Hanson has described a list of several conditions which must exist: 1) Populations of susceptible animals most of which are free of antibody must be present, 2) the weather must be favorable, which means mild temperature, usually 70°F, and 3) the necessary moisture conditions are not easy to define. In arid climates epizootics follow the rainy season. In moderate climates they may occur during periods preceded by heavy or light rain or no rain at all. Good pastures with some tall grass are almost invariably found on farms on which herds become infected indicating that reasonably good moisture conditions are probably a prerequisite for an epizootic. By the examination of records from outbreaks in the U.S., Hanson and his coworkers4 have advanced the theory that epizootics originate from a point source that extends outwards. In areas where the disease is enzootic, numbers of wild animals have been found to have antibodies.

Some workers have proposed that the virus of VS may be arthropod-borne. Several workers have demonstrated mechanical transmission of VS virus (VSV) by several species of mosquitoes and biting flies. Investigators in Venezuela, Panama, and the U. S. have isolated VSV from biting gnats and mosquitoes. On the other hand, Jonkers in a recently published paper, questions whether biting arthropods play a significant role in the dissemination of VSV. He bases this conclusion on several factors, one being that no arthropod has been incriminated in the natural transmission of VSV type New Jersey and a simultaneous appearance of the disease in an entire herd or many herds would mean access to an infective source of major dimensions. He suggests that the virus exists in the pasture and some event renders it infectious.

The virus could be in or on the forage and the event could be an abrasion inducing situation. Even though Jonkers did not spell out alternative means of transmission, the mere fact that he has raised the question probably will stimulate re-thinking of the subject.4,5

The isolation of New Jersey VSV was reported from eye gnats (Hippelates
VESICULAR DISEASE

pusio) which were trapped on a farm in Canon City, Colorado in 1966. VS was occurring in cattle on the farm at the time of collection of the gnats. The workers were uncertain of the significance of the single isolation of New Jersey VSV from the gnats since they feed on mucus from the eyes of infected cattle and are not blood sucking insects, however, the isolation of the virus points up the possibility that insects may play a role in mechanical transmission of the virus rather than develop a carrier state in which the virus multiplies in its tissues.

Shelokov and Peralta working in Middle America, found VSV Indiana type in sandflies caught in the tropical rain forest. These same workers had demonstrated a surprisingly high overall proportion of people in Middle America with VSV Indiana antibodies in their serum. The close association between the high incidence of antibodies in people in areas where there is a high occurrence of phlebotomus sandflies suggested that sandflies participate in the biologic cycle of infection with the Indiana type VSV involving animal and human populations of the American tropics.

Brody et al. also working in Middle America, studied an outbreak of VS due to New Jersey type of the virus in a dairy herd. In a survey of humans in this area, he found that the presence of neutralizing antibody to this virus was higher among people with a history of having worked with cattle than among those who had not. These workers suggested the possible mode of spread in humans either by direct contact with infected animals or arthropods. While both types of spread probably occurred, contact appeared to be more important.

West and Labzoffskey have studied the cellular site of synthesis of VSV through the use of various chemicals, immunofluorescence and electron microscopy. Following the various treatments, virus infectivity was assayed in chick embryo cells and African green monkey kidney cells. The results obtained indicates that the replication of VSV takes place in the cytoplasm with probably no participation of the cell nucleus.

Recently, VSV has caused some interest because of its unusual structural features and because it apparently is recognized as a prototype of a new family of viruses designated Stomatoviridae or Rhabdoviruses. Other viruses morphologically similar to VSV are found in plants, insects, and vertebrates.

Although the main structural features of the virion of VSV have been established, there is still some uncertainty about the configuration of the internal nucleoprotein. Nakai and Howatson concluded that the nucleoprotein is in the form of a ribbon-like strand consisting of a series of regular rod-like subunits of dimensions approximately 90 x 30 x 30 angstroms. The continuity of the strand is maintained by the subunits being attached to a thread of nucleic acid. In the intact virion the strand is in the form of a helix of about 30 coils. In the disintegrating particles, the helix unwinds to form an undulatory ribbon which may assume a configuration of an irregular helix.

Brown et al. have studied the various components of the purified virion of VS. These workers referred to the largest of the four particles which they obtained following concentration, purification, and disruption as the skeleton because it has the same overall shape and size as the intact virion but without the outer fringe structure. This particle possesses low but significant infectivity and fixes complement with hyperimmune serum. Rosette-like particles, similar to those
found in unfractionated viruses mentioned are also present in the disrupted VSV preparations. These particles are found mainly in the 16S fraction of the sucrose gradient and account for a considerable proportion of the immunogenic activity of the preparations. In addition to these two well defined structures, there is also a slowly sedimenting fraction 3 to 6S which possesses complement-fixing and immunogenic activity. This fraction produces two precipitin bands in agar diffusion; consequently, the authors have concluded that there are two different particles in this fraction but they have as yet been unable to separate them.

Halonen et al, have reported on the hemagglutinin of VS prepared in suspension cultures of BHK-21/13S cells. Other workers have previously reported negative results which the present workers attribute to some special requirements for the demonstration of hemagglutinin, including a critical need for the control of temperatures and the use of goose erythrocytes. The development of methods for preparation of hemagglutinin of both Indiana and New Jersey VS is considered to be a significant development which should lead to the development of sensitive hemagglutinin tests for VS viral antibodies. The current demonstration of the development of hemagglutinin for rabies and VS viruses as for other bullet-shaped viruses suggest a common biological property and indicates that perhaps these viruses should be placed in the same family Stomatoviridae based primarily on common physical characteristics.

**FOOT-AND-MOUTH DISEASE** - Since the last Vesicular Diseases Committee report, Great Britain has undergone one of the worst epizootics of foot-and-mouth disease (FMD) ever experienced in that country. This has caused the establishment of a special committee in Great Britain to inquire into the recent epizootic. The Duke of Northumberland's committee is expected to take a year to complete its study. The British Veterinary Association has recommended that if the government continues to import meat and offal from countries where FMD exists, the only way to insure that epizootics of the disease do not occur is to supplement the present measures with a well organized program of systematic vaccination. This would be used as an adjunct to the present policy.

It remains to be seen what the Duke of Northumberland's committee of inquiry will recommend when they review the whole problem of FMD control. The report is anxiously awaited by livestock control officials in many parts of the world for it may well have a profound influence on the course of action which will be followed in other countries.

The World Reference Laboratory for FMD at Pirbright, England, has issued a revised list of subtype reference strains of the virus. Fifty-three subtypes of the virus have been identified at the World Reference Laboratory. Within subtype 0, 10 have been identified, within type C, 4, within type A, 23; 7 subtypes within type SAT-1, 3 within type SAT-2, 4 within type SAT-3, and 2 subtypes have been identified within type Asia-1. The number of types and subtypes of FMDV which have been identified, clearly indicates the complexity of the immunization of animals in a country where the disease does not exist. The question arises as to which types and subtypes of the virus are to be included in the vaccine. During the past year, additional experimental evidence has been advanced concerning the existence of biological carriers of FMD in cattle which have recovered from infection. Sutmoller et al at the Plum Island Animal Disease
Laboratory have continued to study this problem and their results include two facts of interest to epizootiologists; 1. FMDV can infect cattle and multiply in the pharynx regardless of their immune status, and 2. infection and multiplication can occur in the pharynx in the complete absence of clinical signs of the disease.

Others have continued to study the carrier problem and Hedger at the Animal Virus Institute, Pirbright, England, has reported on the isolation and characterization of FMDV in clinically normal herds of cattle in a country where FMD is enzootic (Botswana). This worker surveyed the incidence of FMDV carriers in three localities of a FMD enzootic area. Type SAT-3 virus was isolated from up to 20% of the animals sampled at periods between 7 and 12 months after natural infection. Serums were tested from all the animals from which virus was recovered and compared with similar numbers of serums from the virus negative animals. No correlation was found between the carrier state and the serum antibody titers. Antigenic differences were described which indicated variation of some carrier viruses isolated from the outbreak strain and also antigenic differences between strains of carrier virus from different animals in the same herd. These results also suggested transference of carrier virus from animal to animal may occur in the absence of clinical infection. This study also added evidence to the fact that the appearance of new subtype strains of FMDV result from the spread of the virus through a partially immune population.

There is much evidence in the literature on FMD which indicates that animals are capable of spreading the infection before the appearance of lesions and the virus may be recovered from various secretions and excretions from such animals. Burrows at Pirbright, has studied this problem by placing experimental groups of cattle, sheep, and pigs in an isolation compound with animals of the same species which had been inoculated. The exposed and normal animals were housed in separate animal rooms within the main area and were examined daily for the presence of lesions, and samples were taken of blood and milk, and from the pharynx, rectum, prepuce, and vagina. Virus was recovered from the pharyngeal samples from the majority of animals several days before clinical disease was evident. Virus was also recovered from the blood, milk, rectal, and preputial or vaginal swabs before clinical signs were evident. These findings show that some animals are possible sources of infection for periods up to five days and in pigs up to 10 days before the disease could be diagnosed. The worker also showed that considerable virus multiplication takes place in the mammary gland, accompanied by changes in the volume and character of the milk from the infected quarter. In an animal inoculated in the mammary gland, vesicular lesions characteristic of FMD did not develop in the animal until 3 or 4 days later.

Several years ago, Cottral et al., Plum Island Animal Disease Laboratory, showed that the semen of bulls experimentally infected with FMDV contained the virus in the semen in as soon as 12 hours after inoculation which was 6 to 12 hours in advance of clinical signs of the disease in the animal. Semen from infected bulls was shown to contain the virus for as long as 10 days post-infection. Foot and mouth disease was transmitted to heifers by artificial insemination with semen from infected bulls.

The swine cell line IBR-S2 has been shown to be useful for propagating FMDV and for isolations of FMDV from samples collected in the field. This particular cell
line which was derived from the kidneys of pigs has been maintained in a laboratory in Brazil for several years and may be useful for mass production of FMDV along with other cell lines such as one derived from baby hamster kidney cells. Suttmoller and Cottrel have described improvements in methods used to assay the oesophageal-pharyngeal (OP) fluid specimens from cattle which are carriers of FMDV. The specimens are first treated with trichloro-trifluoroethanol (TTE) prior to assay. The TTE treatment eliminates bacterial and fungal contaminants and breaks down the FMDV neutralizing antibody and other inhibitor complexes. Specimens treated in this manner are then assayed for virus content by inoculation of primary calf kidney cells in prescription bottles or in Povitsky bottles for assay of greater volumes. Aliquots of OP fluids may be stored at -20°C for more than 2 months without appreciable loss in titer when they are removed and treated with TTE. The addition of 50% glycerine to the original OP fluids prolongs storage at -20°C.

Workers at the Plum Island Animal Disease Laboratory have reported on methods for producing purified FMDV. The method consists of first producing large amounts of virus in BHK cell cultures followed by concentration and purification of the virus. FMDV which results from these procedures is pure by critical biological, chemical, and physical tests and production may amount to 150 mg. per week. The current weekly production of crude virus from 400 two liter bottles of BHK cells cultures, yields approximately 1.5 gm. of crude FMDV.

Polatnick and Bachrach have studied the effects of ionizing radiation on infectivity of FMDV. Purified virus added to harvested fluid from cell cultures which contained cellular products, was many times more resistant to inactivation by ionizing radiation than was pure virus. The infectivity of pure virus preparations was shielded from radiation damage by the cellular and growth medium components. Free ribonucleic acid (RNA) was more readily inactivated by radiation than intact virus. The absorbance temperature measurement showed that loss of infectivity by pure virus was accompanied by degradation of the virus particles as well as hydrolysis of the RNA chain. Arlinghaus, et al have studied the site of FMDV ribonucleic acid synthesis in BHK cells and have determined that the virus is synthesized in the cytoplasm. These workers identified three classes of virus-specific RNA by sedimentation rates in sucrose gradients: 37S viral RNA, a 20S ribonuclease resistant RNA, and heterogeneous RNA which has a peak sedimentation rate between 18 and 20S. All three classes were isolated from the cytoplasm. The 20S and 37S are infectious. When extracts are made from FMDV-infected cells and nucleosidetriphosphates are added, RNA synthesis continues in vitro without living cells being present. This is an important step forward in studying the mechanism of FMD RNA replication.

Graves, et al have studied and characterized an RNA-free virus-like particle. During routine purification of various types of FMDV for immunologic studies, serologic evidence from infectious fractions indicated the presence of an antigen not previously described. This new 75S antigen was found to be a characteristic of FMDV strain A-CANEF-1 rather than the effect of the substrate environment. Properties of this antigen suggest that it is the empty shell of FMDV, is similar in size to 140S virus particle, contains no detectable RNA, does not disrupt into the 12S subunits and forms an intracellular crystalline array. The 75S antigen is
apparently produced during replication of the virus. There is no evidence that 75S antigen results from 140S virus extruding its RNA core. The 75S particle of FMDV has features similar to those of the empty protein shell of poliovirus. At present, however, the workers have indicated that there is evidence of a naturally occurring 75S particle in only this virus which appears to be a mutant strain. The complex antigenic nature of this particle and its relationship to 140S and 12S particles remains to be precisely determined.

Wild and Brown have studied the physical properties of the immunizing antigen of FMDV. They inactivated virus with three well known inactivants - Acethylethyleneimine (AEI), Beta propiolactone (BPL), and fomaldehyde. They found that the immunizing activity of the formaldehyde-inactivated virus was much lower than the AEI or BPL-inactivated preparations. This difference in immunizing activity suggested some alteration in the structure had occurred during inactivation with formaldehyde. This was confirmed by measuring the buoyant density of each preparation with cesium chloride. Whereas the peaks of complement-fixing activity of the AEI or BPL-inactivated preparations coincided with the position of untreated virus, the peak in the formaldehyde-inactivated preparation was at a higher density indicating deterioration of the inactivated virus particle due to the formaldehyde treatment. When AEI-inactivated preparation was stored at 25°C, there was a progressive decrease in the immunogenic activity. This loss of biological activity was correlated with an increased density of the particle. Fractionation of AEI-inactivated virus preparations in cesium chloride was associated with considerable loss in immunogenic activity and complement fixing activity. The peak of immunogenic activity corresponded approximately with the density of fresh virus. These results show that retention of the entire protein structure of FMDV is necessary for the preservation of immunogenic activity. In such preparations, the loss of immunogenicity is proportional to the change in density and is probably due to the progressive loss of protein from the virus coat.

Research on inactivated FMD vaccine has continued. In one study, Graves et al. reported on cattle and swine exposed to virulent, homologous FMDV 3 to 14 days after vaccination with oil emulsified inactivated virus vaccine. These animals were found to be resistant to infection. This vaccine was made from virus produced in BHK cells, inactivated with AEI and emulsified with oil adjuvant. Immunity and neutralizing antibody levels produced by this vaccine were superior to those produced by vaccine containing aluminum hydroxide gel adjuvant. A high percentage of cattle exposed as late as 9 months after a single vaccination resisted challenge inoculation with a virulent virus or exposure to clinically diseased cattle.

McKercher and Farris studied similar preparations in swine with equally good results. In both of these studies it was observed that peak neutralizing antibody titers were not reached until 60 to 80 days after vaccination with oil adjuvanted vaccine, as compared with approximately 18 to 30 days when aluminum hydroxide gel was used.

Research is continuing in a number of laboratories on inactivated FMD vaccines and some improvements over vaccines in general use are expected.

Recommendation

The Committee urgently recommends that a study of the epidemiology of Vesicular
Stomatitis be conducted. Reasons for the study were presented in the 1967 report and the recent explosive outbreak of FMD in Great Britain further supports the urgent need. The coexistence of an unpredictable vesicular disease in the United States during an FMD outbreak would seriously delay as well as increase the cost of an eradication program.

REFERENCES


VESICULAR DISEASE


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COMMITTEES FOR 1968-1969

World Veterinary Association

V. A. Seaton (establish a section on diagnostic medicine)

Wildlife Disease Committee

L. A. Griner, Chairman
L. Karstad
D. Trainer
M. E. Macheak
D. C. Gigstad
R. Houk
Ted Kistner

Biological Safety Committee

D. Smith, Chairman
D. Johnson
E. J. Bicknell
E. C. Sharman
Andy Gray
Eric Broughton

Equine Piroplasmosis Committee

A. Holbrook, Chairman
D. E. Cooperrider
T. O. Roby
W. W. Kirkham
W. H. Martin

Recognition Committee

To be selected by D. M. Bedell

Committee for Annual Survey of Laboratories

W. L. Sippel, Chairman
K. D. Weide
D. E. Lundholm
K. K. Keahey
V. A. Seaton
E. P. Pope

Representatives to the National Academy of Science on Veterinary Microbiological Standard Methods

H. W. Dunne
W. L. Sippel

Executive Advisory Council

V. A. Seaton (past Chairman)-1969
L. W. Turner – 1970
N. W. Rokey – 1971
K. D. Weide – 1969
H. L. Chute – 1972

Accreditation Committee

J. G. Miller, Chairman
Executive Advisory Council

Animal Disease Reporting

V. A. Seaton, Chairman
N. E. Hutton
D. E. Cooperrider

Salmonella Committee

L. C. Grumbles, Chairman
E. M. Ellis
J. E. Williams
B. S. Pomeroy
W. E. Lyle
J. C. Olson, Jr.
R. W. Winterfield
W. J. Martin

Program Committee

W. E. Lyle, Chairman
J. F. Frank
S. B. Hitchner
R. W. McIntyre
J. T. Tumlin

Committee for Establishing Uniform Rabies Examination Procedures and Techniques in Laboratories

D. E. Lundholm, Chairman
R. V. Chance
Keith Sikes

Officers for 1969

Dr. D. M. Bedell ... Chairman
Dr. W. E. Lyle ... Chairman-Elect
Dr. E. P. Pope. Secretary-Treasurer
1968-69 OFFICERS OF THE C.V.L.D.

DR. D. M. BEDELL
Chairman

DR. W. E. LYLE
Chairman-Elect

DR. E. E. POPE
Secretary-Treasurer
CONSTITUTION AND BY-LAWS

of the

AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

CONSTITUTION

ARTICLE I–NAME

The name of the Association shall be the American Association of Veterinary Laboratory Diagnosticians.

ARTICLE II–UNITED STATES ANIMAL HEALTH ASSOCIATION AFFILIATION

The American Association of Veterinary Laboratory Diagnosticians shall be an affiliate of the United States Animal Health Association in accordance with a memorandum of agreement mutually agreed upon between the two parties and approved by a two-thirds majority vote of those voting and present at any annual business meeting.

ARTICLE III–PURPOSE

The purpose of this Association shall be the dissemination of information relating to the diagnosis of animal diseases, the coordination of the diagnostic activities of regulatory, research and service laboratories, the establishment of uniform diagnostic techniques, the improvement of existing diagnostic techniques, the development of new diagnostic techniques, the establishment of accepted guides for the improvement of diagnostic laboratory organizations relative to personnel qualifications and facilities, and to act in a consultant capacity to the United States Animal Health Association on uniform diagnostic criteria involved in regulatory animal disease programs.

ARTICLE IV–MEMBERSHIP

Any laboratory worker engaged in the field of disease diagnosis in animals is eligible for membership.

ARTICLE V–MEETINGS

The meetings of the Association shall be annual and special.

ARTICLE VI–OFFICERS

The officers of this Association shall be: Chairman, Chairman-elect, and Secretary-Treasurer.

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CONSTITUTION AND BY-LAWS

ARTICLE VII—EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the Chairman, chairman-elect, and Secretary-Treasurer.

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies.

The Chairman of the Association shall be the Chairman of the Executive Committee.

ARTICLE VIII—PROGRAM COMMITTEE

The Program Committee shall consist of the Chairman-elect and four other members, one each, respectively, from the four districts of the United States, appointed by the Conference Chairman. Said districts shall be known as the *Northeast*, consisting of the states of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; the *North Central*, consisting of the states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; the *Southern*, consisting of the states of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico and the Virgin Islands; and the *Western* district, consisting of the states of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming.

ARTICLE IX—DUTIES OF THE OFFICERS

1. Chairman: It shall be the duty of the Chairman to preside at all meetings of this Association and to appoint all committees. The Chairman shall be an ex officio member of all committees and Chairman of the Executive Committee. (Also see Article II).

2. Chairman-elect: In the absence of the Chairman, the Chairman-elect shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the Chairman, he shall perform all duties of the Chairman. He shall be a member of the Executive Committee and Chairman of the Program Committee.

3. Secretary-Treasurer: The Secretary-Treasurer shall keep an accurate record of the meetings of the Association. Whenever authorized by the Executive Committee, he shall publish newsletters and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the meetings of the Executive Committee and shall furnish a copy to each member of said Executive Committee.

He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman a list giving the name and address of each member and an annual financial report. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be the Secretary of the Executive Committee, also an ex officio member of the Program Committee.
ARTICLE X—AMENDMENTS

The Constitution of this Association may be amended by a two-thirds vote of the members of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting.

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to order.
Report of Secretary-Treasurer.
Chairman’s Address.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers.
Adjournment.

A suspension of the By-laws may be made by a two-thirds majority vote of those present and voting for the purpose of changing the order of business or to facilitate important business.

ARTICLE II—APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary-Treasurer.
An individual member may be expelled for cause upon recommendation of the Executive Committee and a majority vote of a regularly scheduled meeting of the membership.

ARTICLE III—MEETINGS

The annual meeting of the Association will be held the two days preceding the dates of the annual meeting of the United States Animal Health Association and at the same location.

ARTICLE IV—QUORUM

Twenty members of the Association shall constitute a quorum.
Two members of the Executive Committee shall constitute a quorum.

ARTICLE V—PROCEEDINGS

(The articles for publication shall be assembled by the Chairman of the
Program Committee and forwarded by him to the Secretary of the United States Animal Health Association immediately following each annual meeting.

All articles for publication in the United States Animal Health Association Proceedings shall conform to the style requirements of the Journal of the American Veterinary Medical Association.

Abstracts of the articles to be presented at the annual meeting shall be assembled by the Chairman of the Program Committee and submitted to the Secretary of the United States Animal Health Association prior to thirty days in advance of the scheduled annual meeting dates).

ARTICLE VI—AMENDMENTS

The By-laws may be amended by a majority vote of the members of the Association present and voting at an annual meeting.

ARTICLE VII—ELECTION OF OFFICERS

All elective officers (Chairman-elect and Secretary-Treasurer) shall be nominated by a nominating committee appointed by the Chairman, and by nominations from the floor of the annual business meeting. The terms of office for the above shall be for one year following their election. Interim vacancies shall be filled by appointment by the Executive Committee and such appointees will serve until the next regularly scheduled business meeting.

ARTICLE VIII—DUES

The amount of registration fees and/or dues shall be determined by a two-thirds majority vote at any regularly scheduled annual business meeting.

ARTICLE IX—STANDING COMMITTEES

The Executive Advisory Council shall be composed of five members, one member from each of the four districts and the immediate past Chairman. The four district members shall have staggered terms of office not to exceed four years each. Vacancies are to be filled through appointment by the Chairman. The immediate past chairman’s term of office shall be for one year. The duties of the council is to act as an advisory administrative body to the Chairman and actively implement the AAVLD goal to achieve the establishment of a National Reference Assistance Laboratory for State-level veterinary diagnostic laboratories.

ARTICLE X—MINIMUM STANDARDS FOR VETERINARY DIAGNOSTIC LABORATORIES

The Association shall develop minimum standards for the certification of veterinary diagnostic laboratories.
CHAIRMAN'S MESSAGE

Vaughn A. Seaton, D.V.M.

CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS
New Orleans, Louisiana
October, 1968

I welcome you to New Orleans and to the eleventh Annual Meeting of the CVLD. The initial portion of our program is set aside to provide the Chairman an opportunity to discuss items which he deems of interest to the CVLD. Immediately, I'm reminded that we may have a new name in a minute, should you as members of this organization vote to confirm the name change recommended last year at the Annual Meeting in Phoenix. As you may recall, the name accepted last year was the American Association of Veterinary Laboratory Diagnosticians. We shall have an opportunity this morning to confirm that action of one year ago.

I would like to report to you some of the events of this last year, - what items of business have been discussed and what proposals might be considered for our mutual betterment. It is my opinion that over the last ten years, since this organization was started, that the CVLD has come a long way. As is true in any worthwhile venture, there are successes and failures, ups and downs, and often considerable frustrations, and yet I believe we have traveled along rather quickly.

This group is (1) now recognized as the organization which represents and in general, speaks for Veterinary Diagnostic Laboratories over the United States; (2) this organization is asked for advice on all types of technical matters concerning laboratory diagnosis; (3) we are asked to set up recommended procedures for certain laboratory diagnostic functions; (4) we are expected to conduct surveys as to diagnostic functions available over the United States and to assist in the evaluation of diagnostic laboratory competence as a whole.

In the last year, I've experienced this phenomenon of the CVLD being looked to for advice, answers, recommendations, and being looked to for leadership in many areas of diagnostic veterinary medicine. This is good; it is welcomed. And as far as the maturity of the CVLD is concerned, it is a sincere compliment. Let me caution, however, that it also demands some wisdom and judgment and real leadership. It is my hope that the efforts of the officers and membership over the last ten years can be further "built upon" to provide that "extra effort" necessary to excel.

Last year in Phoenix, a set of minimum standards for Diagnostic Laboratories was adopted. This important step was a milestone in the efforts to assist Laboratories to improve themselves and provide impetus to a general upgrading. It was, of course, recognized that "standards" alone are a rather "sterile" approach unless some method exists to implement them or at least encourage laboratories to be able to meet the "standards" which we adopted.

Therefore, Laboratory certification appeared to be a way to provide incentive
and motivation to meet the standards. I was instructed as the new Chairman of this organization to work with the Executive Advisory Committee to study the implementation of a system for Laboratory Certification. We have met twice since the Phoenix meeting – once in Chicago at the AVMA office and once in Boston at the AVMA meeting. We do not yet have a final plan in mind as to how all the details can be carried out but real progress has been made and I believe we can very soon embark on a voluntary laboratory certification program. I am asking that the members of the Executive Advisory Committee who have worked on this problem during the last year be named to a Committee on Laboratory Certification to insure continuity of effort and to maintain the momentum already initiated. Questions concerning funding, site visits if necessary, when and how, and other rather mechanical problems must be resolved. I would charge this committee to proceed full speed on these items.

Recently, Dr. Sipples' committee circulated a questionnaire which many of you have answered and about which Dr. Pope will comment in Dr. Sipple's absence. The purpose of this questionnarie was to gain some insight into current diagnostic capabilities over the United States as a whole.

Other activities of the year included the appointment of two Hog Cholera Committees. It seemed advisable as the Hog Cholera eradication programs progress to have recommended diagnostic procedures in mind for the tissue culture-fluorescent-antibody diagnostic technique (the indirect method) and also recommended procedures for the tissue fluorescent antibody technique (direct method). We may soon be arriving at the stage where the diagnostic competence of individual laboratories could be questioned as it becomes increasingly more difficult to diagnose the less typical and harder to find cases. With this in mind, I felt it prudent for the CVLD to study and recommend acceptable fluorescent antibody procedures which could be followed by laboratories which so desire. Both of these committee reports will be presented this morning.

Last year, as you recall, the CVLD Committee on Recommended Procedures for the Isolation of Salmonella Organisms, which was chaired by Dr. Grumbles, reported their recommendations. It is my understanding that those recommendations are now in use and distributed rather widely by the USDA as the official recommended procedure. It has been suggested that the isolation and identification techniques be studied on a continuing basis and therefore I have again appointed that committee to function under the leadership of Dr. Grumbles.

Again, in an effort to evaluate diagnostic techniques of interest to the CVLD, Dr. Lundholm was chairman of a committee to evaluate fluorescent-antibody diagnostic techniques for rabies dianosis. He will make a report this morning.

As for future committees, I am recommending that our new Chairman, Dr. Bedell, should appoint a committee to study and recommend ways and means to promote Diagnostic Laboratory competence and provide assistance in the area of wildlife diseases. In many states, there is great need for diagnostic assistance in this area. The wildlife people are interested and want assistance and are publishing a journal called "Wildlife Diseases." I believe such a CVLD committee could catalog diseases common to wildlife, domestic animals, and man, serve as a reference source for zoo veterinarians and in general promote veterinary diagnostic medicine in wildlife.
I am also recommending that a committee be established to study foreign animal diseases and biological safety with a specific interest in how diagnostic laboratories should react to suspect foreign animal diseases, who should be alerted to these possibilities, how specimens should be collected, preserved, and transmitted. Should foreign animal diseases occur in the United States, I believe they will likely be discovered first by diagnostic laboratories rather than by practicing veterinarians or regulatory officials outside ports of entry and quarantine stations. We, as diagnostic laboratories, should be better informed on our responsibilities in this area.

One item of business which will be presented today involves a constitutional change. It has been my observation in my year as Chairman-Elect and this year as Chairman, that one of the CVLD's prime needs is for a membership of some identity and of some stature. We now have individual memberships on a rather loose basis which is determined primarily by whomever attends an annual meeting. There is merit to this arrangement and I propose that we keep individual membership on that basis. However, in order to arrive at an organizational basis for membership, I propose that we change Article IX of our constitution to form an Executive Board which would be legislative in function and which would be comprised of one delegate from each State in the Union. This, I believe, would provide a truly legislative body and at the same time provide a demonstrable membership comprised of laboratory diagnosticians from each State. It is recommended that a delegate, or member of the Executive Board, be designated by whatever means desirable by the diagnostic laboratory personnel in each respective state.

Finally, I wish to report that for some time it has seemed desirable that the World Veterinary Association which meets each four years should have a section on their program devoted to Diagnostic Veterinary Medicine. Surely the problems which concern the World Veterinary Association must include some of those of under-developed nations and what I consider to be their primary need for diagnostic facilities, personnel, techniques, and procedures. I sincerely believe that the CVLD has a lot to offer to the World Veterinary Association by the contributions of our members. I am making representation in the World Veterinary Association for a section on their program entitled “Diagnostic Medicine.” To date, the matter is being studied by them and our efforts in this endeavor will continue. The next meeting of the World Veterinary Association will be in Mexico in 1970. I sincerely hope we may be successful in gaining representation on that program and in that organization.

Gentlemen, I have rambled along on current topics which I feel are pertinent to our organization and to Diagnostic Laboratories.

I have greatly enjoyed these two years and have great confidence in the CVLD and its membership.

I wish to thank Dr. Lundholm, who preceded me in the office, for his help, and thank Dr. Bedell for his assistance this year. He has been entirely responsible for the scientific program which we will be hearing today and tomorrow.

I wish to acknowledge a great amount of time, patience, and assistance from our Secretary-Treasurer, Dr. Ed Pope. His chores are monumental and he carries them off in an admirable manner.
RECOMMENDED MINIMUM STANDARDS FOR DETECTING HOG CHOLERA VIRAL ANTIGEN BY THE FLUORESCENT ANTIBODY TISSUE SECTION TECHNIQUE

David Bedell, Jackson, Mississippi, Chairman; H. A. McDaniel, Ames, Iowa; C. D. Clark, Harrisburg, Pennsylvania; A. P. Grey, Manhattan, Kansas; John Aikin, Glenvil, Nebraska.

Specimen Collection and Shipment

Tonsil, spleen and cervical lymph nodes are the tissues of choice in most instances. When chronic hog cholera is suspected two to three inches of the terminal ileum should also be collected. When dead or sick animals are not available for necropsy, tonsil biopsies should be collected. Tonsil biopsies are ideal for this procedure. When other tissues cannot be obtained one inch ear tips may be submitted. Additional tissues such as kidney, lung, adrenal gland and other lymph nodes may be collected in special cases. Each piece of tissue should be placed in separate plastic bags and identified.

Tissues should be preserved by refrigeration if the tissue can be processed within eight hours after collection. If longer time is required the tissues should be preserved by freezing. Frozen coolant cans provide adequate cooling for refrigerating specimens in transit, but dry ice is necessary for shipping frozen specimens.

Procedure for Conducting Test

1. Trim specimens so the tissue blocks are approximately 1/8 inch thick. These blocks are frozen on a freezing microtome chuck using OCT compound* or water to adhere the tissue to the chuck and to form a supporting matrix collar around the tissues.

2. Cut frozen sections eight microns thick. Tissue sections should be mounted directly on glass slides from the freezing microtome blade. Frosted or plain glass slides may be used, however, all frosted slides have the advantage that the tissue sections are less likely to wash off during subsequent processing.

3. Mounted tissue sections are immediately fixed 10 minutes in reagent grade acetone at room temperature.

4. Fixed sections are flooded with anti hog cholera conjugate and incubated 30 minutes in a moist chamber. Conjugate should cover the entire tissue section. An applicator stick can be used to spread the conjugate over the section.

A simple moist chamber can be made from a 25 x 100 centimeter petri dish by placing two short pieces of an applicator stick and a moist ball of cotton on the bottom of the dish. The slide is placed on the piece of applicator stick to prevent capillary attraction adhering the slide to the petri dish.

*Ames Company, Division Miles Laboratory, Inc., Elkhart, Indiana.
DETECTING HOG CHOLERA VIRAL ANTGEN

5. Pour off the anti hog cholera conjugate remaining on the slide and wash thoroughly in buffered saline. Excellent results have been obtained by washing actively 10 minutes through three changes of carbonate-bicarbonate buffered saline, pH 9. Clean classware and buffer should be used for each case to make sure cells are not inadvertently transferred from one slide to another during washing. Carbonate-bicarbonate buffered saline may be prepared as follows:  

Preparation of Carbonate Buffer

Solution A - Na₂CO₃
Distilled water to make 1000 ml.

Solution B - NaHCO₃
Distilled water to make 1000 ml.

Theoretically, a pH of 9.0 should result from mixing 44 ml. of Solution A with 1000 ml. of Solution B, but experience has shown 800 ml. of Solution B (4.2% NaHCO₃) and 215 ml. of Solution A (5.3% Na₂CO₃) usually produce a buffer of approximately pH 9.0. The final pH should be checked on a meter and adjusted to pH 9.0.

Preparation of Carbonate-Buffered Saline

Carbonate-bicarbonate Buffer pH 9.0 1000 ml.
Sodium chloride 8.5 gm.

Keep buffered saline tightly stoppered when not in use. Prepare small amounts frequently rather than large amounts infrequently.

6. Rinse the slides quickly in distilled water. This step is necessary to remove buffer salts that would otherwise dry on the slide.

7. Apply coverslip using buffered glycerine to hold the coverslip over the section. (Buffered glycerine is prepared by combining 9 parts glycerine and 1 part carbonate-bicarbonate buffered saline pH 9).

Controls

The ever present possibility of unidentified fluorescing material in the tissue section and the possibility of degenerating cells absorbing some of the fluorochrome dye necessitates control sections. Two additional control sections should be cut and fixed in acetone.

Control sections are processed in the same manner as the test sections except one control section is flooded with equal parts of hog cholera antiserum and conjugate and the other is flooded with equal parts of normal porcine serum and conjugate. The antiserum or normal serum should be mixed with the conjugate before the solution floods the tissue section. This mixing can be carried out in separate glassware or on the slide adjacent to the tissue section. Subsequent incubation, washing, and cover-slipping are conducted in the same manner as for the test section.

It is not necessary to prepare controls from each piece of tissue processed. Routinely control sections are only prepared from tonsilar tissue. However, if tissue other than tonsil such as spleen or lymph node are found to be positive and tonsil is negative, controls should be carried out on the positive tissue.

Known hog cholera positive section should be processed with each case. This serves as a control for the procedure and conjugate as well as the microscope. Tissue sections known to be negative for hog cholera antigen should be processed any time it appears the fluorochrome in the conjugate may be producing undue nonspecific staining.

**Interpretation**

Typical fluorescing cells containing hog cholera antigen appear light green or apple green with the fluorescence confined to the cytoplasm. Usually several positive cells occur together to form plaques and individual positive cells are scattered throughout the tissue section. Occasionally only plaques or individual positive cells are observed.

Sections treated with undiluted conjugate have the greatest amount of fluorescence. Fluorescence is usually slightly reduced in the section treated with conjugate plus normal porcine serum. In the section treated with conjugate plus unconjugated anti hog cholera serum fluorescence should be completely blocked or significantly less than that seen in the section treated with normal serum plus conjugate.

In actual practice the most troublesome cases are those in which small focal areas in the tissue section exhibit dull fluorescence, but neither of the control sections exhibit any fluorescence. The cause for this reaction is unknown. However, it may be due to fluorochrome staining degenerating cells. Cases of this type may be interpreted as suspicious of hog cholera, but the term "positive" should be reserved for cases in which unconjugated anti hog cholera serum reduces or blocks fluorescence.

**Microscopic Examination**

Darkfield condenser and ultraviolet illumination are necessary. The eye pieces should be 10X. The objectives should be 10X and 25X. The condenser should be high dry darkfield. Oil immersion objective and condenser are not necessary, but may be used according to individual preference. The light source should be a HBO-200 mercury vapor lamp or equivalent. An OG 12 exciter and a blue absorbing barrier filter or the equivalent are satisfactory. An incandescent light source should be utilized to enable switching back and forth from UV to incandescent light. By utilizing both light sources in this manner it can be determined if fluorescence observed is due to light refractive unidentified fluorescing material in the tissue section. Nonspecific fluorescing material is frequently light refractive under incandescence light whereas fluorescence due to conjugated antibodies attaching to viral antigen completely disappears under incandescence light.
Source of Reagents

Diagnostic Services, NADL, is prepared to furnish conjugate, anti hog cholera serum, normal porcine serum and hog cholera positive tissues to any laboratory conducting diagnostic examinations for hog cholera.
RECOMMENDED MINIMUM STANDARDS FOR THE
ISOLATION AND IDENTIFICATION OF HOG CHOLERA
BY THE FLUORESCENT ANTIBODY-CELL CULTURE TECHNIQUE


INTRODUCTION:

The need for a standard technique for the diagnosis of hog cholera using cell culture technique is readily apparent. The fluorescent antibody cell culture technique (FACCT) developed by Coons, et al.,1,2 provides a rapid sensitive viral diagnostic tool in those instances where it has been applied.5 This technique has already been described for hog cholera3,4,6,7 and is presently in use by some veterinary diagnostic laboratories in the national hog cholera eradication program.

It is the purpose of this paper to incorporate these procedures and establish a standard acceptable method for the isolation and identification of the hog cholera virus.

PROPAGATION OF PK 15 CELL LINE:

If standardization of procedures is desirable, there is no better way than to start with standardized supplies and reagents produced under more rigid quality control specifications than would be the case in individual laboratories.

Reliable dehydrated or complete culture mediums for use by bacteriologists has long been commercially obtainable. Very few bacteriologists, no matter how self-respecting, would seriously consider the routine production of culture mediums "from scratch" in their own media kitchens. The commercially available products are competitively priced and routinely more reliable than those the individual can concoct. They have also provided a significant influence in the direction of standardization of culture methods.

The same argument can be applied in favor of using uniformly processed standardized cell culture media, cell culture containers, and auxiliary supplies and reagents.

A tissue cell culture which will propagate hog cholera virus is required. The PK cell line (Cutter) is recommended. The following method for care of the PK 15 cell line has been used: Eagle's medium with Earle's salts or Earle's balanced salt solution may be used for propagation of PK 15 cells - with either medium .5% lactalbumin hydrolysate and 10% bovine calf, or lamb serum, free of bovine virus diarrhea antibodies, are added. Contaminant inhibitors per ml. of final medium include the following: Buffered penicillin G potassium, 100 units, crystalline dihydrostreptomycin sulfate -.1 mg., fungizone - 2.0 mcg., and kanamycin -.1 mg.

Good quality glass and non-toxic stoppers should be used. For propagation of the PK 15 cell line disposable prescription bottles may be rinsed in ion exchange water, used once, and discarded. The Leighton tubes employed for coverslip tissue cultures should be made of hard glass and if washed properly can be used repeatedly.
Sterile disposable plastic culture bottles are available and may be utilized. A number of suitable detergents are available for cleaning tissue culture glassware.

The maintenance of the PK 15 cell line involves the continuous passage of cells in prescription, milk dilution, or plastic bottles, 4-8 oz. size, and volume production of cells in bottles for transfer to Leighton tubes to grow coverslip preparations. The PK 15 cell line should be passaged regularly at least twice a week, as soon as the cell layers in the bottles become confluent. Leighton tubes may be seeded for coverslip tissue cultures at this time. A liberal supply of clean sterile glassware, capped with aluminum foil, should be kept on hand.

To remove cells from the glass, versene, or a versene-trypsin, solution is used. The tissue culture medium is poured off the bottles and the cell sheet is washed once with a small volume of versene solution, 2 ml. for 4 oz. prescription bottle. And sufficient versene solution, 8 ml., for 4 oz. prescription bottle and incubate for 5 to 15 minutes at 37°C. The cells should detach readily with light shaking, but occasionally longer periods of incubation are required. The cell suspension is pipetted gently to disperse the cells and centrifuged at 600 to 800 rpm for 10 minutes on a 20 cm. head without refrigeration. The supernate is discarded and the cells are resuspended with gentle pipetting in a small volume of culture medium supplemented with lactalbumin hydrolysate and calf serum.

The cell suspension is diluted out in the freshly prepared medium so that the harvest of cells from one 4 oz. prescription bottle will be used to seed three new bottles or approximately 36 - 45 ml. However, for seeding coverslips in Leighton tubes, a more concentrated cell suspension is prepared by diluting out the cell harvest from one 4 oz. prescription bottle to 24 - 30 ml. The Leighton tubes vary in size and should receive from 1 - 2 ml. of cell suspension. Experience will help determine the correct seeding rate for producing good coverslips.

The bottles and Leighton tubes should be stoppered tightly (twisted in individually) and placed in an incubator at 37°C which is relatively free of vibration. Upon examination the following day, small patches of growing cells should be observed. The cells should produce a confluent growth by the third or fourth day. The medium should be discarded on the third day and fresh medium, as described above, added to the cultures. It may be advisable to reduce the calf serum content to 5% if the cells will not be used in the next couple of days. The prescription bottles having confluent growth on the third or fourth day should be passaged. Leighton tubes with coverslips may be used for inoculation as soon as the cell sheet approaches confluency. If two sets of coverslips are available, the most recently prepared set should always be used.

After PK 15 tissue culture is established an effort should be made to store some cells in the freezer for reserve. For optimum storage, place in liquid nitrogen or at -70°C. This technique involves preparing a suspension of the cells as if for passage. The cell suspension is resuspended at one-tenth of the usual seeding volume and mixed with 20% pure glycerine. Freeze rapidly in an alcohol-dry ice bath and store at -70°C or in liquid nitrogen. For reconstitution, thaw rapidly at 37°C in water bath and dilute out one to eight in medium. Centrifuge cell suspension, discard supernate, resuspend in medium, and seed heavy in prescription bottles. Another method of cell storage is to place a bottle of cells in a 5°C refrigerator. They will survive up to 30 days without passage.
PREPARATION OF INOCULUM:

Approximately 10 gm. of splenic tissue is placed in a chilled blender jar containing 25 - 30 ml. of cold (4°C) cell culture medium. The medium is identical in composition to that used to propagate cell cultures for FACCT. (Contaminant inhibitors may be increased three fold.) The mixture is blended for 4 to 5 minutes and then centrifuged for 5 minutes at 3000 rpm.

INOCULATION OF CELL CULTURES:

Medium is decanted from the Leighton tube cell cultures to be used, and each of 4 cell cultures is inoculated with 2 ml. of inoculum prepared from each sample. After one hour's incubation at 37°C to allow for adsorption of the hog cholera virus, the inoculum is decanted and cultures are washed several times with medium to remove any remaining debris. Following the addition of 2 ml. of fresh medium to each of the cultures, they are incubated at 37°C.

STAINING OF COVERSILIP:

Coverslips are examined following 24 and 48 hours incubation. The cover glasses are removed from the Leighton tubes by sliding the end of a spatula or bacteriological loop under them and pulling them to the edge of the tubes. Each coverglass is grasped at the edge by forceps or attached to wooden applicator sticks and processed as follows: Appropriate methods, such as the use of separate glassware for each specimen to prevent cross contamination is recommended; dip in buffered saline (pH 7.0-7.2), fix in acetone for 5 - 10 minutes, dry thoroughly, flood with hog cholera conjugate (supplied by NADL, Ames, Iowa) and incubate in a moist chamber at 37°C for 30 minutes, shake off conjugate and dip in buffered saline, wash for 3 minutes in buffered saline, dip for 5 minutes in distilled demineralized water, dry thoroughly, mount on slides, cells downward, with 50% buffered saline and 50% glycerine.

EXAMINATION OF COVERSILIP:

The following microscope and accessory equipment are recommended and are suggested as appropriate without, in any way, detracting from the suitability of other microscopes:

a) Ortholux — (E. Leitz Company, 468 Park Avenue, South, New York, N. Y.) with 200 watt Mercury vapor lamp, HBO200, and incandescent lighting so that the two light sources can be interchanged without disturbing the position of the specimen slide.

b) Dry Darkfield Condenser for use without oil between the surface of the slide and the top lens of the condenser. (No. 84, OREBK - WINE).

c) 10X Achromatic Objective.
d) 25X Achromatic Objective.
e) 10X Periplanatic Oculars (wide field).
f) Filters for ultraviolet-blue fluorescence.

   KG-1   Clear (heat absorbing - in lamp housing) 2 mm.
   BG-38  Light blue (red filter) 4 mm.
BG-12 Dark blue (exciting filter) 3 mm.
OG-1 Yellow (barrier filter) for oculars.

The microscope should be located in a dark room and the observer should allow his eyes to become adjusted before examining the preparation. Under ultra-violet-blue light with the darkfield condenser, the negative cell sheet will be a dark brown or dark green. Positive cells which are infected with hog cholera virus will be bright green (apple green or chartreuse) and will stand out sharply against the darker, negative surrounding cells. The nucleus does not stain and appears as a black hole in the center of the cell. The outline and protoplasmic extensions of the infected cell will be well demarcated. Occasionally cells will show a non-specific fluorescence which will have a blue-gray color with a lower intensity. This non-specific fluorescence may be due to dead cells or toxic cells with cytoplasmic granules. To differentiate this non-specific fluorescence the incandescent light source is switched on the preparation. If the fluorescence observed in a cell is specific, the cell will fade into the background and appear no different from its surrounding cells when viewed by incandescent light. However, cells having non-specific fluorescence will appear brighter and stand out from the neighboring cells in incandescent light. In this case many cells should be examined until some positive cells are found which do not give a bright appearance with the incandescent light.

The entire coverslip should be scanned systematically beginning at the upper left hand corner and proceeding back and forth. Suspicious cells should be checked under high power, 25X, and with the incandescent light source.

DISCUSSION:

The described procedure is intended to only be a simplified version of the FACCT. It is somewhat a sophisticated technique, yet practical enough for use in the average veterinary diagnostic laboratory. Difficulties encountered may be resolved by reviewing procedures described in literature or consulting other personnel involved in this type testing. The use of positive controls employing a laboratory adapted culture of hog cholera virus will assure an accurate testing procedure.

REFERENCES

PROBLEMS ASSOCIATED WITH GROSS SPECIMEN PHOTOGRAPHY

H. C. Morgan, D.V.M., M.S.*

Some of our best diagnosticians dwell in obscurity because they lack interest, ability or equipment to photograph their findings. Many have expensive equipment but find it inadequate for gross specimen photography. The photographic industry must share the guilt for their members have been so competitive in recent years that the consumer has often been sold worthless equipment and been left in a confused state. Each company has "the camera" which will do "everything" but in this author's opinion such a camera does not exist and is not anticipated. However, having the best camera does not guarantee success. Other items of major importance are selection of film and type of light; clarity of background; and depth of field.

Camera

At the professional level nothing short of a single lens reflex should be acceptable. Amateur and range-finder cameras can be "adapted" but a compromise in product is usually the result. Nothing can replace the camera which views the specimen directly without parallax or distortion. As for size, the 35 mm. is the most practical to use.

Several 35 mm. cameras are acceptable for gross specimen photography but probably the most versatile and widely used has been the Exakta. Since it was first produced in 1936 it has contributed more to gross specimen photography than any other camera. Originally the latest model Exakta would sell for more than $300 but competition has forced the price down to approximately $150. The standard lens for the Exakta is 50 mm. so if it is necessary to get closer than 18 inches to the subject extension tubes or portra lens must be used. A special lens (55/1.9 Macro Steinheil Quinar Auto) can be adapted to the Exakta and will permit focusing to 1 1/2 inches without use of tubes. Unfortunately the cost of the lens alone is $150.

In recent years many new single lens reflex cameras have followed the lead made by Exakta. Those which seem to offer the most advantages are the Alpa, Pentax, Minolta, Leicaflex, Nikon and Nikkormat. When gross specimen photography is of primary interest the best of these appear to be the Nikon or Nikkormat FTN with a 55/3.5 Micro Nikor lens. This lens allows focusing to 9 inches without adaptation and permits 1:1 reproduction with an M-tube adapter. Cost of the camera and lens is approximately $300. The camera has a built-in light meter, through-the-lens focusing and almost fool-proof operation.

*Dr. Morgan is Associate Professor; Dept. of Pathology and Parasitology; and Institute of Comparative Medicine; School of Veterinary Medicine, University of Georgia, Athens, Ga.
Selection of Film and Lighting

Film and lighting must be considered together and every effort should be made to achieve the proper color balance. The simplest combination is Kodak's Kodachrome II Type A Professional film (KIIA) with standard photoflood (3400°K) lighting. Use of Type A film has an added advantage since addition of an 85 filter will permit its use in daylight on field trips. Use of daylight film with filters under photoflood is possible but not as satisfactory. The most satisfactory lighting is achieved with two photofloods placed at 60-75° angles about 42 inches from the specimen.

Clarity of Background

The background should not have defects that will detract from the specimen. A photo table should mainly be used for small organs and specimens. Larger organs should be photographed in situ. When colored backgrounds are used a color should be selected which is not present in the tissue. Green seems to have the greatest application.

When glass is used considerable care must be taken to insure that the specimen is clean and free of excess fluid. Movement of the specimen after it is placed on the glass usually results in a smeared background.

Another defect encountered with glass backgrounds is camera reflection. Cameras can be purchased which have no chrome but these usually cost extra. When shiny cameras are used two steps may be taken to reduce the effect. First, no-glare or Tru-Site glass should be used to support the specimen. Some "light spots" will still be evident but it will not be a well-defined reflection. Secondly, a black cardboard may be placed between the camera and table to prevent camera reflections. The bottom from an 8" x 10" box of Kodak printing paper is excellent color, strength and size. A hole is cut for the camera lens and it is attached to the lens with adapter rings, tape or by simply cutting for a "snug" fit.

Some pathologists prefer a transilluminated background (back lighting) but this adds tremendously to reflection problems. For routine work a transilluminated background is not necessary but is good for photographing materials suspended in fluids (fetus, inside membranes).

Depth of Field

Assuming standard procedures are used for determining proper exposure a decision must still be made concerning the desired depth of field. If specimens are shallow (e.g. intestine) then depth of field is not important and any compatible f stop:shutter combination will produce a satisfactory picture. However in close-ups of thick organs (e.g. heart, liver) it is necessary to use all possible methods in an attempt to get all the important areas in focus. A rule of thumb for this is to "shoot" with as high an f stop as is possible while refraining from slowing the speed to more than 1/5 second. Speeds slower than 1/10 second alter the color balance. (Note: Use of KIIA at 1/10 second requires the addition of a CCo5R filter; at 1 second requires CC10R and at 10 seconds requires a CC20R.)

Poor photographic depth of field is usually most obvious when a ruler or other measuring device is placed in the picture. Many times it is placed on the table and
the camera is focused on the prominent lesion several inches higher. The result is a blurred scale which distracts from what could be an excellent picture. To avoid this some type of stand should be used to place the ruler in the edge of the picture and at the same focal point as the subject of major interest.

Discussion

Many books and papers have been written on gross specimen photography so it would be foolish to attempt completeness in this short paper. In fact, after years of counseling individuals on their photographic problems the author is firmly convinced that the only way to learn is by “trial and error”. We always remember our great mistakes and try to avoid their reoccurrence. Loss of pictures documenting “the case” is a hard way to learn so the diagnostician should make his mistakes as early as possible on less important subjects.

Simple statements have been made concerning cameras, film and other subjects. The “best camera” has been suggested but any single lens reflex can be used satisfactorily. Excellent cameras have been left on the shelf while one of the “best” cameras is justified (?) for purchase on the proposed budget. Often “the best”, because of its complexity, simply shares the shelf. The point to be gained here is: Re-examine your holdings – run a few rolls of film through the “old” camera. This may prove to be much cheaper than a new camera.

Other than the cost of the camera, gross specimen photography is inexpensive. Time, energy and experience are necessary to produce consistently satisfactory results. However, necessary experience cannot be gained by reading, viewing or listening. Trial, error and patience are important steps on the road to successful gross specimen photography.
A Gross Specimen Table

A.C. MORGAN, D.V.M.

Purpose of raised glass is to cast shadows from under specimen.

Note: The safety glass is needed for strength in supporting tissues, but unfortunately is highly reflective. A non-glare or Tru-Site glass should be used under the individual tissue.

Stack of 8"x10" and 11"x16" non-glare glass plates can be very helpful when several tissues must be photographed.
CELLULAR IDENTIFICATION IN BODY FLUID ANALYSIS

L. H. Ellington, Jr., D.V.M.
Athens, Georgia

I. Identification of the types of cells frequently found in fluids from the body cavities.

A. Types of cells present

![Images of Mesothelial, Histocyte, Monocyte, Neutrophil, Lymphocyte]

B. Three general criteria for identifying the types of cells (Pleural and Peritoneal)

NOTE: These are very general criteria and are offered only as a foundation for cellular differentiation. One may become as proficient as he desires through repeated and careful examinations of all fluids in abnormal quantities and locations.

1. **Size of Cell**
   When attempting to identify a particular cell, first compare its overall cell size with other cells in the field (including erythrocytes). Are there cells that are larger than the cell in question? Are there any cells that are smaller? It is best to learn to recognize the relative size a particular cell type such as the neutrophil for purposes of comparison. Remember that, in most cases, there will be no cells larger than mesothelial cells and histiocytes (tissue macrophages) and no cells smaller than the lymphocyte.

2. **Segmentation or “Lobing” of the Nucleus**
   After noting the relative size of the cell, the nucleus should then be examined to see if it is “lobed” or segmented. One should note that, generally, the nucleus of the monocyte is “lobed” and the nucleus of the neutrophil is segmented. If the cell in question does not fall into this category then it logically must be a mesothelial cell, histiocyte (tissue macrophage), or a lymphocyte. It would be difficult to confuse the mesothelial cell and the histiocyte with a lymphocyte because of the size difference alone. One would not confuse a silver dollar with a dime! Thus the problem is now how to differentiate a mesothelial cell from a histiocyte as they are about the same size. This leads us to our final criterium.

3. **Presence of Vacuoles in the Cytoplasm (Phagocytosis)**
   After accomplishing the first two criteria, inspect the cytoplasm of the cell for vacuoles. The cell most characteristic of this criterium is
the histiocyte (tissue macrophage) and the tremendous number of vacuoles usually gives the cell a “moth-eaten” appearance. These large phagocytes are frequently found to have engulfed lymphocytes, neutrophils, and even monocytes. The other cells that may have vacuoles are the monocyte and the neutrophil.

C. Summary of identifying features of the five cells commonly found in body fluids.

1. **Mesothelial Cell**
   a. General
   1. Size - large
   2. Nucleus - not “lobed” or segmented
   3. Vacuoles - none
   b. Specific
   1. Round cell.
   2. Nucleus - single or double and in center of cell

2. **Histiocyte (tissue macrophage)**
   a. General
   1. Size - large
   2. Nucleus - not “lobed” or segmented
   3. Vacuoles - abundant
   b. Specific
   1. Nucleus small in relation to total cell size and usually located next to cell wall

3. **Monocyte**
   a. General
   1. Size - intermediate
   2. Nucleus - "lobed"
   3. Vacuoles - few
   b. Specific
   1. Cytoplasm appears gray

4. **Neutrophil**
   a. General
   1. Size - intermediate to small
   2. Nucleus - segmented
   3. Vacuoles - few (if cytoplasm visible)
   b. Specific
   1. Cytoplasm usually not visible

5. **Lymphocyte**
   a. General
   1. Size - small
   2. Nucleus - not “lobed” or segmented
   3. Vacuoles - none
   b. Specific
   1. Nucleus occupies most of cytoplasm

D. Suggested Reading

INTERPRETATION OF FLUID ANALYSIS

A. TRANSUDATE*

1. SP. GR. - 1.017 OR LOWER
2. TOTAL PROT. - 3% OR LOWER
3. RELAT. ACELLULAR
   A. PREDOM. LYMPHOCYTES AND MACROPHAGES (HISTOCYTES)
4. COLORLESS TO PALE YELLOW
5. CLEAR (USUALLY)
6. CAUSES:
   A. PASSIVE CONGESTION
   B. VASCULAR OR LYMPHATIC OBSTRUCTION
   C. HYPOPROTEINEMIA
   D. CHRONIC ANEMIA

B. EXUDATE*

1. SP. GR. - 1.018 OR HIGHER
2. TOTAL PROT. - GREATER THAN 3%
3. VERY CELLULAR
   A. USUALLY NEUTROPHILS-ACUTE
   B. POSSIBILITY OF HISTOCYTES IN CHRONIC INFLAMMATION.
4. YELLOW TO RED
5. TURBID
6. CAUSES:
   A. BACTERIA
   B. TRAUMA
   C. MISC.

C. ABNORMAL PRODUCTS*

1. BLOOD
   A. WILL CLOSELY APPROXIMATE VALVES OBTAINED FROM PERIPHERAL BLOOD e.g. PCV, DISTRIBUT. OF WBC'S ON STAINED SMEAR, ETC.

2. CHYLE
   A. MILKY: POSSIBLY PINK DUE TO PRES. OF RBC'S.
   B. APPROX. 90% SM. LYMPHOCYTES.

3. BILE
   A. YELLOW-GREEN
   B. SEVERE IRRITANT; NEUTROPHILS; ICTOTEST

4. URINE
   A. MONONUCLEAR CELLS
   B. WARM AND CHECK TO SEE IF AMMONIA IS LIBERATED.

5. GASTRIC OR INTESTINAL CONTENT
   A. INGESTA; NEUTROPHILS

CELLULAR IDENTIFICATION

D. DIFFERENTIATION OF NEOPLASTIC CELLS

1. ON CELL TYPE:
   A. LYMPHOSARCOMA
   B. MAST CELL TUMOR (METASTASIZED)
   C. (MELANOMA-METASTASIZED)
   D. (HEMANGIOSARCOMA-BLOOD)

2. ON CELLULAR GROUPING (“BALLING UP” OF CELLS)
   A. CARCINOMAS - MOST LIKELY ADENOCARCINOMAS
      1. OVARY, PROSTATE, BILE DUCT, MAMMARY GLANDS,
         PHYLORUS, INTESTINES, AND BRONCHI
   B. MESOTHELIOMA

3. OTHER NEOPLASMS THAT MAY BE PRESENT:
   A. OSTEOSARCOMA, CHONDROSARCOMA, SERTOLI CELL TUMOR
OUTBREAK OF MALIGNANT CATARRHAL FEVER IN KANSAS

A. P. Gray, D.V.M., Ph.D., and
H. D. Anthony, D.V.M., M.S.

INTRODUCTION

The purpose of this paper is to report the occurrence, clinical signs and pathologic alterations of malignant catarrhal fever (MCF) in a herd of Kansas cattle. This disease has been recognized in many countries of the world. Roderick reported on the occurrence, clinical signs, pathologic changes, transmission, and cattle and sheep association. He also cited the reports of numerous investigators throughout the world. Gross and microscopic lesions were described and discussed in detail by Jubb and Kennedy.

In an experimental investigation by Plowright et al., an agent or agents isolated from several wildebeest produced a disease in cattle similar to, if not identical to, MCF when inoculated into susceptible bovine. They believed the isolates were viruses since passage of agents was possible on cell monolayer with cytopathogenic effect on the ninth day. Transmission of the disease was successful using either bovine heparinized blood or lymph gland suspension.

Blood described and discussed the field procedures essential for the differential diagnosis of mucosal diseases in cattle, including the head and eye and also the intestinal forms of malignant catarrhal fever.

FIELD INVESTIGATION

Investigation of the Kansas outbreak revealed that a young farmer, over the past several years, was in the process of establishing a herd of Charolais cattle. However, at the time of the outbreak, the herd also contained animals of other breeds. A total of 67 head of cattle constituted the herd, with ages ranging from young calves to adult animals several years of age. Ninety-five head of adult sheep, some of which lambed during late winter and early spring, were housed or pastured with or in close proximity to the cattle.

Eleven animals, all under two years of age, died during late winter and spring months during this outbreak. Three young calves that died were not examined or necropsied by a veterinarian. However, according to the owner's description, clinical signs of all three unexamined calves were similar to clinical signs of other necropsied, in which gross and microscopic lesions were typical of MCF. Three additional animals were destroyed by the owner, after treatment by local veterinarians failed to produce improvement in the health of the individuals. The remaining five head were either necropsied by local veterinarians or by personnel of the Veterinary Diagnostic Laboratory. Typical gross and microscopic lesions of malignant catarrhal fever were demonstrated in all five animals.

*Veterinary Diagnostic Laboratory, Department of Pathology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas.
CLINICAL SIGNS

The first clinical signs of illness in the animals detected by the owner consisted of sudden onset of inappetence, noticeable lacrimation of both eyes, increased respiration and standing alone or away from other cattle. During the next several days the previous clinical signs became more pronounced and additional signs consisted of slight to complete corneal opacity beginning at limbus of both eyes. Increased lacrimation discolored the hair of the face and photophobia was observed in some of the infected animals. Hypersensitivity was noted when the back was touched and the animal would jump, followed by staggering and inability to maintain its equilibrium for a few minutes. A slight to copious mucopurulent nasal discharge was present in all of the animals with MCF. Temperatures were elevated from 105 to 108°F at various times during the illness. All eleven animals died, or were disposed of, between the third and seventh day of illness.

In the terminal stages the sick animals would stand dejected, away from other animals, head extended, with labored and rapid respiration, and no desire for feed. A hemoglobinuria was noted in the last animal to die.

NECROPSY EXAMINATION

The gross lesions found during necropsy of two animals by Diagnostic Laboratory personnel consisted of marked swelling of all the visceral lymph nodes. Examination of the digestive tract revealed a gastroenteritis characterized by congestion, resulting in a diffuse reddening of the mucosa of the abomasum and small intestine. A large part of the mucosal surface was covered with a gelatinous mucoid exudate. Ulcers were not detected in these organs; however, small focal areas of deep reddening indicated possibly small hemorrhages were present in mucosa of the abomasum.

Examination of the spleen revealed no obvious engorgement or extensive enlargement. The uncut surface of the liver and kidney revealed a grayish to white mottling in the subcapsular tissue. These changes were more striking in one animal than in the others.

Ventral parts of the anterior lobes of the lungs were deep reddish purple and indicated atelectasis in these parts. A frothy, foamy exudate was present in the large bronchi and trachea. No necrotic changes were detected in the epithelium of pharynx, larynx, and trachea.

Many small, irregular ulcers (Fig. 1), approximately 2 to 3 mm. up to 1 or 2 cm. in diameter, were present on the hard palate of one animal. Also, ulceration of the anterior dorsal surface of the tongue was noted in this animal. The muzzle was encrusted and the removal of the crust left a raw, denuded area.

External nares and turbinates were congested and deep red in color. From the ventral posterior turbinate to the pharynx, a mucopurulent plug of exudate appeared to completely fill the area. The plug of exudate was well formed with a glistening surface and was not attached to the epithelium. The purulent exudate, congestion and swelling of surrounding tissue were probably the primary causes for some of the labored breathing.

No striking gross lesions were detected in the brain other than severely congested meningeal vessels.
Fig. 1. Numerous variable sized ulcers on the hard palate of a 14-month old bovine with malignant catarrhal fever.

MICROSCOPIC LESIONS

Histopathologic studies of the major organs revealed alterations in the tissues characteristic of those described for malignant catarrhal fever and, on these findings, this disease was distinguished from other bovine diseases. Similar lesions were found in tissues from all animals examined. The only difference was in the amount of mononuclear cell infiltration in various organs. Cellular infiltration of brain, liver and kidney was more extensive in one organ than another and also varied between individual animals. The infiltrating cells were of various size and reported by Jubb and Kennedy\textsuperscript{2} to be of reticuloendothelial (RE) and lymphocytic origin.

Vascular lesions which Jubb and Kennedy\textsuperscript{2} described as pathognomonic for MCF were demonstrated in the brain, lung, spleen, liver and kidney. This vasculitis appeared to involve arteries of various size, most generally medium and smaller vessels. The changes were found to involve only part of some vessel walls, while in others all segments were disrupted. Pathologic changes of the vessel wall were similar to those described in equine viral arteritis.

Microscopic changes in the vessel consisted of fibrinoid degeneration in which the involved areas appeared to be composed of a homogenous eosinophilic material with interspersed nuclear fragments. The adventitia and muscularis media of some
vessels were also infiltrated with numerous mononuclear cells, some of which were also fragmented.

Massive infiltration of mononuclear cells were found in the examination of kidney sections from one animal. Areas of infiltration most generally were in the cortex of the kidneys. They were located around small muscular vessels and glomeruli (Fig. 2) which in some instances resulted in destruction of the tubules by pressure atrophy. Characteristic vasculitis (Fig. 3) was found involving numerous vessels and consisted of fibrinoid degeneration, nuclear disintegration and mononuclear cell infiltration of muscularis media.

Fig. 2. Mononuclear cell infiltration around vessels and glomeruli. Also note the infiltration of cells into interstitial tissue resulting in pressure atrophy of tubules.
Fig. 3 Vasculitis of artery in the kidney. Observe the infiltration of cells into the adventitia and muscularis media of the vessel. Note the nuclear disintegration of nuclei in muscularis media.

Fig. 4 Infiltration and proliferation of mononuclear cells in the portal triads of the liver. Vacuoles are also present in hepatic cells, probably indicating fatty changes.
Pathologic changes in the liver consisted of cellular infiltration around vessels and bile ducts of the portal triads (Fig. 4). Close observation of many of the small vessels revealed a vasculitis similar to those observed in slightly larger vessels of the kidney. Moderate fatty changes were also present in the hepatic cells. Destruction of parenchymal cells of the liver by cellular infiltration was not as extensive in sections of liver examined from various animals as in corresponding kidneys.

Examination of splenic tissue revealed a vasculitis of trabecular arteries (Fig. 5). These changes consisted of fibrinoid degeneration, nuclear fragmentation and vacuolation of muscle fibers in the muscularis media. There appeared also to be a disruption in the endothelium. Mononuclear cells also infiltrated the adventitia. No pathologic alterations were found in examination of other areas of the spleen other than the large number of immature lymphocytes in the white pulp and adjacent area.

Meningoencephalitis was a constant lesion in brain sections of animals examined. Microscopic studies revealed an infiltration and possibly a proliferation of mononuclear cells in the leptomeninges and around vessels of the brain parenchyma (Fig. 6 and 7). Many of the cells were exceptionally large, indicating they were not lymphocytes but possibly originated from fixed RE cells. A vasculitis was not as obvious as in other organs since many of the vessels do not have a wide muscular segment. However, vascular damage was evident as indicated by disruption of endothelium and the presence of an eosinophilic serum-like coagulum, especially
in the meningeal spaces. The remaining segments of the brain tissue were not altered other than early degenerative changes in some neurons, which could be expected when blood circulation was impaired.

Fig. 6. Meninges of brain with infiltration and proliferation of mononuclear cells and plasma-like exudate.
Atelectasis and an early bronchopneumonia was found in sections of anterior ventral lobes of some lungs. These alterations were not extensive and not found in all animals. A vasculitis similar to those previously described in other organs was also present in medium sized arteries of sections examined from two animals.

Many mononuclear cells were found in the lamina propria and submucosa of the small intestines and they were exceptionally extensive in some areas. Focal dilated crypts contained an eosinophilic homogenous material interspersed with degenerate and desquamated cells. Lymphoid tissue resembling Peyer’s patches contained some germinal centers depleted of lymphocytes, while other follicles contained large numbers of neutrophils indicating an acute lymphadenitis.

Fig. 7. Parenchyma of the brain with moderate cuffing of vessels with mononuclear cells.

SUMMARY

For an accurate diagnosis of malignant catarrhal fever, as well as other diseases, it is essential to have a good history that includes size of herd, morbidity, mortality, clinical signs, and gross lesions found at necropsy. Also, some diseases such as malignant catarrhal fever have microscopic lesions that are described as pathognomonic and, consequently, it is always helpful to have tissues for histopathologic examination.

Malignant catarrhal fever has a high mortality and low morbidity and only a few animals affected at one time which aids in distinguishing this disease from infectious bovine rhinotracheitis, mucosal disease complex, and para-influenza.
Microscopic demonstration of described pathognomonic lesions consisting of a vasculitis and meningoencephalitis further confirm a diagnosis of malignant catarrhal fever.

A diagnosis of malignant catarrhal fever in this outbreak was made on the basis of history, gross lesions and microscopic examinations. Microscopic lesions consisted of a vasculitis characterized by fibrinoid degeneration of vessel walls, nuclear fragmentation and cellular infiltration in vessels of the kidney, liver, spleen, lung and brain. Also, mononuclear cell proliferation and infiltration was observed in close aposition to vessels of above organs and intestines, which further substantiated the final diagnosis of malignant catarrhal fever.

ACKNOWLEDGMENTS

The author expresses appreciation to the local veterinarians, Dr. A. W. Brecheisen and Dr. J. J. Bailey of Garnett, Kansas, for their assistance during field investigation and collection of specimens.

REFERENCES

FOOT-AND-MOUTH DISEASE ERADICATION IN ENGLAND

By Keith Sherman, D.V.M.*

During the fall and winter of 1967-68, the livestock industry of England experienced its most extensive epizootic of foot-and-mouth disease (FMD). Previous infections with FMD has been promptly controlled by slaughter with minimal loss and spread of the disease.

The rapid spread of this epizootic was caused by many factors of which three were self-evident. First, the area in which the disease occurred has the densest animal concentration of any other place in England or possibly the world. Dairy cattle were predominant among the farm animals which included sheep, swine, and goats. Second, the virus, Type O1 was highly virulent and seemed to have an unusual ability to spread. All susceptible species were readily infected and the disease was transmitted from one species to another without difficulty. Third, it appears that several farms were infected simultaneously by the initial dissemination of the virus.

The first case of FMD was reported on the afternoon of October 25, 1967 from Shropshire County near the border between England and Wales. The disease spread rapidly through Shropshire and adjacent counties. One month later the peak incidence was found; 81 new infected premises were reported in a 24-hour period.

The merit of the slaughter program was questioned and vaccination was considered. Eradication would have been extremely difficult if not impossible if FMD vaccines had been employed. Therefore, regulatory officials decided to continue with the slaughter program.

Twelve veterinarians from the Animal Health Division, ARA, USDA, were detailed to England. The Director of the Animal Health Division believed the first-hand knowledge and experience gained would provide the United States veterinarians with extremely valuable training in the eradication of FMD.

The veterinarians were briefed at the American Embassy and granted membership in the Royal College of Veterinary Surgeons before performing official duties. They were assigned in pairs to FMD centers throughout the infected areas.

Temporary FMD centers were established where the infection was most prevalent and closed as soon as the disease was brought under control. All the centers were accountable directly to Ministry of Agriculture personnel at Tolworth. During the outbreak, over 20 FMD centers were established. A brief description of the most important operations performed by the different sections of a FMD center is as follows:

1. Supply — This section maintained materials and equipment needed by field personnel. Rubber wear, disinfectants, brooms, brushes, cleaning and disinfecting equipment, and numerous other supplies were stocked. This section

*From Pathology Section, Diagnostic Services, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa.
also furnished fuels for the cremation fires used to dispose of slaughtered animals. Large quantities of coal, railroad ties, rubber tires, straw and other materials used to burn carcasses were made available for fast delivery to infected premises.

(2) Equipment and Labor — Bulldozers, draglines, hydraulic back-hoes, tractors, wagons, portable light plants, were some of the heavy equipment items needed. These machines were leased from construction companies. Most of the labor force was obtained on loan from construction companies; however, some workers were supplied by the English military service. The equipment and labor requirements were extensive. At one center 35 newly infected premises were reported in one day. Several pieces of heavy equipment plus a labor crew were required for each premise. When large numbers of animals had to be slaughtered, labor crews and the equipment remained on the farm for a week or more until cleaning and disinfection was completed.

(3) Mapping and Licensing — Maps of areas surrounding infected farms were maintained. These maps were based on a national grid system and were maps of local areas which had been enlarged to provide detailed information. The boundary of each farm was shown as well as the shape and size of each field on the farm. The mapping section attempted to account for every farm and field, and to provide information on which fields held animals and which ones were vacant or under cultivation. When infection with FMD was detected on a premise, the boundaries of the farm were outlined on the map. Animals on contiguous farms were inspected daily. If a permanent natural barrier such as river or public road separated the infected premise from the adjoining premise, it was not considered contiguous. A large force of veterinary officers were required to adequately inspect animals on contiguous premises. Additional officers located and inspected the animals on all other farms throughout the infected area. Another duty of the mapping and licensing group was to make recommendations as to which animals should be destroyed because of their proximity to the infected premise.

Once an area was designated as infected, all animals were immediately quarantined and a license was required to move any animal. Although many farmers had animals ready for market and others had animals located in remote pastures, a strict policy of no movement was maintained. Restrictions on issuing licenses to move quarantined animals to slaughter were reduced as warranted when the number of new cases decreased.

During the FMD campaign farmers were required by law to report any suspected disease condition to the police or to the local FMD disease center. When a report was received, a veterinary officer was dispatched to the farm where he made a thorough examination of the sick animals. If the clinical signs and lesions were positive for FMD, the premise was immediately quarantined. A strict protocol was followed. (Fig. 1).

Case history, clinical observations, and gross lesions were reported to headquarters personnel at Tolworth for official confirmation of the diagnosis. In general, the decision was based on whether animals had elevated temperatures and vesicles on at least two different locations of the body, such as tongue and foot. If vesicles were present in only one location, the veterinary officer might be requested to remain on the premise and reexamine the animal a few hours later. In questionable cases, another veterinary officer might be asked to examine the animal
When personnel at Tolworth confirmed the diagnosis of FMD, they authorized the veterinary officer to appraise and slaughter the affected animals immediately. Arrangements for the destruction of the remainder of the herd were made through the local FMD center which assigned equipment and personnel to the farm. The local police were notified and an officer was assigned to the farm. He stayed near the main gate and controlled movement on and off the premise. The officer required anyone leaving the farm to follow strict disinfection procedures and permitted no one to enter unless they had permission and wore rubber clothes that could be readily decontaminated.

Animal appraisers were detailed to the farm by the FMD center. Animals to be slaughtered were appraised and indemnities were paid to owners on the basis of their market value. Registered animals were appraised in accordance with their value as breeding stock. The veterinary officer accompanied the appraisers when they evaluated the animals.

Commercial slaughterers killed the remaining susceptible animals on the farm with pistols, rifles or captive-bolt guns. The euthanasia was performed in barns or holding pens. The veterinary officer supervised the procedure to make certain that the animals were destroyed humanely and that each animal was pithed to assure death. He also supervised the disposal of the animals. As soon as the animals were buried or placed in position for cremation, cleaning and disinfection procedures were initiated. The veterinary officer supervised the decontamination unless a lay field officer was assigned to take over at this point. After the cleaning and disinfection operation was completed, a representative from the FMD center inspected the premise to make sure a thorough job was done.

Although the veterinary officer on the infected premise was responsible for all phases of the eradication duties, he was greatly assisted by an efficient FMD center that dispatched equipment, supplies, and personnel to the farm in an orderly sequence. When the veterinary officer completed his work at the farm and returned to the FMD center, his automobile and equipment were washed and disinfected and his clothing dry cleaned. Every precaution was taken to assure that FMD virus would not be carried to a new premise.

Two methods were employed to dispose of the dead animals, cremation and burial. Burial, which was employed whenever possible, was less expensive and could be completed more rapidly. However, burial was often made impractical on some premises by heavy rock formations, sandy soil, low marsh land, and the danger of contaminating public water sources. Cremation was most costly since large amounts of fuel were required. An unofficial estimate of $25 was given as the unit cost of burning one large cow.

When the animals were disposed of by burial, a deep hole rather than a long, shallow trench was preferred. Digging machines such as back-hoes and draglines were used. The hole had to be large enough to hold the entire herd with at least 6 feet of soil covering the carcasses. The abdominal cavity and rumen were opened before burial to prevent bloating. The infected animals were placed at the bottom of the burial pit. All burial pit locations were recorded by the mapping section and were periodically covered with fresh soil as settling was produced by carcass decomposition.
When disposal was by cremation, 3 feet of fire bed was required for burning each cow. A narrow, draft trench was dug along the center of the fire bed to allow air to circulate beneath the burning carcasses and fuel material. Railroad ties were placed across this trench at approximately 18 inch intervals. Baled straw was packed tightly on the ties and was covered with a layer of used rubber tires. Ten to 12 inches of coal was placed on top of the ties and a magnesium powder that burned with intense heat when ignited was spread over the coal. The animals were lifted and placed very carefully on the prepared fire bed to get maximum utilization of the fire. Cattle were placed on the fire bed with their feet in the air and calves, sheep, and pigs were placed between the legs of the cattle. When all the animals were on the fire bed, they were covered with loose straw, soaked with diesel fuel, and ignited. The fire was tended continuously, 24 hours a day, until cremation of the herd was complete. The fire usually burned for 2 to 4 days, but more time was required for cremation of a large herd of cattle. When the fire burned out, a hole was dug nearby and the ashes were buried.

Summary

The English veterinarians based their eradication program on sound principles which included early clinical diagnosis of the disease, effective quarantine methods, immediate slaughter, rapid disposal of the herd, and thorough cleaning and disinfection of the infected premise. Competent personnel, adequate equipment, educated and cooperative livestock owners, and sufficient funds to support the program were required for such drastic eradication procedures. The English have proved again that FMD can be eradicated by slaughtering all infected and exposed susceptible animals.

Veterinary diagnosticians throughout the United States should be alert for the signs and lesions of FMD. Prompt reporting of suspicious cases is essential to protect our livestock industry.
FIGURE 1
Flow Chart of Duties Performed by the Veterinary Officer and Support Functions of Foot-and-Mouth Disease Center and Head Office at Tolworth.

HEAD OFFICE TOLWORTH

1. Confirms diagnosis.
2. Assigns identification number to premise.
3. Authorizes appraisal and destruction of sick animals.
4. Authorizes appraisal and destruction of all susceptible animals on the premise.

SEQUENCE OF EVENTS ON FMD INFECTED PREMISE

1. Farmer reports.
2. Veterinary officer assigned from pool.
3. Examine animals.
4. Diagnosis of FMD.
5. Complete official forms.
6. Telephone report.
7. Appraise and slaughter sick animals.
8. Accompany appraisers.
10. Pith animals to assure death.
11. Supervise burn or burial.
12. Appraise and destroy contaminated feed.
13. Supervise cleaning and disinfecting.
14. Veterinary officer returns to pool for reassignment.

FOOT & MOUTH DISEASE CENTER

1. Notifies police.
2. Arranges for appraisers.
3. Hires slaughter team.
4. Hires laborers and heavy equipment for earth moving and disinfection.
5. Obtains fuel for burn.
FLUORESCENT ANTIBODY PROCEDURES
FOR BOVINE VIRUSES

By C. L. Brown, B.S.; E. W. Jenney, D.V.M.;
L. R. Lee, B.S.*

Prior to the development of fluorescent antibody (FA) for the detection and identification of viruses in tissue culture, the Bovine Viruses Unit of Diagnostic Virology at the National Animal Disease Laboratory (NADL) required an additional week to render a diagnosis by conventional methods. With this thought in mind, it was hoped that FA procedures would provide a more rapid diagnosis of bovine viruses.

The purpose of this report is to describe the development of a FA test for bovine viruses in tissue culture and a comparison of three host cell culture systems for their ability to propagate bovine viruses.

MATERIALS AND METHODS

*From the Bovine Viruses Unit, Diagnostic Virology, Diagnostic Services, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, National Animal Disease laboratory, Ames, Iowa 50010.
incomplete adjuvant. The last two injections were given i.v. in increasing dosage but without adjuvant. All animals were bled out 10 weeks after the first injection (Table 1).

**Conjugate Preparation.** — To remove crude protein, all antiserums were fractionated with half saturated ammonium sulfate. Percent protein was determined by the Biuret method. Protein solutions were adjusted to contain one percent protein and then reacted overnight at 4°C. with fluorescein isothiocyanate (FITC) at a rate of 1 mg. dye to 20 or 40 mg. of protein. The resulting conjugates were passed through a G-25 coarse Sephadex column to remove unreacted dye. As a final step each conjugate was treated with rabbit liver tissue powder to absorb out nonspecific fluorescence. Preliminary tests were conducted on all FA preparations to determine titer and specificity before using them in routine testing.

**Tissue Culture**

Comparative studies were made of two continuous passage cell cultures and one primary cell culture for their potential to isolate and identify bovine viruses.

**Bovine Turbinate.** — A continuous passage cell culture was started from tissues submitted as part of a glossitis case. Turbinate cells were started in Hank’s balanced salt solution (BSS) with 20 percent specific pathogen free (SPF) calf serum. The cell cultures were passaged in Eagle’s medium containing 10 percent SPF calf serum.

**Embryonic Bovine Trachea (EBTr).** — This cell line, identified by code CCL 44, was obtained from the American Type Culture Collection Cell Repository, 12301 Parklawn Drive, Rockville, Maryland 20852. EBTr cells were passaged in Eagle’s medium containing 0.5 percent lactalbumin hydrolysate (LAH) and 5 percent SPF calf serum.

**Embryonic Bovine Kidney (EBK).** — This was a primary cell culture initiated in Hank’s BSS with 10 percent SPF calf serum. Tissue culture medium was changed to Earle’s BSS containing 0.5 percent LAH and 5 percent SPF calf serum after confluent monolayers were obtained.

All tissue culture cells were grown in monolayer cultures on coverslips in Leighton tubes for the FA procedures.

**Preparation of Specimen for Inoculation**

Tissues from each of 76 cases were weighed to make up 20 percent suspensions in tris buffered tryptose broth (TBTB) or Hank’s or Earle’s BSS. Tissue suspensions were fortified with 200-300 units/ml. of streptomycin, penicillin, achromycin, and fungizone (SPAF). Tissues were ground in a 50 ml. Lourdes homogenizer cup for 2–5 minutes at high speed. A variety of tissue specimens were processed, such as spleen, lung, trachea, lymph node, blood, intestine, tongue, muzzle and bone marrow, and nasal and tracheal swabs. Some tissues were ground and inoculated immediately into tissue culture and others were ground and frozen at -80°C. until tissue cultures were available for inoculation.

**Tissue Culture Inoculation**

Tissue culture medium was discarded from each tube culture before inoculation with tissue suspensions. One ml. of tissue suspension was inoculated onto each
coverslip and allowed to absorb for one hour at 37°C. Two EBK, one EBTr, and one turbinate cell cultures were routinely inoculated with each tissue. Following absorption the inoculum was poured off and the coverslip cultures rinsed three times with BSS containing antibiotics. One ml. of medium containing 5 percent SPF calf serum, 0.5 percent LAH and 200 units/ml. of SPAF was restored in each tube culture. Tube cultures were incubated at 37°C. and observed every day with a microscope for cytopathic effect (CPE). In the absence of CPE each tube culture was passaged three times and stained for BVD by FA to detect non-cytopathogenic strains of BVD virus.

**Coverslip Staining**

As soon as CPE was observed in tube cultures, coverslips were removed and processed for staining. Coverslip cultures were rinsed in individual beakers containing 0.01 M phosphate buffered saline (PBS). Excess BBS was drained off and coverslips were fixed in acetone 5-10 minutes. Wood applicator sticks facilitated the handling of coverslips and were coded for identification. For staining with FA preparations, coverslips were removed from acetone and placed on staining racks in petri dishes. Conjugates (fluorescent antibody) were diluted to optimum dilution and layered over coverslip cultures. Cultures were incubated at 37°C. in a moisture chamber for 20 minutes. Following incubation coverslips were drained and washed 10 minutes in PBS. Coverslip cultures were rinsed lightly in distilled water, dried, and mounted cell side down on regular microscope slides using a solution of equal parts glycerine and PBS. Positive and negative controls were processed along with case specimens. Stained preparations were placed on the microscope and examined for fluorescence.

**Fluorescent Microscopy**

Microscopic examinations were performed with a Leitz Ortholux microscope. The filtering system consisted of a BG-12 ultraviolet-blue-passing primary filter and a OG-1 ultraviolet-blue-stopping secondary filter. An HBO-200 watt mercury lamp was used as the source of light. Mounted coverslips were examined with a 10x objective and a 10x ocular along with a dry dark field condenser.

Employing this light source and filter system, virus infected cells fluoresced a bright yellow-green against a dark background of normal cells. Depending on the viral agent, fluorescence was observed in individual cells or in a group or plaque of cells. With cytopathogenic strains of virus fluorescence was always observed at sites of cell destruction.

**RESULTS AND DISCUSSION**

**Serums and Conjugates**

* BVD. — Calf serums 4940 and 7479 both had neutralization titers of 1:512. Conjugates prepared from these serums produced dim fluorescence at the 1:32 dilution. Quality stains with bright specific fluorescence were observed at the 1:8 dilution. This dilution was used routinely for the identification of both the cytopathic and non-cytopathic strains of BVD virus. Conjugates prepared from calf serums 4940 and 7479 have been used by other laboratories throughout the country for the diagnosis of BVD.
PI-3. — Conjugates prepared from PI-3 antiserum with a 1:320 hemagglutination-inhibition titer produced bright fluorescence at the 1:8 dilution in PI-3 infected cell cultures. Forty-one tests were conducted on 10 field cases, but only one case was diagnosed as PI-3. This case involved placental tissue with low virus content which produced questionable CPE in tissue culture. The application of PI-3 conjugate to virus infected cells resolved this case as PI-3.

Rinderpest. — The rinderpest serum was obtained from PIADL and the virus was not available for determination of antibody titer of the serum. The conjugate prepared at NADL from rinderpest serum was tested at PIADL to determine its value as a diagnostic reagent for rinderpest. No cross type staining occurred when rinderpest conjugate was used with non-CPE and CPE strains of BVD virus, IBR virus, and PI-3 virus in primary EBK cell cultures. Optimum fluorescence was observed when rinderpest conjugate was diluted 1:8 and layered over rinderpest infected cells. Six field specimens were tested and found negative for rinderpest by FA.

IBR, Rabbit. — Three rabbits had neutralization titers of 1:128 and one rabbit had only a 1:10 titer. Although the titers were not particularly high, in view of the many hypering injections, a conjugate prepared from the pool of two rabbits produced bright fluorescence at 1:8 dilution in IBR infected tissue culture. Fluorescence was still definable at the 1:20 dilution but had become considerably dimmer. However, nonspecific fluorescence occurred when the rabbit conjugate was used on uninfected normal tissue culture cells. The nonspecific staining of conjugates prepared from rabbit antiserum made them unsatisfactory for FA procedures. Unlabeled rabbit antiserums were still useful in neutralization tests.

IBR, Calf and Goat. — The titer of each animal as well as the inoculation and bleeding schedule, the amount of inoculum, and the route of injection are presented (Table 1). The best fluorescence was observed with conjugate prepared with antiserum from goat 74 when diluted 1:16 and some fluorescence could be detected at the 1:32 dilution. Goat conjugate has been used routinely at 1:16 dilution. Optimum staining was observed with conjugate prepared from calf serum 5760 at the 1:8 dilution and in a 1:16 dilution was still capable of detecting IBR virus in tissue culture.

Virus Isolation and Identification. — FA procedures were applied to 76 field cases submitted for virus isolation and identification. Isolates from 22 cases were diagnosed as BVD, five as IBR, and one as PI-3. Fourteen of the 22 positive BVD virus isolates were not cytopathogenic. Before the application of FA procedures for bovine viruses, many cases of BVD went undetected since over 50 percent of BVD outbreaks diagnosed has been caused by non-cytopathogenic strains.

Fluorescent antibody procedures have been used for screening kidney sections of fetal calf kidneys obtained for tissue culture production. Virus contaminated kidneys from uterine infection would invalidate and confuse efforts at BVD virus isolation and cause a 2-3 log drop in virus titer during BVD neutralization tests. Fluorescent antibody procedures have completely replaced the older method of typing virus isolates in tissue culture by this laboratory. The older method required an additional supply of tissue cultures and another week to conduct a virus neutralization test with typing antiserum when virus was isolated. With FA only an optimum dilution of conjugate was prepared. Additional tissue cultures are not
required and a diagnosis is provided in less than 24-48 hours.

_Tissue Culture._ — A comparison was made of two continuous cell cultures and one primary cell culture for the isolation and identification of BVD virus (Table 2). No effort was made to denote the non-cytopathogenic strains of BVD virus. Cases 23022, 28406, 31133, and 23018 represent non-cytopathogenic strains. In some cases CPE has been observed (P) and FA was not attempted. When a virus causes rapid destruction of the cell monolayer, it renders the cell cultures unsatisfactory for FA staining. In these cases the virus was diluted and passaged before staining.

In the study for the diagnosis of BVD, the bovine turbinate and EBK cell cultures served in the isolation and identification of virus from 16 of 18 positive tissues, while only one was missed using the EBTr cell cultures.

A comparison was made of the same cell culture systems with IBR virus (Table 3). Comparative studies have shown that using the EBTr and EBK cell cultures virus was identified from six of eight positive tissues, while all but one was detected using the bovine turbinate cultures. Case 27286, an extensively degenerated fetal abomasum, was found to be positive in tissue culture on a previous test; but we failed to reisolate virus during this study.

The EBTr cell culture becomes quite rough in appearance beyond the 50th passage making it difficult to distinguish CPE. The bovine turbinate cell cultures have proven useful for BVD virus and IBR virus isolation up to the 59th passage but failed to grow beyond the 70th passage.

**SUMMARY**

The incorporation of FA procedures by the Bovine Viruses Unit at NADL has provided a rapid means for the diagnosis of bovine viruses. Both time, at least one week, and material were gained by FA procedures as compared to a diagnosis by neutralization tests.

Seventy-six field cases submitted for virus isolation and identification were tested by FA. Twenty-two cases were diagnosed as BVD (14 were non-cytopathogenic strains), five as IBR, and one as PI-3.

A study of three host cell culture systems (EBK, EBTr, and bovine turbinate) has shown that all three were efficient in isolating viruses if used at low passage. The bovine turbinate and EBK cell cultures were useful in isolating viruses from 16 of 18 positive tissues for BVD. The EBTr cell culture missed only one.

In the study with IBR virus, the EBTr and EBK cell cultures were instrumental in the isolation and identification of six out of eight positive tissues, while the bovine turbinate cell culture missed one.

_The authors acknowledge the assistance of the Diagnostic Virology staff and wish to thank Dr. George Lambert, Animal Disease and Parasite Research Division, National Animal Disease Laboratory, Ames, Iowa, for supplying bovine virus diarrhea antiserum; Dr. G. H. Frank, Animal Disease and Parasite Research Division, National Animal Disease Laboratory, Ames, Iowa, for supplying parainfluenza-3 antiserum; and Dr. J. J. Callis, Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, for supplying the rinderpest antiserum and facilities for testing the conjugate._
### TABLE 1

**IBR Hyperimmunization Schedule of Goats and Calves**

<table>
<thead>
<tr>
<th>Goat No.</th>
<th>Bled 1-17-68 (Preinoc.)</th>
<th>Inoculation 1-31-68</th>
<th>Bled 3-11-68</th>
<th>Inoculation 3-11-68</th>
<th>Bled 3-19-68</th>
<th>Inoculation 3-19-68</th>
<th>Bled 4-2-68</th>
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<tr>
<td></td>
<td>i.m.</td>
<td>i.v.</td>
<td></td>
<td>i.v.</td>
<td></td>
<td>i.v.</td>
<td></td>
</tr>
<tr>
<td>Goat No. 70</td>
<td>Neg. 1:4</td>
<td>10 cc</td>
<td>1:50*</td>
<td>6 cc</td>
<td>**&gt;1:256</td>
<td>50 cc</td>
<td>Died 3-22-68</td>
</tr>
<tr>
<td>Goat No. 72</td>
<td>Neg. 1:4</td>
<td>10 cc</td>
<td>1:64</td>
<td>6 cc</td>
<td>&gt;1:256</td>
<td>50 cc</td>
<td>1:512</td>
</tr>
<tr>
<td>Goat No. 74</td>
<td>Neg. 1:4</td>
<td>10 cc</td>
<td>1:50</td>
<td>6 cc</td>
<td>&gt;1:256</td>
<td>50 cc</td>
<td>1:406</td>
</tr>
<tr>
<td>Calf No. 5760</td>
<td>Neg. 1:4</td>
<td>20 cc</td>
<td>1:64</td>
<td>12 cc</td>
<td>&gt;1:256</td>
<td>100 cc</td>
<td>1:400</td>
</tr>
<tr>
<td>Calf No. 5763</td>
<td>Neg. 1:4</td>
<td>20 cc</td>
<td>1:16</td>
<td>12 cc</td>
<td>&gt;1:256</td>
<td>100 cc</td>
<td>1:128</td>
</tr>
</tbody>
</table>

* The serum dilution that neutralized 100-1000 TC ID$_{50}$ of IBR virus in EBK cell cultures.

**> Denotes greater titer.
TABLE 2 – Comparison of Three Cell Systems for the Isolation of BVD Virus

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tissue</th>
<th>Passage No.</th>
<th>Primary EBK CPE</th>
<th>EB Trachea (CCL 44) CPE</th>
<th>Turbinate* CPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>P</td>
<td>0</td>
<td>P</td>
</tr>
<tr>
<td>20571</td>
<td>Intestine</td>
<td>2</td>
<td>N</td>
<td>P**</td>
<td>P**</td>
</tr>
<tr>
<td>23022</td>
<td>Spleen</td>
<td>1</td>
<td>N</td>
<td>P</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P***</td>
<td>N</td>
<td>P**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,96 hrs.</td>
<td>N</td>
<td>P</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,24 hrs.</td>
<td>N</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>23018</td>
<td>Nasal Exudate</td>
<td>1</td>
<td>N</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(second attempt)</td>
<td>2</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>22925</td>
<td>Spleen</td>
<td>1</td>
<td>N</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>22898</td>
<td>Intestine</td>
<td>2</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>22996</td>
<td>Serum</td>
<td>1</td>
<td>N</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>33583</td>
<td>Nasal Crust</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>33467</td>
<td>Blood Clot</td>
<td>1</td>
<td>N</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td>22947</td>
<td>Blood</td>
<td>1</td>
<td>S</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>S</td>
<td>P</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>P</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td>28406</td>
<td>Placenta</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>34218</td>
<td>No. 1 Ileum</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>O</td>
<td>O</td>
<td>N</td>
</tr>
<tr>
<td>34218</td>
<td>No. 2 Ileum</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>S</td>
<td>O</td>
<td>N</td>
</tr>
</tbody>
</table>

CCL 44 Cell line initiated by A. J. Kniazeff
CPE Cytopathogenic Effect
FA Fluorescent Antibody
P Positive
N Negative
S Suspicious
O Not attempted
* From glossitis case at NADL
** Passage made from diluted virus and stained before CPE
# TABLE 3
Comparison of Three Cell Systems for the Isolation of IBR Virus

<table>
<thead>
<tr>
<th>Case</th>
<th>Tissue</th>
<th>Passage No.</th>
<th>Primary EBK</th>
<th>EB Trachea (CCL 44)</th>
<th>Turbinate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CPE</td>
<td>FA</td>
<td>CPE</td>
</tr>
<tr>
<td>27286</td>
<td>Fetal Kidney</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>22929</td>
<td>Bronchial Lymph Node</td>
<td>1</td>
<td>P</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>P</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>22984</td>
<td>Nasal Exudate</td>
<td>1</td>
<td>P</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>P</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>31831</td>
<td>No. 5 Blood</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>27286</td>
<td>Fetal Abomasum***</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>N</td>
<td>O</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>22929</td>
<td>Lung</td>
<td>1</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>27286</td>
<td>Fetal Lung</td>
<td>1</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>N</td>
<td>O</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>34334</td>
<td>Oral Tissue</td>
<td>1</td>
<td>P</td>
<td>O</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

CCL 44: Cell line initiated by A. J. Kniizeff
CPE: Cytopathogenic Effect
FA: Fluorescent Antibody
P: Positive
N: Negative
S: Suspicious
*: From glossitis case at NADL
**: Passage made from diluted virus and stained before CPE
***: Positive results on previous tests
SEQUENTIAL STERILE AUTOLYSIS
IN THE OVINE FETUS

R. C. Dillman, D.V.M., M.S., Ph.D., Assistant Professor
Iowa State University Diagnostic Laboratory
Ames, Iowa

Gentlemen, I’m here today to try and convince you that post mortem autolysis is not a dead subject. As many of you know, lesions seen in freshly aborted fetuses frequently lack the specificity suggestive of a particular pathogen. When such lesions are absent, we find that aborted fetuses share many common tissue alterations (I’m reluctant to use the term “lesion” here).

The true nature of these common alterations has not been established, but they have been presumed to be indicative of antepartum death and subsequent autolysis. Examination of veterinary literature reveals no experimental study of intra-uterine fetal autolysis. McFarlane, evolved a time-of-death classification and divided antepartum deaths into 2 groups; “dead for a long time” and “dead for a short time”. Dennis modified McFarlane’s scheme and studied perinatal mortality in sheep in Western Australia.

Physicians have long recognized the need for a method of determining the duration of intra-uterine death so that such occurrences might be related to prenatal traumatic or infectious incidents and valid statistical data pertaining to antepartum death might be used to determine the etiology. Shanklin et al. have experimentally examined intra-uterine fetal autolysis in the rabbit and applied the sequential autolytic changes in that species to the stillborn infant and successfully established the time of death of 50/63 fetuses.

With the exception of Shanklin et al., these researchers’ examinations have given only a broad estimate of the sequential autolytic changes prior to expulsion or removal of the fetus. The purpose of this experiment was to determine the sequential autolytic changes in the ovine fetus for a period of 7 days during the last trimester (100-150 days) of gestation.

Twenty-five Western ewes in the last trimester of pregnancy were subjected to surgical entry of the uterus, and sterile fetal death was produced by umbilical artery ligation. Amnionic sac, uterus, and abdomen were closed and the sequential autolytic changes were followed by surgically recovering 2 fetuses for each of the following hourly intervals: ½, 1, 2, 4, 8, 12, 16, 24, 36, 72, 96, 144, and 168. The possibility of bacterial contamination was checked by culturing amnionic fluid at the time of ligation and tissues upon recovery of the fetus. Controls were unligated fetuses from amnionic sacs which had been opened and closed surgically.

At the appropriate time interval, the fetuses were removed by cesarean section and immediately submitted to a systematic necropsy. Sections of 13 organs and tissues were removed from each fetus and fixed in 10% buffered neutral formalin for microscopic examination, care being used to select each tissue from the same relative location. All significant macroscopic and microscopic alterations were photographed and observations tabulated.

If we may have the lights turned out I will project some transparencies that
illustrate the more significant sterile autolytic changes observed macroscopically and microscopically. These are summarized for you in a table I have prepared, so those of you that are interested in the pertinent results may obtain them after our discussion. (Table 1)

**TABLE 1**

Significant Sterile Autolytic Changes in the Ovine Fetus

<table>
<thead>
<tr>
<th>Organ or Tissue</th>
<th>ALTERATION</th>
<th>MACROSCOPIC</th>
<th>MICROSCOPIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>12 hrs.+ cloudy cornea.</td>
<td>72 hrs.+ dehydration</td>
<td>..................</td>
</tr>
<tr>
<td>Skin</td>
<td>36 hrs.+ color changes followed by slipping, sloughing and dehydration</td>
<td>..................</td>
<td>½-12 hrs. hydropic becoming vesicular.</td>
</tr>
<tr>
<td>Subcutis</td>
<td>36-96 hrs. blood-tinged gelatinous fluid.</td>
<td>144 hrs.+ dehydration</td>
<td>..................</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>..................</td>
<td>12 hrs. shrinkage.</td>
<td>48 hrs. bundling.</td>
</tr>
<tr>
<td>Myocardium</td>
<td>2 hrs. friable</td>
<td>8 hrs. contraction</td>
<td>48 hrs.+ hematopoietic foci shrinking.</td>
</tr>
<tr>
<td>Liver</td>
<td>2 hrs. soft.</td>
<td>144 hrs. putty-like.</td>
<td>24 hrs.+ biliary epithelium sloughed.</td>
</tr>
<tr>
<td>Kidney</td>
<td>4-8 hrs. cortex friable.</td>
<td>24 hrs.+ progressive softening of cortex and medulla</td>
<td>16 hrs.+ cortex sloughed.</td>
</tr>
<tr>
<td>Abomasum and Contents</td>
<td>0-12 mucoid with flecks.</td>
<td>12-24 hrs. cloudy mucoid, yellowish</td>
<td>16 hrs.+ gastric epithelium sloughed.</td>
</tr>
<tr>
<td>Spleen</td>
<td>36-96 hrs. cloudy mucoid, reddish.</td>
<td>144 hrs.+ absent</td>
<td>½-16 hrs. hematopoietic foci scattering.</td>
</tr>
<tr>
<td>Thyroid</td>
<td>..................</td>
<td>8 hrs.+ no colloid.</td>
<td>24 hrs.+ hematopoietic foci shrinking.</td>
</tr>
</tbody>
</table>

The autolytic changes manifested in the ovine fetus for periods up to 7 days compared favorably with those reported by Shanklin et al16 and used successfully for determining the time of intrauterine death of stillborn infants.7 Additional examinations on ovine fetal tissues established other autolytic sequences. The data reported here supports and adds to that information and may therefore be of comparative biomedical significance.

Of what significance are these findings to veterinary diagnosticians?
1. The strictly autolytic changes in a ruminant fetus in late gestation have been established. It should now be easier to separate autolytic changes from true lesions in fresh fetuses aborted late in gestation. The following fetal changes were seen which are frequently reported as significant "lesions" in infectious ruminant abortions:

1) subcutaneous blood-tinged gelatinous edema.
2) renal-cortical softening.
3) uniformity of color.
4) hepatic friability and mottling.
5) cloudy-yellow to cloudy-red abomasal contents.

2. Having established the sequential autolytic changes in-utero, we now have a tool for assessing the duration of fetal retention after death. The extent of fetal retention might, in itself, prove to be an aid to differential diagnosis in cases of infectious ruminant abortions.

3. Infectious ruminant abortions, experimentally produced, can be more accurately evaluated by research workers.

4. The mummification, subsequent to sterile autolysis, tends to support the contention of some research workers that fetal death alone does not result in abortion.

LITERATURE CITED

AUTOMATED RECORD KEEPING SYSTEMS
FOR DIAGNOSTIC LABORATORIES AND
A ZOO HOSPITAL

Norman E. Hutton, D.V.M., Lynn A. Griner, D.V.M.,
Vaughn A. Seaton, D.V.M., Kenneth D. Weide, D.V.M.*

HISTORY OF COMPUTING

Computing, or mathematical calculations, started about 4,000 years ago when
a gadget called an abacus was developed. In some places in the world this is still in
use today. In 1642 Blaise Pascal, the brilliant French philosopher and inventor,
constructed the first adding machine. His machine was designed around decimal
counting gears — one tooth for each digit 0-9 which were connected in series so that
one full revolution of a gear would automatically forward the gear to its left by one
digit. This in effect was the first machine capable of carrying ten, and Pascal’s gears
established the basic principle used today in adding machines, desk calculators, and
cash registers.

About 1812 Charles Babbage, an English mathematician from Cambridge
University, made the first major advancement in the development of a computer
when he designed an analytical engine. However, in spite of financial aid from
private sources and the British government, the technology of the time was not
equal to Babbage’s advanced ideas and he died without ever realizing the great
worth of his invention. In the 1930’s, Dr. Howard Aiken of Harvard University,
rediscovered the principles of Babbage’s machine and in 1944 built one according
to those principles. He called it the Harvard Mark I.

World War II spurred the development of a new science called electronics and
this, in turn, promoted the rapid development of computers. In 1945 the first true
computer appeared. It combined the new science of electronics, a unique method
of coding information, and a century-old idea for adding memory to a calculator.
Computers are becoming commonplace and there are many, many varied types of
computers from the small computers to large computers with vast memories.

COMPUTERS AND OTHER INFORMATION RETRIEVAL DEVICES

Computers and other information retrieval devices are very important when
one looks toward providing those interested in animal diseases with immediate
retrieval of information. Veterinary Diagnostic Laboratories and Zoos are tre-
mendous sources of information for those interested in animal diseases. It is here
that exotic diseases may first be noted as well as other diseases which require

*Dr. Hutton is an Instructor and Dr. Seaton is Professor and Head of the Veterinary Diagnostic
Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa. Dr. Griner
is a Pathologist and Head of the Health Department at the San Diego Zoo, San Diego,
California. Dr. Weide is Professor and Head of the Department of Veterinary Science, South
Dakota State University, Brookings, South Dakota.
laboratory assistance in diagnoses. These areas may well be our first line of defense in animal disease eradication and control programs.

In building these information retrieval systems, we were interested in both administrative information as well as disease information. Two of these systems are computer based, that is, their primary input is on punched cards. The other system is a TERMATREX system and its primary input is on a TERMATREX card which lends itself to transferring the information to punched cards if one so desires.

SOUTH DAKOTA VETERINARY DIAGNOSTIC LABORATORY

A computerized system for information storage and retrieval has been developed for use in the Diagnostic Laboratory at South Dakota State University. This system provides for storage and retrieval of both management information and disease information and is used to provide data necessary in preparing budget requests, in providing work measurement reports of the laboratory and also provides information concerning public health, regulatory and other disease control programs of the laboratory. Figures 1, 2 and 3 show the basic input to the system. These are abstract sheets used in recording the information in the laboratory. This information is recorded by the veterinarian and subsequently coded by the coding clerk.

Coding systems have been developed for species codes. These are numeric codes and run from 01 to 99. Each veterinarian in the state of South Dakota is assigned a code number and this is also part of the input to the system. All of the information in regard to breed, animal category, age, sex, disease or condition suspected, and various results of the laboratory tests are coded numerically and in all cases a dictionary is set up which will provide numeric codes and English equivalents of these codes. This type of dictionary provides for information to be recorded as input in coded form, however, the output is in English and can be easily read by the administrators and laboratory diagnosticians.

The input to this system is put on three punched cards and the card columns are shown under each particular item in the abstract sheets. As you will note, columns 2 through 12 are recorded consistently on all cards. This is the core information that is necessary to tie the various cards together to the unique diagnostic number. In other words, year, month, diagnostic number and species is coded on all cards.

Figure 4 shows a portion of the administrative information that is output through the use of this system. The disease information is also part of the output and diagnosis by species is shown and each diagnosis is further broken down by county within the state of South Dakota in order to show where each particular disease is occurring. This provides a picture of where pockets of infection may be in the state or in which areas they are having trouble with certain disease problems. Also part of the output of this system is a disease breakdown by the various age groups of animals and one can see in which age group certain problems are occurring most frequently. Serology tests and the results of these tests are part of the output as is the bacterial sensitivity. Bacterial sensitivity tests are conducted on each organism isolated in the laboratory to see if they are sensitive or resistant to each of the particular drugs shown on the input form.
The San Diego Zoo performs a postmortem examination at the time of death on all birds, reptiles and mammals in the zoo collection. A complete pathology report is maintained in permanent binders at the zoo hospital and all tissue slides are processed for histopathologic study and kept in permanent storage. An immense amount of important information has been recorded in the pathology reports. Selected parts of this information are abstracted for entry into the TERMATREX system in use at the zoo.

The TERMATREX system is manufactured by the Jonker Corporation. It is a desk-top system which allows immediate retrieval of specific records and provides the investigator with information as to the types of tumors or other disease entities seen in each species of zoo animal.

The TERMATREX system consists of three basic items of equipment: a semi-automatic drilling device for data input, a calibrated viewer for data output, and plastic cards for data storage. A TERMATREX card has coordinates of 100 by 100 which provides for 10,000 locations. The entire system is based on accession numbers which can run from 0 to 9,999 providing for a maximum of 10,000 accessions in a single file of cards. If one desires a file of more than 10,000 accessions, this can be handled through another file of cards. The recording of an accession number is done by drilling a hole with the precision drilling device in the appropriate cards for that accession. In other words, if a person had an accession number of 500, a hole would be drilled in the card or cards that pertained to that accession number in the 500th coordinate in the system.

TERMATREX systems are based on a vocabulary of specific terms and each term is assigned a color-number code. These color-number codes have no specific meaning. A color-number code is assigned to a specific term and this mechanism provides for an open-ended system and one can assign additional codes as they become necessary. Due to the large number of species of animals at the San Diego Zoo, two vocabularies are required. One is a list of diagnostic terms and the other is a list of animal species.

A list of animal species was prepared and divided into three major classes: avian, mammalian, and reptilian and amphibian. Each class of animal was further subdivided by order, family, genera, and species. A four-digit code number was assigned to each species of animal. The numbers 0000 to 2500 were assigned to reptiles and amphibians, 2501 to 5000 to mammals, and 5001 to 9999 to birds. The assignment of codes to the various species of animals has been in a manner different from the other code assignments. Each species has been assigned a numeric code which is converted to a TERMATREX code in the following manner: white cards 0-9 represent the thousands position of numbers, white cards 10-19 represent the hundreds position of numbers, white 20-29 the tens position, and white 30-39 the units position. This makes it possible to code 10,000 species on 40 cards. The identification of a given species, therefore, requires four TERMATREX cards. Code numbers were reserved in each family to allow for additions of new species.

For example the Wood Turtle was assigned a code of 0021 which, when coded in the TERMATREX system, required the cards white 0, white 10, white 22, and
white 31. The coding of these various entities such as diagnoses or species of animals is accomplished by assigning a color-number code to the particular term. There are ten colors — these being red, white, yellow, purple, green, orange, gray, black, sand, and blue — and each color has up to 100 cards.

**INPUT OF DATA**

The case abstract in Figure 5 represents a sample necropsy abstract. Necropsy number 2243 was assigned to this case and is recorded in the TERMATREX system by pulling the cards checked on the abstract form and drilling a hole in the 2243 position with the precision drill input device. For the sake of clarity, this record will be briefly reviewed. This case was received in August 1968. The class of animal was “Avian” and was a Senegal Parrot, code number 6498. The sex was female and the age was unknown. The diagnostic procedures used were gross pathology and histopathology. The digestive system was affected and digestive tissues were saved for future reference. The diagnosis was “enteritis.” The case is entered into the TERMATREX system by pulling the following cards: black cards 8, 68, and 14; white cards 6, 14, 29, and 38; black cards 23, 30, 58, 59, 86, and 45; and yellow card 23. These cards are put into the precision input drilling device and the accession number 2243 is drilled in all cards and all information is recorded regarding the case.

**TERMATREX RETRIEVAL OF INFORMATION**

The TERMATREX system is based on light intensity and light penetrating holes previously drilled in the card. Wherever a hole is drilled, light comes through a card meaning that a particular accession is associated with the term. The following is an example of how a search might be made. If one would like to know all the cases of enteritis seen in the zoo, one would put on the viewer yellow card 23, the color-number code for enteritis, and the holes allowing light to pass through would indicate both the number of accessions and the accession numbers where enteritis had been diagnosed. If we further wish to know how many birds were so affected, we would add the TERMATREX card black 14, the color-number code for avian, and the light passing through these cards would show the number of cases of enteritis seen in birds at the San Diego Zoo. One could also restrict this to a particular year — let’s say the year 1965 — one could then add the black 65 card, the color-number code for 1965, on top of the other cards and this would then show how many cases of enteritis were seen in birds in 1965. If we wish to go one step further and ask how many cases of enteritis were seen in 1965 in Senegal Parrots, we would then add the white 6, the white 14, the white 29, and the white 38 cards which would stand for the code number 6498 referring to Senegal Parrots and then the light coming through would show you the number of cases seen.

**IOWA STATE UNIVERSITY VETERINARY DIAGNOSTIC LABORATORY**

The information retrieval system in use at the Iowa State University Veterinary Diagnostic Laboratory is computer based and is developed around the abstract
The abstract has been developed so that any institution could use the system by inserting a code for their particular institution. A unique accession number is assigned to the case and the month and year the case is reported is also recorded. The number of animals submitted live or dead is recorded to provide perspective regarding the number of postmortem examinations conducted. The numbers of animals submitted in the form of tissue, blood, serum and other methods is also recorded. The number of days from the time the case is submitted to the time the final report is made is recorded. This provides perspective regarding the amount of time it takes to get the report back to the practitioner or client and when correlated with the diagnostic procedures used in conjunction with the case, may show where added personnel or equipment or a change in procedures is needed.

The input data is punched onto cards and summarized each month and yearly. A sample of the output is shown in Figure 7. There are many other types of output available such as the number of times each veterinarian used the laboratory and the distribution of disease entities diagnosed by county.

Additional abstract sheets are used to record supplementary information. Bacteria and viruses isolated, parasites identified, hematological examination results and fungi isolated are recorded as is the serological tests conducted and the results of these tests. Chemistry and toxicological analyses conducted are recorded along with the results of these analyses. Bacterial sensitivities are conducted and the results are recorded for future study.

**SUMMARY**

We have discussed three information storage and retrieval systems. One of these is the Veterinary Diagnostic Laboratory at South Dakota State University, the other system is in use at the San Diego Zoo Hospital and the third system is used at the Veterinary Diagnostic Laboratory at Iowa State University. It has been shown that there are a variety of ways of recording information and this information is recorded for many different and varied purposes. In all of these systems, however, we have combined the recording and retrieval of management information as well as scientific information. It appears that these systems compliment each other in that many of the pieces of data that are required for those interested in disease information retrieval also provides those concerned with budget requests and work measurement with information that is necessary.

Two of these systems are computer based systems, that is, the primary input is on punched cards and the primary output of the system is computer listings of information. The other system is a TERMATREX system that provides for input by drilling holes into a pre-selected set of TERMATREX cards. The output is a reading of incidence or number of findings as related to various types of entities. Although the primary mechanism of the TERMATREX system is a drilling of holes in related cards, this system is equally adaptable to a computer bases system and the abstract developed for the San Diego Zoo was also developed so that the information could be put onto punched cards for retrieval or analysis of information from several zoos. The cost of these systems varies and is proportional to the type of information that you desire from such a system.
One of the prime sources of information for animal disease reporting and morbidity and mortality information is the veterinarian who is interested in recording this information in some type of method whereby it can be retrieved. These systems are but three of the many methods that can be used, however, the prime prerequisite to a system has been met — those desiring information can be served.

REFERENCES

<table>
<thead>
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<th>VS SDSU NO.</th>
<th>CARD NO.</th>
<th>YEAR</th>
<th>MONTH</th>
<th>DIAGNOSTIC NUMBER</th>
<th>SPECIES</th>
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<table>
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<th>NO. SICK</th>
<th>NO. DEAD</th>
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<th>NO. SPECIAL STAINS</th>
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<td>70 71 72</td>
<td>73 74 75</td>
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<td>78 79 80</td>
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<td>48</td>
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<td>NO. FA BACT.</td>
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**FIGURE 2**
<table>
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<td>30 31</td>
<td></td>
</tr>
<tr>
<td>T3 T4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>15 16</td>
<td>Aureomycin Chloromycetin</td>
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<td>Aureomycin Chloromycetin</td>
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<tr>
<td>17 18</td>
<td>Erythromycin Neomycin</td>
<td>34 35</td>
<td>Erythromycin Neomycin</td>
</tr>
<tr>
<td>19 20</td>
<td>Penicillin Streptomycin</td>
<td>36 37</td>
<td>Penicillin Streptomycin</td>
</tr>
<tr>
<td>21 22</td>
<td>Terramycin Tetracycline</td>
<td>38 39</td>
<td>Terramycin Tetracycline</td>
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<tr>
<td>23 24</td>
<td>Triple Sulfa Furacin</td>
<td>40 41</td>
<td>Triple Sulfa Furacin</td>
</tr>
<tr>
<td>25 26</td>
<td>Tylosin Polymixin B</td>
<td>42 43</td>
<td>Tylosin Polymixin B</td>
</tr>
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<td>27 28</td>
<td></td>
<td>44 45</td>
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<tr>
<td>29 30</td>
<td></td>
<td>46 47</td>
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</tr>
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</table>

**SIGNIFICANT LAB. RESULTS (5 POSSIBLE)**

| 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 |

**DIAGNOSIS (5 POSSIBLE) PRIMARY**

| 62 63 64 65 66 67 |

**NO. DAYS TO FIRST REPORT**

| 68 69 70 71 72 73 74 75 76 |

**FINAL REPORT BY**

| 77 78 79 80 |

**FIGURE 3**
DEPARTMENT OF VETERINARY SCIENCE
ANIMAL DISEASE RESEARCH AND DIAGNOSTIC LABORATORY
SOUTH DAKOTA STATE UNIVERSITY
BROOKINGS, SOUTH DAKOTA

ADMINISTRATIVE INFORMATION

FREQUENCY OF DAYS UNTIL FIRST REPORT REGARDING CASE

<table>
<thead>
<tr>
<th>No. of Days</th>
<th>All Cases</th>
<th>Histopathology</th>
<th>Virology</th>
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<td>34</td>
<td>1</td>
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<td>7</td>
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<tr>
<td>8</td>
<td>18</td>
<td>5</td>
<td>2</td>
<td>16</td>
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<td>8</td>
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LABORATORY ANIMALS USED

<table>
<thead>
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<th>Number</th>
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<tr>
<td>Guinea pig</td>
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<tr>
<td>Mouse</td>
<td>117</td>
</tr>
<tr>
<td>Rabbit</td>
<td>9</td>
</tr>
<tr>
<td>Rat</td>
<td>7</td>
</tr>
</tbody>
</table>

FIGURE 4
# AUTOMATED RECORD KEEPING SYSTEMS

## ZOOLOGICAL SOCIETY OF SAN DIEGO

### Pathology Report Code

**Month** 8  **Year** 68  
**Necropsy No.** 2243 
**(Accession)**

<table>
<thead>
<tr>
<th>Animal Class</th>
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<th>Sex</th>
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<tbody>
<tr>
<td>BK 14</td>
<td>Avian</td>
<td>Male</td>
</tr>
<tr>
<td>BK 16</td>
<td>Mammal</td>
<td>Female</td>
</tr>
<tr>
<td>BK 18</td>
<td>Reptile</td>
<td>Unknown</td>
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**AGE**

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<tr>
<th>Code No.</th>
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**DIAGNOSTIC PROCEDURES**

| BK 30 | Unknown |
| BK 31 | 0-less 1 day |
| BK 32 | 1 day-less than 1 mo. |
| BK 33 | 1 mo.-less than 6 mo. |
| BK 34 | 6 mo.-less than 1 yr. |
| BK 35 | 1 yr.-less than 2 yrs. |
| BK 36 | 2 yrs.-less than 5 yrs. |
| BK 37 | 5 yrs.-less than 10 yrs. |
| BK 38 | 10 yrs.-less than 20 yrs. |
| BK 39 | 20 yrs. and over |

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Tissue Saved</th>
<th>Diagnostic Category</th>
<th>Tissue Saved</th>
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<tbody>
<tr>
<td>BK 81</td>
<td>Skin-Pelage Sys.</td>
<td>BK 40</td>
<td>BK 89</td>
</tr>
<tr>
<td>BK 82</td>
<td>Musculo-Skel. Sys.</td>
<td>BK 41</td>
<td>BK 90</td>
</tr>
<tr>
<td>BK 83</td>
<td>Respiratory Sus.</td>
<td>BK 42</td>
<td>BK 91</td>
</tr>
<tr>
<td>BK 84</td>
<td>Cardiovascular Sys.</td>
<td>BK 43</td>
<td>BK 92</td>
</tr>
<tr>
<td>BK 85</td>
<td>Hemo.&amp;Lymph Sys.</td>
<td>BK 44</td>
<td>BK 93</td>
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<td>BK 86</td>
<td>Digestive Sys.</td>
<td>BK 45</td>
<td>BK 94</td>
</tr>
<tr>
<td>BK 87</td>
<td>Urogenital Sys.</td>
<td>BK 46</td>
<td>BK 95</td>
</tr>
<tr>
<td>BK 88</td>
<td>Endocrine Sys.</td>
<td>BK 47</td>
<td>BK 96</td>
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### System DIAGNOSES Code

#### Digestive

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<thead>
<tr>
<th>Enteritis</th>
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<tr>
<td>Enteritis</td>
<td>Yellow 23</td>
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Multiple Diagnosis __ Red Transparency
Multiple Neoplasia __ Green Transparency

**FIGURE 5**
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<th>Report Date</th>
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<th>Species or Specimen</th>
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<table>
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<th>Age</th>
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<td>Alive and/or dead animal(s)</td>
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<td>No. of animal(s) (tissue, blood, serum, fecal swabs, misc.)</td>
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<td>Non-animal sample(s)</td>
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<td>(1) --Dairy</td>
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</tr>
<tr>
<td>(2) --Beef (pasture)</td>
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<tr>
<td>(3) --Beef (feedlot)</td>
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<td>(4) --Bovine (mixed)</td>
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<tr>
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<td>75 -Form 1</td>
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<tr>
<td>65 -Bacteriology</td>
<td>70 -Chemistry &amp; Toxicology</td>
<td>76 -Form 2</td>
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<td>66 -Virology</td>
<td>71 -Rabies Mouse Inoculation</td>
<td>77 -Form 3</td>
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<tr>
<td>67 -Sensitivity</td>
<td>72 -Field Trip</td>
<td>78 -Form 4</td>
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<tr>
<td>68 -Parasitology or Mycology</td>
<td>-Final Report (days)</td>
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<td>61  62  63</td>
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Form type 1

FIGURE 6
ADMINISTRATIVE INFORMATION

ACCESSIONS REQUIRING A SPECIFIC SERVICE

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<td>Total Accessions for Month</td>
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DISEASE INFORMATION

SEROLOGY BY SPECIES OF ANIMAL

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<td>Cattle</td>
<td>Leptospirosis</td>
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<td>460</td>
<td>9</td>
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<tr>
<td>Pig</td>
<td>Brucellosis</td>
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<td>0</td>
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<td>Pig</td>
<td>Leptospirosis</td>
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<td>177</td>
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<td>Sheep</td>
<td>Brucellosis</td>
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<td>7</td>
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</tr>
<tr>
<td>Sheep</td>
<td>Leptospirosis</td>
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FIGURE 7
DATA STORAGE AND RETRIEVAL
AS USED IN
FLORIDA DIAGNOSTIC LABORATORIES

D. E. Cooperrider, D.V.M., M.Sc., Chief
Diagnostic Laboratories Section
Kissimmee, Florida

Man has been accumulating information of all kinds since the beginning of time and at first the struggle was to find some way to transmit this information to others while retaining portions for the individual. The development of writing was a significant forward step in collecting and transmitting information. Down through recorded history, various filing methods were used and all were adequate for their particular time in history. With the ever-increasing volume of scientific knowledge and the increased animal and human population (3,000 increase each morning), it has become apparent that much better and faster methods of storage and retrieval of information of all kinds are necessary.

There are numerous definitions of an expert and I'm sure I qualify under the one which says "an expert is an individual who can take something simple and make it sound confusing". I shall do my best.

As an example of the need to be able to recall information, this became dramatically apparent to our laboratory and disease control people several years ago, when, after numerous unsuccessful attempts, we finally recovered the organism *Clostridium hemolyticum*, which causes "redwater disease". When this bacteriological recovery was confirmed, we were practically unsuccessful in locating the previous cases where we had good reason to suspect the presence of the disease. This placed us in the position of now knowing where we were with respect to the disease and started us in on a study of the various data storage and retrieval systems.

We had already started to use, in a limited way, the "Nomenclature of Animal Diseases" as prepared by the AVMA Special Committee, so we tended to continue this in more detail.

As originally devised, the Disease Classification and Data Retrieval abstracts in use by the National Cancer Institute, Veterinary Data Program, were directed toward recalling information on a specific individual each time that individual appeared for either treatment or surgery. This means that the same accession would reappear so there would be continuity of treatment. As our laboratories are a vital part of the state disease control system, our primary interests are diagnosis of disease conditions in all animals and the area of the state involved with treatment or retreatment not generally a part of the system. The individual animal is only a part of a larger pattern.

We surveyed the possibilities of several systems and became acquainted with the efforts of the NCI - Michigan State project. They were using the Standard Nomenclature of Veterinary Diseases and Operations (SNVDO) in conjunction with IBM. Since there was already operating within the Florida Dept. of Agriculture a data processing center using IBM equipment, this was the most logical system on
which to concentrate. In the summer of 1964, the MSU facility was visited and the operation surveyed. As a result, definite plans were instituted to start the IBM data retrieval system in our laboratory. Negotiation with NCI enabled us to plan for the operation and an initial abstract form was devised. Since this was to match and interlock with the NCI program, we retained much of the abstract form which was already in test operation at MSU. We did not retain the “Operations” portion, and the remainder of the abstract form was developed with three objectives in mind:

1. Possible use in USA by other diagnostic laboratories.
2. Possible use in USA by Dept. of Agriculture in morbidity and mortality studies.
3. Possible use in other parts of the world to tie in disease reporting and epizootiology.

In our laboratories, it became apparent that the materials received varied widely in types and tests desired, as differentiated from a system where each presentation was a single animal for treatment. As a result, two abstracts were developed, one for single sample accessions and one for multiple sample accessions. The single sample is used for autopsy specimens and most of the other material goes on multiple abstracts. This gives us a very flexible system which can now adequately catalog the various types of tests for the various disease conditions and give us a monthly account of our laboratory activities. Complete two and three digit keys were compiled for diseases and types of tests as well as using previously developed keys for breed, sex, etc. For an example of one problem, there is no way in the SVNDO system to catalog negative tests since all diagnoses are positive. And there was no way to describe several types of tests on the same specimen; e.g., an equine serum sample may be entered with a request for an A/G ratio, A/T ratio, gel-precipitin test for piroplasmosis, plate agglutination test for leptospirosis and chemical tests to determine the amount of calcium and phosphorus in the serum. Since these can be routine on equine serum samples in our laboratory, it was necessary for the abstract to show this information in order for us to summarize our activities and recall the accession for study of comparison results on later specimens. This offers a greater service in adding to the knowledge by follow-up of suspected conditions. We presently request additional specimens on many of our accessions, especially those dealing with serological tests.

The system is wide enough in scope and digits to accommodate the accessions of the main laboratory and those of the four branch laboratories. Since the interests of these branch laboratories are primarily poultry or swine, depending on their location, we adequately cover all the species of animals which are economic factors in our state. The findings of all these laboratories are abstracted and included in the system reports.

There is an entry by which a second abstract form called a “trailer” can be keyed into the system. With this additional card available, though not yet in use, our sectional laboratories within the main laboratory can go into greater detail in cataloguing the work done by them. The possibilities are almost limit-less when one considered all the interconnected data that can be collected, stored, recalled and studied from not only all the laboratories in the country, but world-wide.

As presently devised, data control input is as extensive and thorough as we can make it and our present plans are to use this for cataloguing past accessions and
move forward with a continuing system to take care of current accessions.

This system has now been in operation for well over two and one-half years and we have on IBM cards all current accessions in the main laboratory and are in the retrospect cards to mid-1964.

In the laboratory at Live Oak, all accessions since the opening of the laboratory in 1966 are catalogued and the information is available to us on call from our IBM Data Processing Section in the Department at Tallahassee.

As you can see from the material in your hands, the system is relatively simple and should be adequate for most laboratories. Like all things devised by man, it will not suit all situations. We have made some changes and probably will make more from time to time. We have ceased to use the facilities of NCI and have taken all our operations into the Department. We send NCI duplicates of our single case abstract cards but not the multiple. We are beginning to see the possibilities of the system if it were tied in with a National Reporting System as suggested by the USLSA 1966 Resolution No. 1 in the Report of Delegates to the National Association of State Departments of Agriculture.

It should be noted that at the Veterinary Medical Records Librarians' meeting at Boston in July, there was discussion of the possibility of the substitution of the International Classification of Diseases (ICD) for the SNVDO. This would appear to be an attempt towards greater uniformity between veterinary and human medical terminology and probably would be of great importance to the epidemiologist, but the loss of flexibility and diagnostic detail in our own operation leads me to not favor the change.

I do not maintain that the IBM System is the "only" system or the "best" system. It is best for us in our present situation. I would strongly suggest the common use of the SNVDO, because at some one place our various systems must coincide and the Nomenclature is the logical place. If we are all calling the disease by the same name, then we can all recall that disease incidence by any system devised.

I would like to see a nation-wide system of disease reporting and record keeping instituted as recommended at the 1967 meeting of the USLSA. Any advice or organizational help that may be needed can be found in any of the groups presently working on data storage and retrieval.
## FLORIDA DEPARTMENT OF AGRICULTURE

### DIAGNOSTIC LABORATORIES

#### COMBINED ACTIVITIES

#### MONTHLY REPORT

### AUGUST 1968

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FLUORESCENCE MICROSCOPY FOR DIAGNOSIS OF BABESIA IN THE BLOOD


INTRODUCTION

Babesiasis has so far been diagnosed by demonstration of the parasites in blood smears stained with Giemsa or similar dyes. Occasionally the organisms are numerous enough to be found easily. More often, however, slides have to be searched for a long time and even then it is not always possible to find the parasites in known cases of babesiasis. A thick film technique introduced by Mahoney and Saal2 has the advantage of higher concentrations of the organism, but unfortunately the parasites tend to become distorted and difficult to identify among the cellular debris. Staining is also more difficult to indentify among the cellular debris. Staining is also more difficult and suitable batches of dyes should be selected, preferably by spectrophotometric examination.3 Because of these difficulties many workers still prefer to use smears rather than thick films.

Sometimes blood is examined not only for the presence of babesia, but also to determine their numbers, which gives an indication of the severity of the infection. Also, when infected blood is to be used as a vaccine, counting becomes essential in order to calculate the effective dose. At one time or another, various indirect methods of counting parasites in fixed and stained blood smears were tried. Neither was entirely satisfactory. Counting against white cells was found too inaccurate because of uneven distribution of leukocytes in smears. Counting against a known number of introduced fowl erythrocytes, which were easily recognized because of their nuclei, was found to be tedious for numbers over 100,000/mm³, 1 and unsuitable for numbers over 250,000/mm³. The usual technique now is counting against erythrocytes. As there are always much fewer parasites than erythrocytes in the blood, large numbers have to be counted to obtain a reasonable degree of accuracy. This is tedious and time consuming. Also, this being an indirect method, a separate total erythrocyte count has to be performed, which adds an additional error. For these reasons counting is now being done only when unavoidable, such as in vaccine production, and even then not as often as would be desirable.

This paper is to draw attention to newly developed fluorescence techniques which not only permit a much more rapid and reliable qualitative diagnosis of babesiasis and other protozoan infections, but together with a specially developed counting chamber can be used for direct counting, which is much quicker and more accurate than the indirect methods.

QUALITATIVE DIAGNOSIS

A method of staining air dried blood smears with acridine orange was published previously.4 When examined under the fluorescence microscope a picture as shown in Fig. 1 was seen. The erythrocytes appeared as black discs on a green background. Some of them contained babesia organisms, which were bright red with a contrasting yellow nucleus. The nuclei of the white cells were bright yellow.

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The cytoplasm of lymphocytes was red, while that of mature polymorphonuclear cells showed only a faint green fluorescence or none at all. The color of the white cells serves as a guide for the correct intensity of the stain.

Because they appeared in bright colors on a dark background, the organisms were much more conspicuous than in Giemsa-type stains. Furthermore, with fluorescence, high-dry magnification could be used instead of oil-immersion, as is necessary in Giemsa. This made it possible to search larger areas at a given time.

Extensive comparisons between fluorescence and Giemsa methods showed that the chances of finding babesia with the fluorescence technique are in the mean 64 times greater than in Giemsa stained smears, and 16 times greater than in Giemsa stained thick films. In the course of these examinations vitally stained preparations were also used. They were easily prepared, simply by mixing on a slide one drop each of blood, saline and acridine orange (1:10,000 in saline as for the previous stain) upon which a cover slip was placed. It was then immediately ready for examination. Vital staining was generally less efficient for finding small numbers of parasites than any of the methods mentioned previously. However, it was very simple and fast, and if the parasites were not too rare, they could be found quickly, which made it useful for preliminary screening of blood samples.

**DIRECT COUNTING OF BABESIA**

The technique of counting babesia directly was based on the above mentioned vital staining together with a specially designed counting chamber (Fig. 2). The chamber was made by cementing 3 strips of cover slips onto a microscope slide. The middle strip was 5 mm. wide and the thinnest of the three. On either side of this center strip, but separated from it by about 1 mm. were the two lateral supports which both were of equal thickness but thicker than the center strip. When a cover slip was placed over the two lateral supports a narrow space was formed between the center strip and the cover slip. The depth of this space was measured with a micrometer, about 0.01 mm. was the most suitable depth.

The counting chamber was charged similar to a hemocytometer by placing blood, diluted with saline and acridine orange, on the center strip, at the edges of the cover slip. The diluted blood quickly filled the space between center strip and cover slip by capillary action and could then be examined immediately under the fluorescence microscope. Babesia and white blood cells appeared in the same brilliant colors on a dark background as in fixed smears. In addition, vital staining also revealed the thrombocytes. These were pale green with a slightly irregular outline and often contained small red granules. After a few minutes the thrombocytes tended to rise to the top, while babesia settled to the bottom of the fluid.

Mature erythrocytes were not visible in vital staining. However, with the fluorescence equipment used they could be made visible by moving the lever, which changed fluorescence to normal illumination, to a halfway position. This way the preparation could be observed under mixed light and the fluorescent organisms could be seen in relation to the erythrocytes. Most counting was done with this mixed light.
The magnification of the X40 dry objective with X10 oculars was sufficient to count babesia under the fluorescence microscope. The organisms were counted either in a number of whole fields, or when more numerous, in a number of fields outlined by a grid in the ocular.

The number of organisms per cubic millimeter was calculated as the total number of organisms counted, multiplied by the dilution of the blood and divided by the volume of blood examined:

\[
\text{Total counts} \times \frac{\text{dilution}}{\text{No. of fields} \times \text{area of one field} \times \text{depth of field}}
\]

Ninety-five percent confidence limits of \( \pm 10\% \) were regarded sufficient for the counts, which was much more accurate than any indirect method. Experiments showed that this could be achieved by counting a minimum of 50 fields if at least 250 parasites were found. Otherwise, examination was continued until at least 250 parasites were counted.

**SUMMARY**

Dried and fixed smears of blood stained with acridine orange, as well as vital staining with this dye, were used for the diagnosis of babesiasis by fluorescence microscopy. With dried and fixed smears the chances of finding the parasites were 64 times greater than with Giemsa stained smears and 16 times greater than with Giemsa stained thick films. Vital staining, although less efficient for finding small number of organisms, was a very quick technique and as such valuable for screening and making a diagnosis in blood samples with not too low a parasite burden.

Vital staining combined with a specially developed counting chamber was used for direct counting of babesia. This proved much less tiring and time consuming and more accurate than previous methods of indirect counting, in which the parasites were counted against blood cells in Giemsa stained smears.

Babesia are counted for two reasons. First, as an indication of the severity of an infection. Second, when parasitized blood is used as a vaccine, to avoid ineffectively low - or fatally high - doses.

The advantage of fluorescence of Giemsa techniques is that the organisms stain in very bright colors on a dark background, which makes them more conspicuous. Lower magnifications can be used and thus larger areas can be searched at a given time.

Furthermore, because of the red cytoplasm and the contrasting yellow nucleus of the babesia it is easier to differentiate damaged and distorted organisms from cellular debris.

**REFERENCES**


Figure 1. Bovine Blood stained with acridine orange, seen under the fluorescence microscope. The erythrocytes are black on a dark green background. Some erythrocytes contain Babesia bigemina, which are bright red with a contrasting yellow nucleus.

Figure 2. Chamber for direct counting of babesia under the fluorescence microscope.
INTRODUCTION

Toxoplasmosis is one of the most widespread infections in man, wild and domestic mammals and birds. Although it is considered a zoonosis, definitive proof of animal-to-man transmission is lacking. The present lack of complete knowledge about its life cycle and mode(s) of transmission precludes definitive control programs in man and lower animals. Knowledge of the prevalence of infection in specific geographic areas in man, domestic and wild mammals and birds is a necessary prerequisite to understanding man-animal interrelationships.

Since the original discovery of *Toxoplasma gondii* 60 years ago, numerous papers and books have been published about toxoplasmosis.

The purpose of this paper is to (a) discuss relevant aspects of the disease, (b) review the studies conducted on man and lower animals in Iowa, (c) encourage veterinary diagnostic laboratories to use and evaluate diagnostic tests presently available for toxoplasmosis, and (d) list several books and publications for further reading.

EARLY HISTORY

*Toxoplasma* was first described by Nicolle and Manceaux in 1908 in a North African rodent, *Ctenodactylus gundi* and in the same year in the rabbit, *Oryctolagus cuniculus*, by Splendore in Brazil.\(^1\) In retrospect, the first description of the disease in man was made in 1923 by Janku, an ophthalmologist, who described the organism but was unable to identify it.\(^3\) The first isolation of *Toxoplasma gondii* from a human patient (newborn infant) was reported in 1939 by Wolf, Cowen, and Paige. Subsequent immunological and biological studies established the identity of the causative agent.\(^4\)

ORGANISM CHARACTERISTICS

*Toxoplasma gondii*, a protozoan parasite, is considered the only member of the genus *Toxoplasma*. Two recognized forms occur: the trophozoite in the acute infection and the cyst in the chronic form.\(^5\)

Organisms isolated from mammalian and avian hosts appear immunologically, serologically, and morphologically similar to human strains, the main difference being in the virulence of the strain for different animal species.\(^6,7\)
Toxoplasma lacks host specificity. It requires an intracellular habitat to reproduce.\textsuperscript{8} It is primarily a parasite of the fixed cells of the reticuloendothelial system but has the ability to parasitize nearly every body cell type with the possible exception of the non-nucleated red blood cells; it can be propagated in tissue culture.\textsuperscript{9}

Multiplication is by longitudinal binary fission and by a method described more recently by Goldman, Carver, and Sulzer,\textsuperscript{10} called endodyogeny, a process of internal budding in which two daughter cells are formed from the parent cell. An electron microscope study has confirmed these observations.\textsuperscript{11} According to Jacobs,\textsuperscript{12} endodyogeny can be considered a special form of shizogony in which only two merozoites are formed.

In fresh preparations, the trophozoite form is sharply outlined, crescent shaped, attenuated at one end, and rounded at the other end and measures 4 to 7 microns in length and 2 to 4 microns in width. The cytoplasm is distinct and clear, and the nucleus is well defined. Geimsa-stained organisms have a blue cytoplasm and a red to purple nucleus located centrally or near the attenuated end.

In chronic infections the cyst form may be found principally in the brain, eye, or muscle tissues. The cysts are usually round or spindle shaped, contain hundreds of organisms, and measure 30 to 100 microns in diameter. The wall of the cyst is considered to be a product of the interaction of host and parasite rather than of the parasite alone. Therefore the term “cyst” should be used rather than “Psuedocyst”.\textsuperscript{12} The cyst is surrounded by a membrane that is argyrophilic, and viable organisms within the cyst contain glycogen and are periodic acid-Schiff (PAS) positive.

The trophozoite form is considered to be sensitive to changes in osmotic pressure, to drying, and to exposure to artificial gastric juices.\textsuperscript{13} This form apparently dies rapidly after it leaves the animal host and apparently does not play a major role in the transmission from animal to animal or animal to man. The cyst form, however, appears to be more resistant and is considered by many to play a major role in the transmission of infection, especially in the omnivorous and carnivorous species. The trophozoite forms are destroyed by peptic juice in a few minutes, whereas the cyst forms remain infective for 3 hours. The cysts also lose their viability after heating at 50\textdegree C. for 30 minutes or 56\textdegree C. for 10 to 15 minutes; freezing and drying have a similar detrimental effect on the parasite.\textsuperscript{14}

These two forms are the only forms presently known in the life cycle of Toxoplasma.

EPIDEMIOLOGY

Transmission. The ubiquitous nature of Toxoplasma throughout the world in human and animal populations, including birds, has precluded complete understanding of the methods for its transmission except by congenital means. Errington’s\textsuperscript{15} statement, “Nature’s way is any way that works,” certainly describes the relative efficiency by which Toxoplasma apparently perpetuates itself. Presently, modes of transmission must be assessed with knowledge of two forms: (a) the trophozoite form in acute infection, believed to be too fragile to be of major importance in direct spread from one host to another, including man (it is well
known that mice, acutely ill, do not transmit infections to cagemates, except possibly by cannibalism); and (b) the cyst form which appears to be more resistant to environmental factors and is considered more important in the transmission of the infection, especially in omnivorous and carnivorous species14 with the largest concentration in tissues of the central nervous system and skeletal muscle.16

Raw or undercooked meat containing encysted Toxoplasma has been shown experimentally to result in infection when fed orally to animals.17 The encysted form can withstand artificial pepsin digestion for up to 3 hours. Jacobs et al. suggested that this may be an additional means of transmission to man,14 and have demonstrated the cyst form in the muscle of sheep, swine18 and chickens.19 However, vegetarians in the United States and India have been found positive serologically.20,21

Experimental studies on parasitemias in animals have suggested the potential for arthropod transmission but have shown essentially negative results.16 Experimental transmission has been successful in most animal species by infecting them by commonly used routes.

McCulloch, et al.22 reported a significant association of skin-test positivity and moderate to marked soil contact which gives indirect support to Jacobs’23 hypothesis that an intermediate stage of Toxoplasma may possibly occur in the soil and that geographical variations in prevalence rates of toxoplasmosis may be due to climatic and soil conditions in different areas.

Hutchison is credited with an important clue to a mode of transmission with his experimental nematode transmission of T. gondii via Toxocara cati eggs in Toxoplasma-infected cats.24,25 These studies have been confirmed in experimentally and naturally infected cats by Jacobs and Melton 25 and by Dubey 27 who further discusses feline toxoplasmosis and its nematode transmission.28 Hutchison also experimentally infected one-day old chicks with toxoplasmosis by feeding them orally a portion of the Toxocara cati ova.26 He thus hypothesized that this transmission mechanism could be responsible for avian toxoplasmosis. Hutchison suggested that man could acquire toxoplasmosis via nematode ova originating from domestic animals that live in close association with him and that nematodes of herbivores may also be implicated as vectors.25 He further comments that “the survival of Toxoplasma within nematode ova which remain viable in the external environment for long periods might account for the lack of seasonal prevalence in toxoplasmosis”.25 Because of the frequent occurrence of toxoplasmosis in chickens and its presence in the intestines of the birds, Jacobs and Melton suggest that studies on the transmission of the infection via helminth eggs (Ascarids and Heterakids) are worthy of investigation.19

Intraspecies transmission of T. gondii via nematode ova may have occurred in the “Pig-to-pig” transmission studies of Verma and Dienst29 and the “Sheep-to-sheep” studies by Hartley.30 These investigators did not mention the presence or absence of intestinal parasites in the swine or sheep studies. Walton et al.31 state the following: “Should nematode egg transmission play a significant role in transmission of human infections, or in maintaining a high level of infection in domestic animal reservoirs, any conditions affecting survival of the nematode eggs would influence the human infection rate . . . when more information is available regarding nematode species capable of transmitting toxoplasms, studies to correlate
the prevalence of these nematodes and toxoplasmosis should prove enlightening." Undoubtedly the epidemiology of toxoplasmosis is determined in part by the oral route of entry and variable eating habits of man and animals throughout the world.

Comparisons have been made in the United States and other areas of the world between urban and rural human populations with some showing differences and other showing none. The differences may be due to associated animal contact rather than residence classification of rural versus urban. Several studies give indirect support to the hypothesis that animal contact is an important factor in the transmission of toxoplasmosis to man. 

**Age and Sex.** Serological surveys reveal an increasing proportion of persons with positive reactions as age increases. Although higher prevalence rates have been observed in females in Norway and Poland, significant differences have not been reported in the United States.

**Geographical distribution.** The lowest human serological or skin-test positive rates have been noted in frigid areas such as Alaska and Iceland, whereas the highest rates have been reported from warmer climates—Honduras and Tahiti; none of the Eskimos tested and 68% of the Tahitians tested were positive at a 1:16 dilution or higher. Hot, dry, or high altitude areas have consistently lower prevalence rates than warm, moist areas.

Generally, prevalence rates in various human population groups in the United States are highest in the East, lowest in the West, and intermediate in the central area. Walls and Schultz noted that the high prevalence area roughly coincides with the Appalachian region. They support the possibility that poor dietary and sanitary conditions which exist in some areas of this region might influence the potential for spread of toxoplasmosis.

**Immunity.** A discussion of immunity regarding the obligate intracellular parasite, *T. gondii*, must include the stage of infection and the form—trophozoite or cyst. Acquired immunity depends upon several factors, of which antibody (dye test) is considered one. Antibodies apparently have little effect on the intracellular form of *Toxoplasma*. During the early stages of infection, extracellular forms are found in the blood, can multiply in macrophages, but soon diminish as antibodies appear. In addition to humoral immunity, cellular immunity appears important in the termination of infection.

Cysts form during late stages of infection, usually with no signs of inflammation surrounding them. In animals and probably in man they are usually found in the brain or eye, possibly because of low immunity in these tissues.

Animals surviving the infection may remain chronically infected. In man and animals hypersensitivity mechanisms may be important during the late stages of acute infection and in the chronic stage, causing continued pathogenesis. Jacobs states that immunity to reinfection is partial and that in toxoplasmosis a state of continued host-parasite equilibration exists rather than absolute immunity.

**ANIMAL TOXOPLASMOsis**

Whether in man or lower animals, toxoplasmosis is primarily an inapparent infection as attested by serologic and/or parasitologic surveys of apparently normal
animal populations.\textsuperscript{18,19,39,51-66}

Although overt disease is considered to be rare, especially in the adult, increased interest in this disease has led to the recognition of a variety of clinical forms, primarily because of increasing availability of diagnostic services for medical and veterinary medical clinicians.

The organ system(s) or tissues affected, the age and species of animal, and the strain of \textit{T. gondii}, all determine the spectrum of clinical signs and course of toxoplasmosis.

Naturally occurring clinical toxoplasmosis in ovine, feline and canine species in the United States was first reported by Olafson and Monlux in 1942.\textsuperscript{67} Cole and co-workers described enzootics in cattle, swine, dogs and sheep in Ohio.\textsuperscript{68} They state that the disease was characterized by fever in acute cases, respiratory distress, and central nervous system disturbances. In pregnant animals, premature birth, abortion, and stillbirths were observed. Significant autopsy findings were pneumonia, intestinal ulceration, gray foci and enlargement of the liver, and an increased amount of fluid in the serous cavity.

Ostendorf and Henderson reported the first description of clinical toxoplasmosis in chickens kept for egg and meat production in the United States in 1962.\textsuperscript{69} However, their study was supported only by morphologic studies and transmission experiments. No serologic studies were conducted.

Jacobs,\textsuperscript{70} Weiner\textsuperscript{71} and Siim et al.\textsuperscript{72} review and discuss the literature regarding naturally occurring and experimental studies of toxoplasmosis in mammalian and avian species.

**DIAGNOSTIC METHODS**

Because the protean clinical manifestations of toxoplasmosis preclude definitive diagnosis on clinical grounds alone, it is imperative that direct and indirect laboratory methods be used.

\textit{Isolation and identification of the parasite.} The laboratory-reared mouse is most commonly used by inoculating necropsy material intraperitoneally, subcutaneously, or intracerebrally. It is essential that laboratory strains of mice be free of toxoplasmosis. Preferably, preinoculation serums collected from the orbital sinus should be tested in parallel with postinoculation serums. Tissues used depend upon the clinico-pathological picture of the case but often include spinal or ventricular fluid, peripheral blood, lymph node, or muscle. Body fluids or tissues should be processed as soon as possible because of the fragile nature of the trophozoite forms of \textit{Toxoplasma}. Freezing destroys both trophozoite and cyst forms. Necropsied material is usually ground with a mortar and pestle in physiological saline solution, and a 10 to 20\% suspension is inoculated into mice. The mice are observed for 4 weeks, their sera are tested for \textit{Toxoplasma} antibodies, and brains are checked for cysts by squashing a portion of the brain between a coverslip and a slide and examining the material by microscope.

If the mice sicken during the observation period, impression smears of lung, liver, brain, and spleen are made with Wright's or Giemsa's stain, and portions of these tissues are subinoculated into fresh mice. Staining of peritoneal fluid may also be done. Morphological identification of the trophozoite or cyst forms is
presumptive support but should not be taken as conclusive proof of *Toxoplasma* infection, several other organisms appear similar. They include *Encephalitozoon, Microtus (M)-organism, Sarcocystis, Besnoitia, Globidium*, leishmaniform-stages of *Trypanosoma, Cryptococcus (Torula), Histoplasma*, and *Klossiella.*73 Serologic evidence in mice with supporting cyst or trophozoite forms give positive evidence of infection. Isolation of *T. gondii* is most decisive but concomitant infections must always be suspected (e.g. canine distemper). Because encysted forms of *Toxoplasma* may remain in brain and muscle tissue for prolonged periods, isolation from these tissues should be supported by clinical and serological evidence if it is to be associated with the current illness. A peptic digestion technique for concentrating parasites from muscle tissue has been successfully used.18

**Serological tests.** Blood specimens collected during the acute and convalescent stages should be tested for *Toxoplasma* antibodies. A rising titer should be observed between acute and convalescent specimens to support a clinical diagnosis. A large proportion of the normal human and animal population in temperate and tropical zones of the world have *Toxoplasma* antibodies.

**SABIN-FELDMAN DYE (SF) TEST.**47 The Sabin-Feldman dye test is most commonly used and is specific and sensitive. The dye test usually becomes positive in 10 to 14 days after onset and reaches a maximum titer in 3 to 5 weeks, often persisting in detectable levels for many years, perhaps for life. A micromodification of this test has been described.92

**JACOBS-LUNDE HEMAGGLUTINATION (HA) TEST.**74 The Jacobs-Lunde hemagglutination test is also commonly used. In acute human cases, HA titers generally are detectable later than dye test titers. Jacobs reviews recent comparative HA and SF studies and further states that the HA test cannot be offered as a substitute for the SF test in suspected cases of acute human toxoplasmosis, either congenital or acquired, but it does have usefulness in the diagnosis of chronic infection.75 Fairchild et al. also report results of a comparative study, i.e. HA and SF.76

**COMPLEMENT FIXATION (CF) TEST.**77 Antibodies detectable by the complement fixation test appear later than either the SF or HA test in man and also diminish much more rapidly; it is often used as an adjunct to these tests. Fowls, in general, may show only slightly positive or completely negative serologic responses with the SF or CF test. Harboe and Reenaas have used a CF inhibition test with more success.78 Robertson et al. in Canada add further information on the use of the CF test with sera of experimentally exposed pigeons, chickens and turkeys. They stated that the direct CF test was suitable for use with pigeon sera; the indirect CF method effectively demonstrated antibodies in chicken and turkey sera.79 They also discuss further studies on the use of the CF test on other domestic animal sera.80

**INDIRECT FLUORESCENT ANTIBODY TEST (IFA).**81 The IFA test has been described and compared with other tests for the serodiagnosis of toxoplasmosis and found to be satisfactory when compared with the SF test.82 A statistical evaluation of internal reproducibility of titers in the IFA test indicates that titers can be reproduced on a within-day basis within limits of plus or minus one twofold dilution with 95 percent confidence.83

Ruckerbauer et al. state that the direct method, the inhibition and indirect
modifications of the fluorescein-labelled antibody technique were suitable for demonstrating *T. gondii* in fluid and tissue-impression slides from animals in the acute phase of infection; this method was not applicable with frozen tissue sections.\(^8^4\) The fluorescence inhibition test was also used on the immune sera of selected avian and mammalian species.\(^7^9,8^4\) Nobuto et al. also studies the complement fixation inhibition test in experimental and spontaneous swine toxoplasmosis.\(^8^5\)

**19S-7S GLOBULIN ANTIBODY TECHNIQUE.**\(^8^6\) A technique of identifying 19S and 7S globulin antibody has been developed by Remington and Miller as an additional aid in the diagnosis of acute human cases.

**Skin-test.**\(^8^7\) In man, this test is regarded primarily as a survey tool, primarily because it takes 2 months or as long as a year following infection for sensitivity to develop.\(^1^9\) Nobuto et al. have used a skin test for the diagnosis of past or latent swine toxoplasmosis.\(^8^8\)

**IOWA STUDIES — A REVIEW**

**HUMAN POPULATION GROUPS TESTED (SKIN-TEST OR SEROLOGICAL)\(^2^2,8^9\)**

**Medical and Veterinary Students:**\(^2^2\) During 1960-61, 775 veterinary and medical students were skin-tested for evidence of previous infection with *Toxoplasma gondii*. The students who reported a "more than 70%" farm background had a significantly greater proportion of skin-test positives than did the group with "more than 70%" of lifetime residence in a city of "more than 2500" population. No significant differences were noted in the proportion of skin-test positives among the four academic classes of veterinary students.

Skin-test positive status was significantly associated with moderate or marked contact with swine, horses, sheep, cattle, cats, chickens, and turkeys. Other statistically significant items included marked soil contact, frequent work with farm animals, and a recollection of having had a pet dog in the home prior to age 6. Items showing no significant association were moderate or marked contact with dogs, wild rabbits, geese, domestic ducks and pigeons and a history of animal bites.

**Serologic Survey of Human Population Groups:**\(^8^9\) Between 1961 and 1965 several population groups were tested serologically by the Sabin-Feldman dye test. The proportion positive (1:16 or higher) by group tested follows: wildlife workers (1962), 19%; veterinarians (1962) 20%; veterinarians (1965), 24%; and premarital blood specimens from Iowa residents, 11%. The proportion of positive titers increased with both sexes by advancing age classification.

**ANIMAL PREVALENCE STUDIES\(^5^1,5^3\)**

**Serologic Survey of Toxoplasmosis in Iowa Domestic Animals:**\(^5^1\) A serologic survey was conducted to determine the presence of *Toxoplasma* antibodies in Iowa cattle, horses, swine, sheep, dogs and cats. Blood specimens from cattle, swine and sheep were collected from more than one source. Part of these studies were conducted in cooperation with Seaton and Bennett of the Iowa Veterinary Medical Diagnostic Laboratory. The proportion positive at a 1:16 dilution or higher by the
Sabin-Feldman dye test was as follows: cattle, none; horses, none; swine, 7-86%; sheep, 13-37%; dogs, 16% and cats, 5%.

Wild Mammal Serologic Survey: Following a human leptospirosis outbreak in 1964, wild mammal epizootiologic studies were conducted on several farms. Traps were set and of 75 animals found in the traps, blood specimens were available for Toxoplasma testing on 47 (the majority of animals not tested were mice that were found dead when removed from the traps). The following wild mammals were collected and found to be positive at 1:16 dilution or higher for Toxoplasma antibodies: raccoon, 0 of 5; squirrels (fox) 0 of 4; muskrats, 0 of 11; rabbits (cottontail) 0 of 8; opposum, 1 of 12 (1:256 titer); fox (red) 1 of 4 (1:256 titer); fox (gray) 2 of 2 (both at 1:64 titer); feral cat, 1 of 1 (1:1024 titer).

These data indicate the prevalence of Toxoplasma antibodies in the animals tested but may not necessarily indicate the prevalence of the infection by species in Iowa.

STUDIES ON AN IOWA FARM WHERE AN ADULT CASE OF TOXOPLASMOsis OCCURRED (1962-64)

The first successful isolation of Toxoplasma gondii from a woman in Iowa was made by Green and Remington in Des Moines, Iowa, in 1957. Following the isolation of T. gondii from an adult farmer in 1962, by Smith, McCulloch and Davis, epidemiologic studies were begun on the patient's farm. Blood specimens were collected from his wife and four children, three being positive at a 1:16 dilution or higher (Sabin-Feldman dye test).

Serologic or parasitologic evidence of past or persistent infection to Toxoplasma (1:16 dilution or higher) was found in sheep (42%), swine (68%), cattle (2%) (1 only at 1:16 dilution), and laying hens. Some of the animals were not on the farm during the presumed exposure period for the adult case. Follow-up studies were conducted during a two-year period after the isolation of T. gondii from the farmer.

The laying hens were used for both egg production and as a source of meat for the family. Using the pepsin-digestion-isolation technique of Jacobs and co-workers, tissue isolation studies were initiated. Laboratory white mice were inoculated intraperitoneally with a suspension of the digest material.

Results of Protocol I and II: Of the 24 pools of chickens (2 chickens per pool), evidence of Toxoplasma infection was found in 20 (83%). The tissues used in protocol I and II were brain (not digested) and ovary-oviduct pools.

Attempts were then made to isolate from other pooled organs and tissues. Heart, breast and thigh muscles were pooled from two chickens and processed as previously described. Additionally, the wings (feathers removed, but skin, subcutaneous tissue and meat included) were processes as described — 8 wings from 4 chickens included as one pool.

Results of Protocol III: Of the fourteen pools (heart, breast, thigh) tested, evidence of Toxoplasma infection was found in 14 or 100%. None of the 7 pools (4 chickens each) of wings were positive.

Specific tissues and organs were then studied, these being heart, breast, gizzard, liver and thigh tissues.
Results of Protocol IV: Evidence of *Toxoplasma* infection was found in 11 of 12 (92%) pools of heart tissue; 5 of 12 (42%) pools of breast tissue; 3 of 12 (25%) pools of gizzards; 1 of 12 (8%) pools of livers; and 1 of 12 (8%) pools of thigh tissue. The widespread nature of toxoplasmosis on this farm (serologic and parasitologic) leads to problems in determining the link between any one animal species and the clinical and subclinical evidence of infection in the farm family. The problem of determining the source of infection is further complicated by the lack of definitive information about the modes or methods by which animals contract the infection.

**COMPARATIVE ECOLOGY OF TOXOPLASMA INFECTIONS IN MAN AND ANIMALS ON AN IOWA FARM**

Studies were initiated to obtain knowledge of the mode(s) of transmission of *Toxoplasma gondii* among sentinel chickens placed on the same farm for one year. During the study period (1966-1967), the farm was under surveillance and selected epidemiologic-epizootiologic factors measured. During each of 45 field trips (40 miles from laboratories), the farm family was interviewed and selected specimens (animal tissues, blood, feces, etc.) returned for testing.

On March 29, 1966, 250 one-day old chicks were placed on the farm. Each month (April through October 1966, and March 1967), 24 chickens were examined (serologic and parasitologic) for evidence of toxoplasmosis. Between November, 1966 and May, 1967 serologic and/or parasitologic studies were conducted on the farm family, and on domestic and wild animals (pigs, dairy cows, horses, dogs, cats, a weasel, mice, sparrows and pigeons). Results: (1) None of the chickens were infected with *Toxoplasma gondii*; (2) none of the pigs, dairy cows or horses were positive serologically (1:16 titer or higher). One dog changed serologically from negative to 1:16 between November, 1966 and May, 1967. Two cats (introduced on farm, January, 1967) were positive (1:16 and 1:64), (3) *Toxoplasma* organisms were isolated from a least weasel but none from mice, sparrows or pigeons. (4) One of the two farm children changed serologically from negative to a 1:4 titer.

Fecal specimens were collected from chickens, pigs and dairy cattle and processed by Hutchison's method.25 Helminth eggs were commonly observed in each species. All attempts to isolate *T. gondii* from nematode ova were unsuccessful.

Possible reasons for the apparent recession (comparing 1966-1967 to 1962-1964 farm study) of toxoplasmosis in the major domestic farm animals (chickens, pigs, dairy cows, horses) include: (a) turnover of animals by death/slaughter, (b) no sheep on the farm during 1966-1967 study, (c) age or breed of animals tested, and/or (d) changing ecologic relationships.

**SUMMARY AND CONCLUSIONS**

Toxoplasmosis is a widespread infection in man, wild and domestic mammals and birds. Although primarily an inapparent infection, it can cause mild to severe clinical signs. The disease may also be chronic or latent in form. In this regard, Siim et al. state "with regard to latent infection, one cannot help wondering about the
situation which would arise if the now apparently well-balanced status between *Toxoplasma* and the host were to become disturbed in favor of the parasite, with the consequent development of manifest severe toxoplasmosis.72

Technologic advances in veterinary medicine, animal science and management practices have helped to increase our food supply. Because of some of these changes, animals have more opportunities to become "stressed". Natural and man-made environmental stresses may cause a shift in this host-parasite balance.

Veterinary medical diagnostic services must be provided if we are to assess the full impact of toxoplasmosis on human and animal health and welfare now and in the future.

ADDENDUM

Jacobs (see reference 75, this publication) first reported the possibility that *T. gondii* might be fecally transmitted in *Toxocara*-negative cats. Hutchison, Dunachie and Work feel that the question of nematode transmission of *T. gondii* is still open; they have also reported that *Toxoplasma* can be fecally transmitted in the absence of nematodes (Acta Path. Microbiol. Scandinav. 74:462-464, 1968).

Frenkel, Dubey and Miller have now shown that infectious fecal forms of *Toxoplasma* can be separated from *Toxocara cati* eggs (In press, Science, Spring, 1969). These findings have been confirmed independently by Sheffield and Melton (In press, Science, Spring, 1969).

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TOXOPLASMOSIS


SUGGESTED READING


CIVIC ACTION

Joe T. Williams, Major, USAF, VC
Medical Civic Action Officer
Directorate of Professional Services
Office of the Surgeon General, USAF

Civic Action is not new. While the term may be new, the concept of Civic Action and the activities of military forces have a long history. It goes back to Biblical days: “and the builders had every one his sword fastened around his loins while they were building. . .” (Nehemiah 4). From Roman legions constructing aqueducts and roads to the empire-building armies of Europe dispensing culture, the military has played a part in social and economic development. As America moved across the continent in 1820, General Zachary Taylor wrote, “The ax, pick, saw, and trowel have become more the implements of the American soldier than the cannon, musket, or sword.” Recent American Civic Action abroad stems from World War II and Korea. Congress confirmed the basic authority for US Military Civic Action in a provision inserted in the 1961 Foreign Assistance Act. President Kennedy followed this up with National Security Action Memorandum No. 119. This Civic Action doctrine assumes that the US Military should advise or support what are essentially host country programs. Civic Action simply means using military forces for non-traditional military projects — projects that are useful to the local population in fields such as education, public works, health, sanitation, agriculture — indeed anything with economic or social progress. We know that limited war or counterinsurgency is the expensive way to take care of conflicting interests and pressures of governments and people. The better way to solve these problems is by eliminating the conditions that give rise to instability and revolution. Here Civic Action plays a primary role. Where it fails to do this, Military Civic Action falters.

In 1966, the Air Force deployed to Northeast Thailand, its first specifically designated Civic Action Unit, a part of the 606th Special Operations Squadron. Basically, the 606th Special Operations Squadron is a medical Civic Action unit actively engaged in various Royal Thai Government Public Health/Nation Building Programs from clinical human medicine and dentistry to the construction of short take-off and landing fields for medical resupply and air evacuation. Medical Civic Action strives to improve the health and well-being of the entire community; to remove the causes of disease and malnutrition. The entire program is quite interesting with each facet a story unto itself, however, this morning, I will limit most of my remarks to the Veterinary activities. I feel very fortunate to have been a “charter member”.

The medical, dental, veterinary, bio-environmental, medical service corps, civil engineering and special operations officers assigned to this unit provided a wide range of specialties as well as a varied background of experience to cope with this departure from traditional military operations. After coordination of our planned activities with the U. S. Embassy’s Country Team and with various Royal Thai Ministries such programs as the following began and have developed over the past
two years. These are not unilateral American programs, but are an American input into existing Royal Thai Government programs. They are Thai programs, not American programs.

1. **1st and 2nd Class Health Centers**: The Royal Thai Government has established small medical centers in rural areas. However, there is a shortage of trained medical personnel to man the centers. We furnished medical and dental officers and enlisted technicians to advise and train Thai medical personnel.

2. **Sanitation and Personal Hygiene.** The goal here is to teach both the medical personnel of the 1st and 2nd class health centers and the teachers in local schools, public health, proper waste disposal, insect control, proper food handling, and personal hygiene. We held classes for the school teachers who then in-turn imparted their knowledge to their students.

3. **Potable Water**: The goal here is to improve the local sources of water; to either help the villagers dig wells or improve existing wells; to case and cap and sometimes to help install a pump so that drinking water supplies would be cleaner. We did occasionally have some trouble when a newly cased, capped well was ready for use, mainly because the water was clearer and lacked the "richness and body."

4. **STOL Strips**: The Civic Engineer provided the guidance, indeed he was the catalyst, to construct several short take-off and landing strips. These strips were used for resupply of our teams, for air evacuation and by Thai officials.

5. **Other Programs**: We were also involved in other programs; advising and teaching simple clinical laboratory procedures; teaching oral hygiene; providing Thai Public Health publications in the health centers; conducting a human parasite survey; conducting a survey of the dental needs of school children; and other programs.

6. **Veterinary Activities**: The veterinary activities varied from long range livestock development to almost immediate projects for food procurement. As Northeast Thailand's economy is based on agriculture, primarily livestock production, an improvement in the livestock industry is essential to economic growth. This section of Thailand supports about 50% of the water buffalo and cattle, and about 30% of the swine. Poultry, both chickens and ducks, are raised by the individual farmers. The diseases and parasites affecting livestock and poultry cause an undetermined economic loss.

In livestock development, a joint Thai-American program with a Thai veterinarian as "Project Officer," assisted by a USAF veterinarian, was designed to survey selected areas of Northeast Thailand to collect data on the animal diseases and parasites most prevalent, and to improve the existing Thai veterinary diagnostic laboratories in these areas. Three joint Thai-American field teams are deployed from three of the laboratories. Each deployed team is composed of a Thai veterinarian and laboratory technicians from the Veterinary Diagnostic Laboratories and a USAF veterinarian, a clinical laboratory technician and a veterinary technician. The Thai veterinarian is the "Team Chief." To enhance the Veterinary Diagnostic Laboratory capability, the tests are performed by the Thais assisted by the USAF counterparts. The villagers gather their animals at a central location where a blood sample and a rapid plate test for brucellosis is accomplished on each animal; fecal samples are taken and examined for internal parasites; a blood smear on each tenth animal is examined for blood parasites; each animal is examined for
ectoparasites and evidence of clinical disease. The water buffalo and cattle are also
immunized against hemorrhagic septicemia and in some cases anthrax or foot and
mouth disease. The hogs are not forgotten, they are given hog cholera vaccine. We
also hope to do serum antibody searches if a sufficiently sophisticated laboratory
can be developed or suitable arrangements can be made with other laboratories.

Just what diseases and parasites and at what level of prevalence do we expect to
find them? We don’t know, only experience will tell us that. We do know that a
highly fatal form of hemorrhagic septicemia is present and that the peak prevalence
corresponds to the plowing season – just when the rice farmer needs his water
buffalo and oxen the most. We also know that anthrax, foot and mouth disease,
brucellosis, tuberculosis, leptospirosis, hog cholera, swine erysipelas, trichinosis, and
parasitism are endemic in Thailand. Parasitism is probably the number one problem
in all animals. Hemorrhagic septicemia is probably the most serious and costly
disease of water buffalo and cattle. Hog cholera probably accounts for more direct
herd death losses than any other swine disease. This is truly an exciting program
that has both vast and varied potential and is a direct and essential part of the
intensification of livestock production. The ability to diagnose and the capability to
control animal diseases and parasites is a vital factor in the improvement of the
agricultural sector of the economy.

Another program that we are deeply involved with is rabies control. Rabies is a
continuing public health problem with over 400 human deaths recorded annually.
In a survey conducted several years ago, by the SEATO Laboratory, approximately
4% of the “normal” appearing stray dogs picked up in Bangkok were positive for
rabies. With a problem of this magnitude, rabies control appears to be self-evident.
However, for many reasons, it was felt by both Thais and Americans that effective
rabies control was not possible in Thailand. In the fall of 1966, a joint pilot rabies
control program was developed to test the hypothesis that effective rabies control
was possible both in urban and rural Thailand.

In many ways, the pilot rabies control program was routine and very similar to
those conducted in the United States. However, the differences, the unusual and
unexpected problems are what make pilot programs interesting and challenging.
The fact that dogs have free run of the streets, are under-fed and under-foot, and
fight over the scraps of food that fall to the floor in the open air restaurants of
up-country Thailand, presents thought provoking problems for solution. The
elimination of these roving, uncared for strays presented the most difficult obstacle.
The idea of eliminating them offended the Buddhist sensitivity to destroying life.
The suggestion was made that baits without poison also be offered. In this way, the
dog could make the decision, and in essence, decide his own fate. If he chose the
unpoisoned bait he would live. This overcame the Buddhist religious objection and
was the course of action followed. Extreme care and control of the baits was
exercised to make absolutely sure the baits went only to stray dogs. The pilot
program has been completed. The success of it is evidenced by the fact that the
Royal Thai Government has assigned two Thai veterinarians and four veterinary
technicians to full time rabies control for the next five years. The Thai team
continues to prove that effective rabies control is possible. They have immunized
over 30,000 dogs against rabies and eliminated more than 3,000 strays. This doesn’t
mean that rabies is now passe and that we are not still involved in improving the
Thais capability to control rabies. Rather, our efforts are now directed to developing the capability to perform laboratory diagnosis of animal rabies in the up-country veterinary diagnostic laboratories and to improving veterinary rabies vaccine production.

Another program is to make available to the rural Thais the resources of the animal breeding stations scattered throughout Northeast Thailand. The better bulls, boars, and roosters are already there. It is a matter of obtaining the service of these animals over a wider area. An interesting custom and one of the reasons why it is so important to increase the use of these better breeding animals concerns the selection of the breeding bulls. The animals are raised primarily to be used as draft animals. Therefore, as the better male animal makes a better draft animal, he is castrated, leaving the poorer male as the stud bull. It isn’t a simple matter of transportation to bring them together, there are many other factors involved, such as disease control, holding pens for overnight stops, and feed. But these problems are being resolved.

Developing sources of fresh eggs is another activity that increasingly shows a vast potential. We haven’t limited ourselves to advice on handling, storage, and grading of eggs, but rather have made this a part of the overall program to teach poultry management, disease prevention and control, and marketing of eggs. The Americans stationed up-country provide a ready market and our efforts were to take advantage of this market. Poultry flocks have been developed and eggs are being sold. We hope after this modest start that the supply of fresh eggs will develop so that all fresh eggs can be purchased from the local community. The incentive is the “profit motive” and the “American market.” As an aside, I feel that each poultry farmer/producer who improves his standard of living becomes an effective barrier (the first line of defense) against insurgency propaganda.

Gentlemen, these are some of the Air Force civic action activities in Northeast Thailand. The concept of placing more emphasis on investment in “Human” development to produce “Ideas” which, in turn, create “Wealth” is one I believe in and it is workable.

Finally, the essential thing to remember about a country’s armed forces is that they exist and can be directed to function where needed. They can be a very effective force in nation building through military civic action.
THE OCCURRENCE OF BLASTOMYCOSIS IN MISSISSIPPI:
PROBLEMS ENCOUNTERED IN DIAGNOSIS

Julius E. Thigpen and J. F. Busey*
State Veterinary Diagnostic Laboratory
Jackson, Mississippi

INTRODUCTION

Blastomycosis is a disease occurring mainly in humans and dogs. However, a case has been reported in a horse,1 a sea lion,14 and a cat.9 There are many questions unanswered concerning the source of infection and the habitat of the fungus in nature.

The disease does not appear to be transmissible from humans to humans, from dogs to dogs, or from dogs to humans.

The causative agent of blastomycosis was first isolated in 1894 by Gilchrist5 from a human hand lesion. It was later named *Blastomyces dermatitidis* in 1898 by Gilchrist and Stokes.6 Meyer11 reported the first canine cases in 1912; however, *B. dermatitidis* was first isolated from a dog in 1936 by Martin and Smith.8

*B. dermatitidis* is a dimorphic fungus which grows in the mycelial phase at 25°C and in the yeast phase at 37°C. Temperature alone is responsible for the conversion of the mycelial phase to the yeast phase.

The organism in the yeast phase (8 to 20 microns in diameter) is spherical in shape, has a characteristic thick, refractile-walled cell, and exhibits single budding with a wide junction between cells. The cell wall is sufficiently thick to give these forms a “double contoured” appearance in fresh preparations.

The mycelial phase is composed of short, broad, 3 to 4-celled hyphal segments, with oval to round microconidia (3 to 4 microns in diameter) attached to the hyphae near the septations or attached directly to the end. These terminal spherical to pyriform conidia are called terminal chlamydospores.

Blastomycosis is primarily a pulmonary disease of dogs and humans. However, the lesions may be confined to the skin and subcutaneous tissue or may be generalized (systemic). The disease has occurred most often in humans (between 30 to 50 years of age) associated with agriculture or outdoor work. The young dog appears to be more susceptible, with the majority of the cases occurring before 3 years of age.9,10

The disease is quite prevalent in Mississippi as indicated by Mississippi being second only to Arkansas in total canine cases (64) reported and first in overall rate/100,000 population when ranked by human cases.2 This is shown in Figure 3.

*John F. Busey, Medical Service, Veterans Administration Hospital, Jackson, Mississippi.

The suggestions of Dr. David Bedell, Dr. J. Branson, and the excellent technical help of Mrs. Margaret Hudspeth are greatly appreciated.
The purpose of this paper is to emphasize the incidence of canine blastomycosis in Mississippi, therefore making more people aware of the disease, and to stimulate more interest, statewide, among veterinarians in recognizing the disease. Some of the problems encountered in making a rapid, accurate diagnosis, which is essential for a successful and economical therapeutic program, will be discussed.

EPIDEMIOLOGY AND OCCURRENCE

Blastomycosis is primarily confined to the United States and Canada, but occasional human infections have been reported in Mexico and in northern South America. Recently several cases have been reported from Africa. North American blastomycosis is endemic to the Mississippi-Ohio River Valley and Middle Atlantic states as indicated by the total number of combined human and canine cases. The states with the highest total (canine and human) blastomycosis cases reported as of January 1, 1967, are: (1) Kentucky - 281; (2) Arkansas - 234; (3) Mississippi - 232; (4) North Carolina - 185, (5) Tennessee - 128; and (6) Louisiana - 110.2

CLINICAL SYMPTOMS

The clinical symptoms of blastomycosis in the dog are many and can best be described here in Figure 9. This chart was taken from a review by Menges.9

PATHOLOGY

The pathology of blastomycosis in the dog has many manifestations. The gross lesions observed are described in Figure 10 from a review by Menges.9

PROBLEMS IN DIAGNOSIS OF BLASTOMYCOSIS

1. Chronic nature of the disease.
2. Awareness of the disease.
3. Preparing specific antigen (CF), (skin test).
4. Preparing specific antisera (FA).
5. Serological cross-reactions (agar-gel precipitation).
6. False positives and negatives.
7. Contaminated specimens for isolation.

EVALUATION OF THESE PROBLEMS

I. Chronic nature of the disease: Pulmonary blastomycosis is initiated by inhaling the organism *B. dermatitidis*. The length of the incubation period is questionable since it is difficult to establish the original source of infection in nature. The duration of illness may vary from one week to 3 years. Menges et al.9,10 reported that 86% of 70 dogs with blastomycosis had an illness that lasted from one week to 4 months.

II. Awareness of the disease: The average veterinarian may not consider blastomycosis as a probable diagnosis until his antibiotic therapy has been
unsuccessful or until blastomycosis has been confirmed by the diagnostic laboratory. In some cases this may be as long as 10 days to a month when culturing the organism. By this time, the prognosis is usually grave. This delay of time in diagnosis may mean the difference between successful treatment of blastomycosis or death. Amphotericin B as well as 2-hydroxystilbamadine have been used successfully in several cases.

Selby et al. have reported that 5% of 151 stray dogs in Arkansas were positive to blastomycin antigen when skin tested. Therefore, we may have 5% or more of reactors undiagnosed in stray dog population in Mississippi.

III. Preparing specific antigen or antisera: This is a major problem due to common antigens found in B. dermatitidis and H. capsulatum. Kaufman and Kaplan demonstrated 5 antigenic fractions (A, B, C, D, and E) between the two organisms. These antigenic fractions were either independent or common fractions between the yeast and mycelial phases. The yeast phases of H. capsulatum and B. dermatitidis were shown to contain the respective specific antigens B and D. Antigens A and C were found in the yeast phase in both H. capsulatum and B. dermatitidis. The mycelial phases of both organisms had no specific antigens. Therefore, to produce a specific antigen for B. dermatitidis, one would best use the yeast phase and adsorb out the common yeast-phase antigens (A, C and E) of H. capsulatum, leaving the specific antigen D, providing there are no other unknown antigens that might cross-react. This specific antigen D, when used in a diagnostic test, should provide the antigen for a more highly specific test or tests, such as agar-gel diffusion, skin test, complement fixation, or fluorescent antibody.

IV. Cross-reactions (False positives and negatives): The skin sensitivity, complement fixation, and agar-gel diffusion test have been widely used in humans suspected of having blastomycosis. These tests are less reliable in blastomycosis than in other systemic fungal diseases. The skin test results with Blastomyces antigen are more frequently negative than positive in persons having blastomycosis. The complement-fixation tests are positive in only 30 to 60% of patients with proved blastomycosis. The agar-gel precipitation test results are more reliable than either of these two. Comparisons of these three tests are shown in Figure 11. Comparisons of the fluorescent antibody, culture and histological studies are shown in Figure 12.

V. Problems in isolation: It is difficult to obtain sputum from dogs. In dogs with skin lesions the problem is not so difficult. Most specimens are contaminated with bacteria or other fungi. The saprophytic fungi will outgrow the Blastomyces and make isolation more difficult. Antibiotics such as penicillin and streptomycin should be sued for bacterial contaminants. Actidione or chloramphenicol can be used for the common fungal contaminants; however, actidione will also suppress growth of some pathogenic fungi such as Cryptococcus.

The pH of the media (7.0) is important, especially in converting the phases.

Slow growth of the organism is a problem. It takes 6 to 7 days to have sufficient growth to see at room temperature. This time is often longer when
converting back to the yeast phase. This delay in diagnosis, especially when depending on culture, is time-consuming.

DISCUSSION OF DIAGNOSTIC PROBLEMS

None of the present-day serological tests are accurate enough for a confirmative diagnosis of B. dermatitidis. A positive diagnosis can be made only on seeing the organism in exudates, growing in culture, or demonstration of the organism in biopsy specimens or tissue sections. It is the yeast phase of the organism that is found in the tissues or exudates.

It would appear from these data that the skin sensitivity and complement-fixation tests are of little value as a diagnostic tool. The agar-gel precipitation test seems to offer the best aid as a diagnostic tool, although there is room for improvement in this test.

From the correlation of FA, histological, and culture methods, it would appear that neither alone is sufficient for an accurate diagnosis in all positive cases. All three techniques should be employed for the most satisfactory results.12

SUMMARY

Blastomycosis is more widespread and occurs more frequently in the dog in Mississippi than is generally recognized. Mississippi with 64 cases is second only to Arkansas with 104 in the total number of canine cases reported through January 1, 1967. This is more than any other state has reported during these two years. Mississippi is first among the states in total human cases reported with 7.71 as the overall rate/100,000 population. Busey2 suggests that the incidence of canine blastomycosis may be ten times greater in the canine than in the human population. This was based on the findings in several counties in Arkansas and Mississippi where both the veterinary and medical professions were aware of and on the look-out for the disease. This would suggest that there is more blastomycosis occurring in the dog than has been reported.

These data indicate that Mississippi should be considered a highly endemic area and should lend itself to some interesting studies in the future concerning blastomycosis.
Canine Blastomycosis in the United States and Canada 1912-1960

- 1 case  Total cases: 128

Suspected endemic area, based on reported human cases
UNITED STATES BLASTOMYCOSIS BY STATES
NUMBER OF CASES: HUMAN/CANINE

FIGURE 2 shows the origin of all reported human and canine cases in the U.S. as of January 1, 1967.
FIGURE 3 shows blastomycosis ranked by total canine cases.

UNITED STATES BLASTOMYCOSIS
Overall Rate / 100,000 Population*
Ranked by Canine Rates2

<table>
<thead>
<tr>
<th>Rank</th>
<th>State</th>
<th>Canine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arkansas</td>
<td>40.76 (104)</td>
<td>7.28 (130)</td>
</tr>
<tr>
<td>2</td>
<td>Mississippi</td>
<td>20.57 (64)</td>
<td>7.71 (168)</td>
</tr>
<tr>
<td>3</td>
<td>Kentucky</td>
<td>13.82 (60)</td>
<td>7.27 (221)</td>
</tr>
<tr>
<td>4</td>
<td>Iowa</td>
<td>4.57 (18)</td>
<td>0.54 (15)</td>
</tr>
<tr>
<td>5</td>
<td>Alabama</td>
<td>2.57 (12)</td>
<td>0.95 (31)</td>
</tr>
<tr>
<td>6</td>
<td>Louisiana</td>
<td>2.15 (10)</td>
<td>3.07 (100)</td>
</tr>
<tr>
<td>7</td>
<td>Tennessee</td>
<td>1.96 (10)</td>
<td>3.31 (118)</td>
</tr>
<tr>
<td>8</td>
<td>Illinois</td>
<td>1.74 (25)</td>
<td>0.56 (56)</td>
</tr>
<tr>
<td>9</td>
<td>Georgia</td>
<td>1.60 (9)</td>
<td>0.81 (32)</td>
</tr>
<tr>
<td>10</td>
<td>North Carolina</td>
<td>1.54 (10)</td>
<td>3.84 (175)</td>
</tr>
</tbody>
</table>

*1960 Population Census

FIGURE 4 shows U. S. Blastomycosis ranked by total human cases.

UNITED STATES BLASTOMYCOSIS2
Total Number of Cases
Ranked by Human Cases

<table>
<thead>
<tr>
<th>Rank</th>
<th>State</th>
<th>Human</th>
<th>Canine</th>
<th>Total Human + Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kentucky</td>
<td>221</td>
<td>60</td>
<td>281</td>
</tr>
<tr>
<td>2</td>
<td>North Carolina</td>
<td>175</td>
<td>10</td>
<td>185</td>
</tr>
<tr>
<td>3</td>
<td>Mississippi</td>
<td>168</td>
<td>64</td>
<td>232</td>
</tr>
<tr>
<td>4</td>
<td>Arkansas</td>
<td>130</td>
<td>104</td>
<td>234</td>
</tr>
<tr>
<td>5</td>
<td>Tennessee</td>
<td>118</td>
<td>10</td>
<td>128</td>
</tr>
<tr>
<td>6</td>
<td>Louisiana</td>
<td>100</td>
<td>10</td>
<td>110</td>
</tr>
<tr>
<td>7</td>
<td>Wisconsin</td>
<td>74</td>
<td>8</td>
<td>82</td>
</tr>
<tr>
<td>8</td>
<td>Illinois</td>
<td>56</td>
<td>25</td>
<td>81</td>
</tr>
<tr>
<td>9</td>
<td>W. Virginia</td>
<td>56</td>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>10</td>
<td>Michigan</td>
<td>36</td>
<td>0</td>
<td>36</td>
</tr>
</tbody>
</table>
FIGURE 5 shows U. S. blastomycosis overall rate/100,000 population ranked by human rates.

**UNITED STATES BLASTOMYCOSIS**

*Overall Rate / 100,000 Population*<sup>*</sup>

*Ranked by Human Rates*

<table>
<thead>
<tr>
<th>Rank</th>
<th>State</th>
<th>Human</th>
<th>Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mississippi</td>
<td>7.71 (168)</td>
<td>20.57 (64)</td>
</tr>
<tr>
<td>2</td>
<td>Arkansas</td>
<td>7.28 (130)</td>
<td>40.76 (104)</td>
</tr>
<tr>
<td>3</td>
<td>Kentucky</td>
<td>7.27 (221)</td>
<td>13.82 (60)</td>
</tr>
<tr>
<td>4</td>
<td>North Carolina</td>
<td>3.84 (175)</td>
<td>1.54 (10)</td>
</tr>
<tr>
<td>5</td>
<td>Tennessee</td>
<td>3.31 (118)</td>
<td>1.96 (10)</td>
</tr>
<tr>
<td>6</td>
<td>Louisiana</td>
<td>3.07 (100)</td>
<td>2.15 (10)</td>
</tr>
<tr>
<td>7</td>
<td>W. Virginia</td>
<td>3.01 (56)</td>
<td>0.38 (1)</td>
</tr>
<tr>
<td>8</td>
<td>Wisconsin</td>
<td>1.87 (74)</td>
<td>1.42 (8)</td>
</tr>
<tr>
<td>9</td>
<td>District of Col.</td>
<td>1.44 (11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>10</td>
<td>Alabama</td>
<td>0.95 (31)</td>
<td>2.57 (12)</td>
</tr>
<tr>
<td>10</td>
<td>North Dakota</td>
<td>0.95 (6)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>*</sup> 1960 Population Census
FIGURE 6 shows Mississippi's total human and canine cases by counties from 1963 to January 1, 1967.
FIGURE 7 shows Mississippi's blastomycosis by yearly rate/100,000 population from 1963-67 when ranked by human rates.

**MISSISSIPPI BLASTOMYCOSIS**
Yearly Rate / 100,000 Population
Based on 1963-67
Ranked by Human Rates

<table>
<thead>
<tr>
<th>Rank</th>
<th>County</th>
<th>Human</th>
<th>Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leake</td>
<td>4.57 (4)</td>
<td>8.00 (1)</td>
</tr>
<tr>
<td>2</td>
<td>Lamar</td>
<td>2.72 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>Marion</td>
<td>2.64 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>Jones</td>
<td>2.62 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5</td>
<td>Jasper</td>
<td>2.41 (2)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*1965 Population Estimate

FIGURE 8 shows Mississippi blastomycosis by yearly rate/100,000 population from 1963-67 when ranked by canine rates.

**MISSISSIPPI BLASTOMYCOSIS**
Yearly Rate / 100,000 Population
Based on 1963-67
Ranked by Canine Rates

<table>
<thead>
<tr>
<th>Rank</th>
<th>County</th>
<th>Canine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Newton</td>
<td>21.43 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>Rankin</td>
<td>17.54 (5)</td>
<td>0.51 (1)</td>
</tr>
<tr>
<td>3</td>
<td>Perry</td>
<td>15.38 (1)</td>
<td>2.19 (1)</td>
</tr>
<tr>
<td>4</td>
<td>Winston</td>
<td>14.81 (2)</td>
<td>2.09 (2)</td>
</tr>
<tr>
<td>5</td>
<td>Hinds</td>
<td>13.68 (21)</td>
<td>0.65 (7)</td>
</tr>
</tbody>
</table>

*1965 Population Estimate
FIGURE 9 shows the frequency of symptoms and signs observed in 66 canine blastomycosis cases.9

THE FREQUENCY OF SYMPTOMS AND SIGNS OBSERVED IN 66 CANINE BLASTOMYCOsis CASES9*

<table>
<thead>
<tr>
<th>Symptom or Sign</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnea</td>
<td>27</td>
<td>41</td>
</tr>
<tr>
<td>Cutaneous Abscesses</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>Fever</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>Anorexia</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>Depression</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>Blindness</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Lameness</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Nasal and Eye Discharge</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Weakness</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Enlarged Lymph Nodes</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Chronic Cough</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Tumor-like Mass in Skin</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Draining Lymph Nodes</td>
<td>9</td>
<td>14</td>
</tr>
</tbody>
</table>


FIGURE 10 shows the frequency of tissues with gross lesions observed in 82 canine blastomycosis cases.9

THE FREQUENCY OF TISSUES WITH GROSS LESIONS OBSERVED IN 82 CANINE BLASTOMYCOsis CASES9*

<table>
<thead>
<tr>
<th>Organ or Tissue</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>57</td>
<td>70</td>
</tr>
<tr>
<td>Lymph Node</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>Skin</td>
<td>34</td>
<td>41</td>
</tr>
<tr>
<td>Eye</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Liver</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Spleen</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Kidney</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Bone</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Pleura</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Testicle</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Joint</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
FIGURE 11 shows a comparison of diagnostic aids in blastomycosis.3,4,10

**COMPARISON OF DIAGNOSTIC AIDS IN BLASTOMYCOSIS**3,4,10

<table>
<thead>
<tr>
<th>TEST</th>
<th>Antigen</th>
<th>Precipitation</th>
<th>Complement Fixation</th>
<th>Skin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast and mycelial</td>
<td>31</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Yeast</td>
<td>33</td>
<td>33</td>
<td>--</td>
<td>8</td>
</tr>
<tr>
<td>Mycelial</td>
<td>7</td>
<td>--</td>
<td>8</td>
<td>--</td>
</tr>
<tr>
<td>Total Positive</td>
<td>71</td>
<td>33</td>
<td>8</td>
<td>--</td>
</tr>
<tr>
<td>Neither</td>
<td>9</td>
<td>32</td>
<td>9</td>
<td>--</td>
</tr>
<tr>
<td>Total Proven Cases</td>
<td>80</td>
<td>65</td>
<td>17</td>
<td>47%</td>
</tr>
<tr>
<td>%</td>
<td>88.7%</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 12 shows a comparison of fluorescent antibody, histopathology, and culture in 372 animals examined for *Blastomyces dermatitidis*.12

**COMPARISON OF FLUORESCENT ANTIBODY, HISTOPATHOLOGY, AND CULTURE IN 372 ANIMALS EXAMINED FOR BLASTOMYCES DERMATITIDIS**12

<table>
<thead>
<tr>
<th>Fluorescent Antibody</th>
<th>Histopathology</th>
<th>Culture</th>
<th>No. of Animals</th>
<th>Proved Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>338</td>
<td>0</td>
</tr>
</tbody>
</table>

Total no. of animals tested 372; no. of proved cases 34.

FA - 25 of 34 = 74%
Histo. - 32 of 34 = 94%
Culture - 11 of 34 = 32%
REFERENCES


STUDIES OF BOVINE MASTITIS:
A COMPARISON OF THE EFFICIENCY OF TWO METHODS OF CULTURING MILK FOR THE DIAGNOSIS OF BOVINE UDDER INFECTION – THE FRESH MILK-BLOOD AGAR METHOD VS. THE INCUBATED MILK-TELLURITE-THALIUM, CRYSTAL VIOLET METHOD

G. E. Morse, D. V. M.*

A fresh milk-blood agar method of culturing milk for the detection of udder infection has been described recently. It was shown that this is a simple, accurate and economical method for the detection of most bacterial infections associated with bovine mastitis. The method will also accurately reflect the absence of udder infection.

From its extensive use, both as a research procedure and in field studies, generally in connection with mastitis control programs; it has been established that the streptococci and staphylococci are the most frequently detected forms of udder infection accounting for approximately 98% of all udder infections in the cow. The fresh milk-blood agar method readily detects these two principle forms of udder infection (Figs. 1 and 2), and reveals the presence of Gram negative bacilli such as the coliforms and pseudomonads. It also detects the uninfected state (Fig. 3) despite the fact that milk samples must be collected through a teat canal that cannot be sterilized and under conditions that predispose to contamination of the milk samples with organisms arising from the cow's environment. The milk samples must be carefully collected to minimize contamination and the incubation of fluid milk samples (i.e., before streaking on a solid media) must be avoided if accurate results are to be obtained for the diagnosis of udder infection. The reliability of the fresh milk-blood agar method in studying udder infection over a long period is shown in Fig. 4. Note the regularity with which the infection forms are repeatedly isolated on successive weeks in both the lactating and dry periods.

A second basic method of culturing milk with numerous modifications has been widely used to detect udder infections. This method is based on a principle involving prior incubation of milk samples in order to increase the numbers of bacteria to more detectable levels.

The current interest in accurate bacteriological methods for detecting udder infections prompts the present report of a study made by the author and the late Dr. James M. Murphy in 1957. The purpose of the study was to compare the efficiency and the accuracy of the two basic methods: fresh milk vs. incubated milk.

Prior to 1930, the bacteriological culturing of milk was generally based on the sound principles of Koch who discovered and developed the use of solid culture media for the study of bacteria in pure culture. Between 1930 and 1940, other methods for detecting udder infection were developed and widely promoted. It was

*Bovine Mastitis Research Unit, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania 19348.
generally assumed that most mastitis was caused by the streptococci and investigators were looking for short cuts that would avoid the tedious and time-consuming procedures of pour-plating. Bryan\textsuperscript{1} turned to the microscopic examination of milk films and recognized that fresh milk did not usually contain sufficient numbers of bacteria to be consistently detected by this method. For this reason, he incubated the milk sample before preparing the milk film. In 1936, Hotis\textsuperscript{4} developed a test which made it possible to examine milk for bacterial content without using a microscope. Reed,\textsuperscript{9} using incubated milk without inhibitory media, came to the erroneous conclusion that all udders had a “normal” bacterial flora. Such methods, which failed to recognize the principle of the “doubled unknown,” have been responsible for much of the laboratory muddle described by Murphy.\textsuperscript{6} Fortunately, they have fallen into disuse, if not disrepute, by most investigators.

During this same period, two other groups, Edwards\textsuperscript{3} in England, and Plastridge \textit{et al}\textsuperscript{8} in Connecticut, devised methods of using incubated milk and inhibitory media primarily for the detection of streptococci, particularly of \textit{Str. agalactiae}. These methods are suitable for \textit{Str. agalactiae} detection because this organism, being an obligatory udder pathogen, is seldom found as a contaminant in carefully collected milk samples. The principle deficiency of these procedures is the inability to make a clear-cut distinction between contaminating and infecting streptococci other than \textit{Str. agalactiae} and in masking the presence of other forms of udder infection.

Using the same model as was used for the fresh milk-blood agar method, we compared the results obtained when culturing milk from uninfected cows using:

1. the fresh milk-blood agar procedure;
2. enrichment, milks incubated without inhibitors;
3. selective enrichment, milks incubated with inhibitors, potassium tellurite-boric acid broth* and then streaked on additional inhibitory media, i.e., thallium-crystal violet agar* (TCV).\textsuperscript{2}

Milk samples from 5 cows, known to have uninfected udders by long-term twice-daily cultural examinations, were examined culturally by the 3 methods for 5 consecutive days.

The results of the cultural examinations are shown in Figs. 5, 6, and 7. Seventeen percent (17\%) of the samples cultured by the fresh milk-blood agar method showed some bacterial growth as small numbers of colony forming units (CFU) without the appearance of similar CFU’s in succeeding samples thus making it possible to clearly identify them as contaminating bacteria. Aliquot portions of the same milk samples when cultured on blood agar following incubation of the milk sample (enrichment) revealed 38\% of the quarter samples with bacterial growth in such numbers as to preclude identification as contaminating organisms except by the failure of the same specie to appear on successive samples. A 17\% level of contaminating growth was observed on the TCV agar. Although this level was the same as detected on the fresh-milk-blood agar cultures, the growth was of obviously different forms and CFU’s were present in sufficient numbers to suggest

\*See appendix for formula
an actual infection. The similarity to actual infection could be a cause of serious error if diagnosis was based on a single cultural examination as is often the case.

A second series consisted of culturing three successive samples collected at one milking from the same 5 cows with uninfected udders. The samples were cultured in the fresh state on blood agar and then incubated (enriched) and again cultured on blood agar. (Figs. 8 and 9). Although the samples were carefully collected, 15% of the quarter samples showed the presence of contaminating CFU in very small numbers, with 30% of the enriched samples showing bacterial growth that could not be distinguished from actual infection by a single cultural examination.

To show the effect of teat canal contamination which cannot be removed by teat end scrubbing, syringe samples7 were collected before the regular samples from 4 left-front teats and 4 right-front teats of each of 4 cows for 5 consecutive days. The front teats only were used because of the greater difficulty in syringe sampling rear teats. Immediately following syringe sampling, milk samples were collected from the same teats in the usual manner by milking through the teat canals. Both sets of fresh samples were streaked on blood agar and then incubated and streaked on blood agar. Results are shown in Figs. 10 and 11.

Two of the syringe samples out of 40 (5%) had contaminating CFU's present on blood agar while 4 out of 40 (10%) in the incubated (enrichment) series showed contaminating growth. Samples collected through the teat canals from the same glands at the same time showed 10 out of 40 (25%) and 16 out of 40 (40%) with contaminating CFU's for the fresh and enrichment procedure respectively. A graphic summary of the two sampling and cultural methods is shown in Fig. 12.

The data thus far have shown that there is an inherent error in using results obtained by the prior incubation of milk samples since false positives are obtained on milks collected from cows known to be uninfected.

The proponents of using incubated milk often claim that it is more efficient in detecting Str. agalactiae. The efficiency of the two methods for the detection of this form of infection was compared. The enrichment procedure consisted of transferring a 1 ml. aliquot of a given milk sample which had previously been streaked on blood agar to a vial containing 3 ml. of potassium tellurite-boric acid broth. The milk broth mixture was incubated for 18 to 24 hours at 37°C. A loopful (0.01 ml.) of the thoroughly mixed broth culture was plated on one quadrant of the surface of a TCV agar plate exactly as with the fresh milk-blood agar procedure. The inoculated plates were incubated at 37°C and examined for bacterial growth after 24 and 48 hours. The bacterial growth was usually heavy and confluent, although occasionally individual colony forming units could be recognized. Str. agalactiae growth was identified by an intense blue-violet color which is typical only for this organism. Bacterial growth other than Str. agalactiae was grouped by appearance into 3 general color groups: brown, green and white based on colony appearance and the color of the adjacent media. A comparison of the two methods is summarized in Table 1.

A total of 2,429 quarter samples were cultured on both blood agar and TCV agar plates. There were 1,715 quarters free of pathogenic bacteria on blood agar media, 1,319 (77%) of these were also negative on the TCV media. Of 158 quarter samples identified with Str. agalactiae present on blood agar, 142 showed the typical blue color on the TCV plates (90%). Two quarters (1%) were negative due
to over-inhibition, while 14 quarters (9%) were masked by bacterial growth of the brown, green or white forms. Four (0.2%) of the 1715 quarters which were negative on blood agar showed the characteristic growth and blue color on TCV which is indicative of Str. agalactiae. The potassium tellurite-boric acid broth and TCV media effectively inhibited 336 (60%) of the 556 quarter samples which had shown some form of bacterial growth other than Str. agalactiae on blood agar. The principal form of growth in the 220 quarter samples which were not sufficiently inhibited by the tellurite-boric acid broth and TCV agar was of the green category.

As the tellurite-boric acid broth–TCV media is primarily designed to permit the growth of streptococci and particularly to identify Str. agalactiae the relative efficiency of the two methods in this area is of particular interest. It is possible that the additional isolations on the plates were the result of contamination of samples from uninfected quarters with Str. agalactiae organisms from infected quarters. However, if one assumed that all the TCV isolates were, in fact, true infections, then there were a total of 166 Str. agalactiae infected quarters as revealed by both methods, of which 158 (95%) were detected by the blood agar method and 150 (90%) by the TCV methods. Four quarters were shedding too few organisms to be detected by the fresh milk-blood agar method and 4 quarters which should have yielded Str. agalactiae colonies were masked by the presence of other bacterial growth. The fresh milk-blood agar method failed to detect a total of 8 (5%) of the 166 Str. agalactiae infected quarters.

The incubated milk-TCV method prevented the growth of Str. agalactiae in 2 of the 158 quarter samples showing Str. agalactiae on blood agar and masked the presence of Str. agalactiae by overgrowth with other organisms in 14 of the 166 quarter samples in which Str. agalactiae was detected by either method. It failed to detect 16 (10%) of the 166 Str. agalactiae quarters.

In summary, both methods identify Str. agalactiae infection reasonably well with a 95% and 90% efficiency for the fresh milk-blood agar vs. the incubated milk-tellurite-TCV method respectively. The ability of the TCV enrichment procedure to detect the presence of Str. agalactiae present in numbers too low to be detected by the blood agar method could be of considerable value in the final stages of a Str. agalactiae eradication program. Although the differences were not particularly marked, they were consistently in favor of the fresh milk-blood agar method when one considers only the detection of Str. agalactiae. The fresh milk-blood agar method is clearly superior in detecting the other forms of infection.
### TABLE 1
**COMPARISON OF THE EFFICIENCY OF TWO CULTURAL METHODS**

<table>
<thead>
<tr>
<th>FRESH MILK-BLOOD AGAR MEDIA (FM-BA)</th>
<th>MILK INCUBATED IN FLUID STATE TELLURITE BROTH, THALLIUM-CRYSTAL VIOLET MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QUARTER</strong></td>
<td><strong>COLONY COLOR</strong></td>
</tr>
<tr>
<td><strong>CULTURES</strong></td>
<td><strong>NEGATIVE</strong></td>
</tr>
<tr>
<td>Negative</td>
<td>1,715</td>
</tr>
<tr>
<td>Streptococcus Agalactiae</td>
<td>158</td>
</tr>
<tr>
<td>Other Streptococci</td>
<td>129</td>
</tr>
<tr>
<td>Hemolytic Staphylococci</td>
<td>174</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>236</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^a\) Intense blue-violet color indicative of *Str. agalactiae.*  
\(^b\) = too few bacteria per ml. to be detected on BA - 4/1,715 (0.2%)  
\(^c\) = TCV media too inhibitory - 2/158 (1%)  
\(^d\) = enrichment cover-up - 14/158 (9%)  
\(^d\) = blood agar cover-up - 4/556 (0.7%)  

Total *Str. agal.* "error": BA 8/166 - 5%; TCV 16/166 - 10%
POTASSIUM TELLURITE – BORIC ACID BROTH

Ingredients:
- Dextrose infusion broth (BBL 01-129) 30 g.
- .1% Potassium tellurite solution 50 ml.
- 4% Boric acid solution (Dissolve boric acid crystals fresh for each preparation) 50 ml.
- Distilled water 900 ml.

Add dextrose infusion broth to flask. Add distilled water and the two solutions and heat until all ingredients are dissolved. Dispense in 3 ml. quantities per vial, cap loosely and autoclave at 15 lbs. for 15 min. Remove promptly to prevent overheating. Tighten caps and store in refrigerator.

THALLIUM, CRYSTAL VIOLET MEDIUM (TCV)

Ingredients:
- Blood agar base (Difco 9945-01) 40 g.
- Esculin 1 g.
- 3% Thallium sulfate solution 10 ml.
- Crystal violet solution (0.1 g. per 75 ml. distilled water) 1 ml.
- Distilled water 1,000 ml.

Add the blood agar base to a 2 liter flask and add part of the distilled water. Measure esculin, add to flask. Add the thallium sulfate and the crystal violet solutions. Add remaining distilled water, swirl flask, melt and dispense by 200 ml. quantities into 300 ml. flasks. Autoclave at 15-lb pressure for 10 min. Add 10 ml. sterile bovine blood to each flask and mix thoroughly prior to pouring. Cool and pour plates as for blood agar preparation. Incubate poured plates 18 hours to dehydrate and then store in refrigerator.
CONSECUTIVE SAMPLES COLLECTED
FROM AN INFECTED UDDER
PLATED ON BLOOD AGAR

STREPTOCOCCI

COW 104 AM PM

MONDAY

TUESDAY

WEDNESDAY

THURSDAY

FRIDAY

QUARTER KEY [RF LR RR LF]

FIGURE 1
Fresh milk-blood agar cultural results from foremilk samples collected twice daily for five consecutive days from cow 104 infected with Str. agalactiae in the LR, LF and RF quarters. Note the consistent recovery of streptococci in the successive samples.
The fresh milk-blood agar cultural results from foremilk samples collected twice daily for five consecutive days from cow 37 infected with hemolytic staphylococci in the RR quarter. Note the consistent recovery of the staphylococcus and the presence of alpha and beta hemolytic zones.
CONSECUTIVE SAMPLES COLLECTED FROM AN UNINFECTED UDDER PLATED ON BLOOD AGAR

COW 128

AM  PM

MONDAY

TUSSDAY

WEDNESDAY

THURSDAY

FRIDAY

FIGURE 3

The fresh milk-blood agar cultural results from the foremilk samples collected twice daily for five consecutive days from cow 128 with an uninfected udder. Two quadrants (Monday PM and Tuesday AM) have a single CFU which does not appear on successive samples; an indication that they are contaminating bacteria rather than an actual infection.
Fresh milk-blood agar cultural results from foremilk samples collected from 10 cows at weekly intervals for 40 weeks. Solid circles = hemolytic staphylococci, open circles = non-hemolytic staphylococci, Y = yeast-like organisms, C = coliforms, S = streptococci, T = intramammary infusion of antibiotic. Areas enclosed in boxes denote dry periods. Note the consistency of cultural results.
CONSECUTIVE DAILY SAMPLINGS
Plated fresh on blood agar

LORIE  ELEANOR  BLANCHE  FRANCINE  ARLENE

MONDAY

TUESDAY

WEDNESDAY

THURSDAY

FRIDAY

1/20  3/20  8/20  1/20  4/20
5%    15%   40%   5%    20%

17/100 = 17%

FIGURE 5
Fresh milk-blood agar cultural results from foremilk samples collected for five consecutive days from 5 cows with uninfected udders. Numerical notations indicate ratio of samples with contaminating CFU’s vs. total samples cultured.
CONSECUTIVE DAILY SAMPLES
Plated on blood agar after enrichment

LORIE  ELEANOR  BLANCHE  FRANCINE  ARLENE

MONDAY

TUESDAY

WEDNESDAY

THURSDAY

FRIDAY

6/20  8/20  9/20  10/20  5/20
30%  40%  45%  50%  25%

38/100 = 38%

FIGURE 6
Cultural results from aliquots of the foremilk samples depicted in Fig. 5 following incubation of the milk prior to streaking on blood agar. Numerical notations indicate ratio of samples with contaminating CFU's vs. total samples cultured.
CONSECUTIVE DAILY SAMPLES
Plated on TCV agar after enrichment

LORIE ELEANOR BLANCHE FRANCINE ARLENE

MONDAY

TUESDAY

WEDNESDAY

THURSDAY

FRIDAY

2/20 4/20 5/20 5/20 1/20
10% 20% 25% 25% 5%

17/100 = 17%

FIGURE 7
Cultural results from aliquots of the foremilk samples depicted in Fig. 5 following incubation of the milks in inhibitory broth and culturing the broth-milk mixture on TCV agar. Numerical notations indicate ratio of samples with contaminating CFU's vs. total samples cultured.
CONSECUTIVE SAMPLINGS SAME DAY

Plated fresh

First | Second | Third
---|---|---
LORIE | 4/20 | 4/20
ELEANOR | 20% | 20%
BLANCHE | 5%
FRANCINE
ARLENE | 9/60 = 15%

FIGURE 8
Fresh milk-blood agar cultural results from 3 milk samples collected successively at one milking from each of 5 cows with uninfected udders. Numerical notations indicate ratio of samples with contaminating CFU's vs. total samples cultured.
CONSECUTIVE SAMPLINGS SAME DAY
Plated after enrichment

First  Second  Third

LORIE

ELEANOR

BLANCHE

FRANCINE

ARLENE

7/20  6/20  5/20
35%  30%  25%

18/60 = 30%

FIGURE 9
Blood agar cultural results from aliquots of 3 milk samples collected successively at one milking from each of 5 cows as depicted in Fig. 8 following incubation of the milk prior to streaking. Numerical notations indicate ratio of samples with contaminating CFU's vs. total samples cultured.
STUDIES ON BOVINE MASTITIS

TEAT CANAL SAMPLES

Four LF Teats  Four RF Teats

Fresh  Enriched  Fresh  Enriched

MONDAY

TUESDAY

WEDNESDAY

THURSDAY

FRIDAY

6/20  10/20  4/20  6/20

Enriched 16/40 = 40%

Fresh 10/40 = 25%

FIGURE 10

Cultural results from milk samples collected by syringe through the wall of the teat for five consecutive days from 8 front teats and streaked in the fresh state on blood agar. An aliquot portion was incubated prior to streaking on blood agar. Numerical notations indicate ratio of samples with contaminating CFU's vs. total samples cultured.
Blood agar cultural results from milk samples collected in the regular manner through the teat canals of 8 front teats immediately after the syringe samples depicted in Fig. 10 were collected. Aliquot portions were streaked fresh and following incubation on blood agar. Numerical notations indicate ratio of samples with contaminating CFU's vs. total samples cultured.
Graphic representation of the degree of contamination obtained by the two methods of sample collection and two cultural procedures. Samples were from udders known to be uninfected. CE = canal sample, enriched (by prior incubation). CF = canal samples streaked fresh. SE = syringe samples enriched (prior incubation). SF = syringe samples streaked fresh.
REFERENCES


A DIRECT FLUORESCENT ANTIBODY TECHNIQUE FOR THE EXAMINATION OF ANIMAL BYPRODUCTS, FOODS AND FEEDS FOR SALMONELLA. A PRELIMINARY STUDY

Edwin M. Ellis and Rube Harrington, Jr.
Diagnostic Services, Animal Health Division
Agricultural Research Service
United States Department of Agriculture
Ames, Iowa

INTRODUCTION

The presence of Salmonellae in a wide range of feeds and animal byproducts is causing great concern among feed manufacturers, livestock producers, and Animal Health officials. A rapid method for the detection of these organisms in feeds and animal byproducts is a very desirable technique and one that is needed.

Coons and his associates in 1942 introduced the fluorescent antibody technique which made possible a new rapid method for detecting microorganisms. Thomason, Cherry and Moody were the first to apply the FA technique to the Salmonellae. Later, Thomason, Cherry and Edwards, Haglund, Insalata, Georgala and Caldwell found that non-specific staining was the principal problem associated with the rapid fluorescent antibody technique. Samples containing debris were also a serious problem.

The purpose of this preliminary study was to develop a procedure for the rapid detection of Salmonellae in rendered byproducts and feeds using FA techniques.

This paper describes the formulation and use of a Salmonella, genus specific conjugate composed of 38 salmonella H antigen agglutinins. (See Figure 4.) The procedure employed combines bacterial concentration, selective enrichment, and the FA method.

MATERIALS AND METHODS

Sources of Salmonellae

Animal byproduct samples were obtained from rendering plants by Minnesota Animal Health Division field personnel. Feed samples were provided by the Animal Services Unit of the National Animal Disease Laboratory.

Salmonella and E. coli serotypes and other bacterial species were provided by the Diagnostic Bacteriology Unit and the Salmonella Reference Center, Diagnostic Services, NADL.

Serums

Salmonella polyvalent H antiserums were obtained from the Biological Reagents Section, Communicable Disease Center, Atlanta, Georgia. (These serums were produced following the protocol listed in the references.) Serums are not difficult to produce providing the recommended cultures are used and the method
of inoculation and bleeding of rabbits is strictly adhered to. A polyvalent H antiserum may be obtained from Difco* but it lacks 16 H antibodies which were present in the serum labeled in this study. In these preliminary studies, the serums produced by the Biological Reagents Section, CDC, were used to conserve time. The serum contained 50 percent glycerine as a preservative so it was necessary to dialyze it against distilled water for 4 to 6 days or until tests for glycerine were negative. The original volume was then restored by pressure dialysis. Similar serums without glycerine are now being prepared by Diagnostic Services at NADL.

Preparation of Globulins

Globulin fractions containing active antibody against 38 H antigens were prepared by precipitation of polyvalent H serum with half saturated ammonium sulfate.

The final precipitate was dissolved in an equivalent volume of cold distilled water and dialyzed at 4 degrees C. against physiologic saline solution to remove the ammonium sulfate. The globulin was conjugated according to the method of Coons. Unconjugated fluorescein was removed by dialysis for 3-5 days using .01 M phosphate buffered saline pH 7.6 at 4 degrees C. or by passing the labeled globulin through a G25 Sephadex column. When the conjugate appeared weak in staining, concentration by pressure dialysis increased the intensity by concentrating the labeled globulin.

Preparation of Slides

Slides were prepared by taking a loop of culture or bacterial sediment and making a circular thin smear within an inscribed area on a clean, non-fluorescing glass slide. Smears were allowed to air dry at room temperature and were heat fixed. After fixation, a drop of conjugate was placed on the smear and the slide was incubated in a moist chamber for 30 minutes at 37 degrees C. After incubation the slides were washed by passing through two changes of phosphate buffered saline, 30 minutes each, with a final rinse in distilled water. Counterstain was applied according to the method of Hall with the chelated azo dye, flazo orange. Smears were examined using the 54 X oil objective. Buffered glycerine was applied to the smear and a coverslip was added.

Microscopy

Slides were examined for fluorescing organisms with a Leitz Ortholux microscope. The filters consisted of a BG 12 blue-exciting filter and an eyepiece blue absorbing filter. The light source was an HBO-200 watt mercury lamp. Salmonella fluoresced a bright yellow-green against a dark field. Other organisms or debris stained an orange color with flazo orange counterstain.

Specificity of Staining Reaction

The specificity of the staining reactions was determined by pretreatment of known salmonella smears with unlabeled specific antibody and by adsorption of

*Difco Laboratories, Detroit, Michigan.
labeled antibody with known salmonella cells. The use of either of these techniques prevented the staining of known salmonella cells. An attempt to stain salmonella strains possessing an H phase, for which the polyvalent H antiserum lacked agglutinins, seemed of interest. It was reasonable to assume that these serotypes would not fluoresce. A Salmonella sp. (unnamed) having the antigenic formula 39:z481,5 in the z48 phase and S mikawashima, 67:y,e,n,z15 z50 in the z50 phase were stained with the conjugate. The counterstain flazo orange was then applied. The slides were examined under the fluorescent microscope.

Enrichment and Concentration

Figure 1 illustrates the method used to enrich and concentrate bacteria from feed and animal byproduct samples. The culture method used was the ARS 91-68, revised 1968, hereafter referred to as the standard method. Brilliant green broth was substituted for tetrathionate in the technique because of the strong reducing effect of tetrathionate which reduces the fluorescein to a colorless compound. The failure of Salmonellae to stain following culture in tetrathionate broth has been reported. The reason for failure of fluorescein staining was not given. A 30-gram sample of feed or animal by-product was placed in a wide mouth jar to which 100 ml. of buffered brilliant green broth was added. After eight hours or overnight incubation at 37 degrees C., approximately 10 ml. of the liquid portion was removed, placed in a tube, and centrifuged at 3,000 r.p.m. for 30 minutes. After centrifugation, the supernatant liquid was discarded and 1.5 ml. of physiologic saline was added to resuspend the sediment.

In the technique described here, brilliant green broth culture is discarded if no growth is apparent after 8-12 hours incubation.

The use of potassium tartrate gradient is illustrated in Figure 2.

A 20 x 100 ml. tube was layered from top to bottom with 40%, 34%, and 26% potassium tartrate and 1 ml. of the bacterial sediment. The percentages of potassium tartrate were not made up by weight-volume. Solutions were prepared according to a special table or by checking optically. After centrifugation at 3,000 r.p.m. for 30 minutes, the Salmonellae and other bacterial species formed a dense layer between the 26% and 34% concentrations of potassium tartrate. The organisms found were mostly gram negative.

The top layer of debris was discarded and 1 ml. of the bacterial layer was suspended in a tube containing 3.5 ml. of saline and 1 ml. of pentane. (See Figure 3). After mixing and centrifugation at 3,000 r.p.m. for 30 minutes, the top layer of solution containing debris was discarded, and slides were prepared from the sediment.

Examination of samples that were FA positive but negative on routine culture was done by extensive culture using several samples and numerous cultures.

Results

Preliminary results on 319 mixed and pure cultures of bacteria are summarized in Table I. Of the 143 salmonella strains examined, only three failed to stain. The three fluorescent antibody negative serotypes were Salmonella pullorum rough antigen strains. Included in the 140 positive staining serotypes were non-motile species Salmonella pullorum, Salmonella gallinarium and 45 additional salmonella
serotypes which are commonly associated with salmonellosis in animals. With the exception of Arizona 7:1,7,8 and 7:1,2,6, which are of interest as animal pathogens and are considered by some workers as subtypes of Salmonella, no non-salmonella bacteria stained. Eight arizona cultures failed to stain. Additional cultures examined included 30 E. coli serotypes, Citrobacter, Proteus, Pseudomonas and 20 other bacterial species.

The fluorescence technique was applied to 200 animal byproduct and feed samples. A sample was considered positive if a bright fluorescing rod-shaped bacterium was observed in more than one field. The presence of Salmonellae was confirmed by the standard method and by culturing a portion of the bacterial sediment used to prepare fluorescent antibody slides by the same method. A comparison of these is summarized in Tables II and III.

In Table II is seen a comparison of the fluorescent antibody technique and the two culture trials. Of the 200 samples examined, 21 percent were positive by the fluorescent antibody technique, 14 percent were positive by the standard method, and 13 percent were positive by sediment culture. (See Figure 3.) The possible lack of homogeneity of the Salmonellae in the sample was considered.

In Table III is seen a further breakdown of the results obtained from the fluorescent antibody and sediment culture trials. Of the 200 samples examined, 24 were positive by both methods, 18 were positive by FA only, 2 were positive by sediment culture only, and 156 were negative by both methods.

To determine the value of the potassium tartrate gradient, pentane, and counterstaining with flazo orange, the supernatant layer from positive tankage samples was divided into three equal amounts. Smears were prepared and examined. Three experiments were performed to illustrate the effect, one using each aliquot. A bacterial smear was prepared without the use of potassium tartrate concentration, pentane, or counterstain. When this was done, large fluorescing particles interfered with observation of bacterial cells to such an extent that it was impossible to determine the presence of Salmonellae. The second slide was prepared using sediment resulting from the potassium tartrate gradient and pentane extraction. The Salmonellae fluoresced and were easily identified. Only a few non-specific fluorescing particles were observed in the slide prepared in this manner. A third slide was prepared from sediment in which the potassium tartrate gradient, pentane and flazo orange counterstain were used. The non-salmonella bacteria and background material stained orange to light red with the flazo orange whereas the Salmonellae appeared a typical yellowish-green.

The contrasting background produced by the flazo orange made it possible to detect small numbers of organisms among large amounts of debris and other bacteria. It was further desired to attempt to prepare a slide from bacterial sediment without the use of pentane or the potassium tartrate gradient. Various species of bacteria and debris were stained by the flazo orange. Only the Salmonellae were observed not to take the flazo orange counterstain and were easily observed to fluoresce the yellow-green color.

Four samples that were negative on routine culture but FA positive yielded Salmonella upon extensive culture attempts.

The Salmonella sp. (unnamed) having the antigenic formula 30:z48 1,5 in the z48 phase and S. mikawashima, 6,7:y-e,n,z15, 250 phase were examined. The
antigen z48 was not stained while *S. mikawashima* having the z50 antigen stained well.

**Discussion**

The failure of three strains of *S. pullorum* to stain was not surprising as these were rough antigen strains that also failed to agglutinate. The fact that any *S. pullorum* or *S. gallinarum* stained is of considerable interest, since these *Salmonellae* are not flagellated and lack H antigen. Studies are now underway to determine the presence of “H” like antigens in these serotypes or the possibility that even low titers (less than 1:10) of “O” agglutinins in the polyvalent “H” antiserum was enough to cause the organisms to fluoresce specifically. More work needs to be done before any conclusions can be reached.

Some *arizona* cultures stained due to crossing to certain H antigens with the salmonella H agglutins. Table IV4 indicates the H antigens of the Arizonae and cross reactions that occur with the *Salmonellae*. Eight arizona cultures failed to stain. This failure could have been due to (1) no cross reacting agglutinins in the conjugate, (2) agglutinins present but at too low a titer to react, and (3) culture having poor motility. No effort was made to assure good motility prior to staining. The cross reaction of the salmonella H agglutinins with the Arizonae is considered important in that these organisms are a common cause of enteritis in man and animals and are therefore clinically related to the Salmonellae. If it is agreed that a conjugate should detect Arizonae in samples of animal byproducts and feeds, a conjugate could be prepared to include all of the arizona a agglutinins that do not cross react with the *Salmonella*. Adsorption of the salmonella polyvalent H antiserum to remove arizona agglutinins that would cross react with *Salmonella* would decrease the staining ability of the conjugate. Attempts are being made to test this.

In order to be uniform and to make the work as reproducible as possible, the standard method was used for routine isolation attempts, with the exception mentioned. More extensive techniques were employed when it was necessary to prove the presence of *Salmonella* in cases where the standard method failed.

Where materials to be examined for *Salmonella* have been heated so that dead organisms might be present in the sample, the question arises whether the technique would detect dead *Salmonella*. Dead *Salmonella* take the fluorescent stain specifically providing they have not been heated to temperatures in excess of 100 degrees C. *Salmonellae*, in rendered byproducts processed in a properly operated cooker, lose their immunologic specificity due to the degradation of the protein. *Salmonellae* that have been autoclaved do not stain with the conjugate.

The presence of fluorescein-staining debris has been a serious problem. Haglund8 reported that debris in egg powder prevented examination of this material for *Salmonella*. Centrifugation and filtering removed the material but allowed the organisms to remain in the filtrate.

Rendered byproduct samples offered a more serious problem in that very small particulate matter auto-fluoresced and made detection of the *Salmonella* impossible. Use of pentane flotation and/or flazo orange counterstain removed the difficulty and allowed debris-free preparations. The use of flazo orange was very useful even without pentane flotation. The counterstained debris did occasionally
make observations of the *Salmonella* difficult. In no case was it impossible to find the salmonella organisms.

The selection of a serum from which to extract the globulin (agglutinins) was based upon several reports in the literature\(^2\),\(^7\),\(^8\),\(^11\) where H agglutinins were used in obtaining varying degrees of specificity for the genus *Salmonella*. The Reference Center, Diagnostic Services, NADL, which is part of Diagnostic Bacteriology, has had extensive experience in the area of salmonella agglutinins. For many years no non-salmonella organisms have been known to agglutinate when tested using a polyvalent H antiserum containing 38 H agglutinins. It was conceivable that if these agglutinins were labeled with FITC, the same specificity would result. Testing the serum for O agglutinins against all O antigens revealed only slight amounts, no level being over 1:10 dilution.

The method of Coons\(^3\) was selected for producing the conjugate after more sophisticated techniques such as dialysis labeling\(^1\) produced poor results.

The fact that it was not found necessary to absorb the final conjugate with bacterial organisms closely related antigenically with the *Salmonellae* (except the Arizonae) was interesting and somewhat surprising. It is possible that the preliminary dialysis of the polyvalent H antiserum against distilled water to remove the glycerine preservative had some effect on the final results.

Results of other workers\(^2\),\(^7\),\(^8\),\(^11\) who used H agglutinins for labeling have reported "specific" staining with certain interpretations placed on the observation of the slide. Fluorescent antibody tests, where a clear cut determination cannot be made, are not very satisfactory for diagnostic purposes. Some have reported the "indirect" test as superior to the "direct." Haglund\(^7\) stated this but did little work on the direct technique. The O-H serum employed in the indirect technique lacked some important agglutinin factors which would mean that the organism being stained would be observed only by its O staining agglutinin. (For example, the H serum lacked agglutinins r and z as well as some of the higher z components.) This would mean that if the O agglutinins for a specific serotype were present, and the serotype was in a given H phase, the organism would be stained only by the O agglutinin and no H staining would be expected to occur. Such would be the case with *S. remo*, *S. bradford*, *S. heidelberg*, and *S. infantis* existing in the r phase. If the z agglutinins were omitted, a salmonella such as *S. indiana* would fail to be stained by H agglutinins. When working with fluorescent conjugates against *Salmonella*, it is important to be very aware of the agglutinins included in the starting serum. Examples of this are given above. When an O-H antiserum is employed, the value of the combination is somewhat lost if factor agglutinins for any anticipated serotypes are lacking in either O or H.

If staining is weak, it is possible to increase intensity by concentrating the labeled globulin through pressure dialysis.

Brilliant green broth was used as an enrichment. Tetrathionate broth was found acceptable by one worker\(^7\) providing the culture was transferred to a mannitol broth (Hajna & Damon, 1950) and then examined by the FA technique. They found that when cultures were examined directly from tetrathionate broth, poor staining resulted. This phenomenon was probably due to the strong reducing action of tetrathionate, reducing the dye fluorescein, to a colorless compound.

For confirmation of FA results, the standard method was followed. It was
recognized that when numbers of *Salmonellae* in a given sample were low, the standard techniques for isolation of *Salmonella* failed. Levels of detection for various bacterial organisms have been established and it is recognized that when too few are present, the chance of finding them is appreciably reduced. Studies are under way in which it is hoped to relate the number of salmonella organisms found per 54 X field to the number in the original sample prior to enrichment culture. Where FA was positive and the standard method was negative, 1-5 salmonella organisms were seen per 54 x field. It is estimated that the number of organisms in these samples was below the level of detection by cultural methods. Vigorous efforts resulted in some recoveries of *Salmonella* where the standard method had failed and the FA technique was positive. The authors have little doubt that the FA technique will find more *Salmonellae* positive specimens than can be found by conventional culture methods. More work is being done to support this observation.

**Summary and Conclusions**

Studies including 319 pure and mixed cultures indicate that the conjugate prepared from a polyvalent H antiserum containing 38 H agglutinins was *Salmonella* genus specific, including the related Arizonae. Examination of 200 animal byproducts and feed samples indicated that the fluorescent antibody method was more sensitive than culture methods employed. This was attributed to the fact that *Salmonella* were observed in samples in numbers below the level of detection by cultural methods. This procedure provided a rapid method for detecting *Salmonella* in feeds and animal byproducts, reducing the time required to approximately 9-12 hours.

**Acknowledgements:** The authors would like to thank Dr. Billie O. Blackburn, Leader, Salmonella Reference Center, Diagnostic Services, NADL, for his assistance, Mrs. Velma Karns and Mrs. Mariam Fetters for technical help.

### TABLE I

CONJUGATE STAINING REACTIONS WITH VARIOUS BACTERIA

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. of Cultures</th>
<th>No. of FA Results</th>
<th>% of Total Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
<td>1,143</td>
<td>140 4 3</td>
<td>98.1</td>
</tr>
<tr>
<td>Arizona spp.</td>
<td>32</td>
<td>24 8</td>
<td>75</td>
</tr>
<tr>
<td>E. coli</td>
<td>244</td>
<td>0 44</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>10</td>
<td>0 10</td>
<td>0</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>9</td>
<td>0 9</td>
<td>0</td>
</tr>
<tr>
<td>Other spp.</td>
<td>381</td>
<td>0 81</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>319</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Includes 46 *Salmonella* serotypes.

2 Includes 30 *E. coli* serotypes.

3 Includes 20 different bacterial spp.

4 *Salmonella pullorum* antigen strains.
TABLE II
COMPARISON OF FA AND CULTURAL PROCEDURES

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive</th>
<th>Negative</th>
<th>% of Total Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent antibody</td>
<td>42</td>
<td>158</td>
<td>21</td>
</tr>
<tr>
<td>Conventional (ARS 91-68) (from raw material)</td>
<td>28</td>
<td>172</td>
<td>14</td>
</tr>
<tr>
<td>Sediment culture only</td>
<td>26</td>
<td>174</td>
<td>13</td>
</tr>
</tbody>
</table>

TABLE III
FLUORESCENT ANTIBODY (FA) AND CULTURAL RESULTS

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>FA Positive</th>
<th>Culture Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>156</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>42</td>
<td>26</td>
</tr>
</tbody>
</table>

TABLE IV
RELATIONSHIPS OF THE H ANTIGENS OF ARIZONA AND SALMONELLA

<table>
<thead>
<tr>
<th>Arizona H Antigen</th>
<th>Related Salmonella H Antigen</th>
<th>Arizona H Antigen</th>
<th>Related Salmonella H Antigen</th>
<th>Arizona H Antigen</th>
<th>Related Salmonella H Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>z4</td>
<td>21</td>
<td>z35</td>
<td>30</td>
<td>1,5</td>
</tr>
<tr>
<td>2</td>
<td>z23</td>
<td>22</td>
<td>k</td>
<td>31</td>
<td>z</td>
</tr>
<tr>
<td>3</td>
<td>z24</td>
<td>23</td>
<td>L z13</td>
<td>32</td>
<td>c</td>
</tr>
<tr>
<td>5</td>
<td>z26</td>
<td>24</td>
<td>r</td>
<td>33</td>
<td>i</td>
</tr>
<tr>
<td>10</td>
<td>z32</td>
<td>26</td>
<td>z52</td>
<td>35</td>
<td>a</td>
</tr>
<tr>
<td>13</td>
<td>g,p,z51</td>
<td>27</td>
<td>z10</td>
<td>39</td>
<td>z47</td>
</tr>
<tr>
<td>16</td>
<td>z29</td>
<td>28</td>
<td>e,n</td>
<td>42</td>
<td>z50</td>
</tr>
<tr>
<td>20</td>
<td>z36</td>
<td>29</td>
<td>k</td>
<td>43</td>
<td>b</td>
</tr>
</tbody>
</table>

NOTE: See reference 4.
SALMONELLA IN ANIMAL BYPRODUCTS, ETC.

Figure 1.

30 Gm. Sample

Add 100 ml. Brilliant Green Broth (buffered).

Incubate 37º C., 8 hours. Remove supernate.

Centrifuge 3000 rpm, 30 minutes

Pour off supernate.

Figure 2.

Resuspend sediment in 1.5 ml. saline.

Layer 1 ml. sample on top of 26% K. tartrate gradient.

Centrifuge 3000 rpm, 30 minutes

Remove debris and bacteria then next ½ inch above and below the 26% layer.

DEBRIS

26%

BACTERIA

34%

40%

Place 1 ml. from previous tube in a 20 x 100 ml. tube.
Add 3.5 mL saline and 1.5 mL Pentane. Mix well.

Centrifuge 3000 rpm, 30 minutes. Decant.

Remove bacterial sediment and prepare smear.

Air dry and fix.
Stain with FA conjugate.
Examine for fluorescing bacteria.

Figure 3.
ORGANISMS USED IN THE PREPARATION OF SALMONELLA DIAGNOSTIC SERA, FLAGELLAR

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Serotype or Strain</th>
<th>Antigen</th>
<th>Serotype or Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>S. paratyphi A</td>
<td>24, 223</td>
<td>S. cerro</td>
</tr>
<tr>
<td>b</td>
<td>S. paratyphi B, phase 1</td>
<td>24, 224</td>
<td>S. dusseldorf</td>
</tr>
<tr>
<td>c</td>
<td>S. cholerae-suis, phase 1</td>
<td>24, 232</td>
<td>S. tallahassee</td>
</tr>
<tr>
<td>d</td>
<td>S. typhi</td>
<td>26</td>
<td>S. kentucky, phase 2</td>
</tr>
<tr>
<td>e, h</td>
<td>S. reading, phase 1</td>
<td>210</td>
<td>S. illinois, phase 1</td>
</tr>
<tr>
<td>e, n, x</td>
<td>S. abortus-equ 2</td>
<td>227</td>
<td>S. simsbury (S. senftenberg, phase 2)</td>
</tr>
<tr>
<td>e, n, z15</td>
<td>S. sandiego, phase 2</td>
<td>270</td>
<td>S. tennessee</td>
</tr>
<tr>
<td>f, g</td>
<td>S. derby</td>
<td>235</td>
<td>S. chittagong, phase 2</td>
</tr>
<tr>
<td>g, m</td>
<td>S. enteritidis</td>
<td>236</td>
<td>S. wescaco</td>
</tr>
<tr>
<td>g, m, s</td>
<td>S. montevideo</td>
<td>237</td>
<td>S. wickha, phase 2</td>
</tr>
<tr>
<td>g, p</td>
<td>S. dublin</td>
<td>238</td>
<td>S. lille</td>
</tr>
<tr>
<td>g, p, u</td>
<td>S. rostock</td>
<td>239</td>
<td>S. quimbamba, phase 2</td>
</tr>
<tr>
<td>g, q</td>
<td>S. moscow</td>
<td>241</td>
<td>S. karanjina, phase 1</td>
</tr>
<tr>
<td>g, s, t</td>
<td>S. senftenberg, phase 1</td>
<td>242</td>
<td>S. bunnick, phase 2</td>
</tr>
<tr>
<td>g, (p)251</td>
<td>S. wayne</td>
<td>243</td>
<td>S. senftenberg, 2170-58, phase 2*</td>
</tr>
<tr>
<td>i</td>
<td>S. bonariensis, phase 1</td>
<td>244</td>
<td>S. quinhon, phase 1</td>
</tr>
<tr>
<td>k</td>
<td>S. thompson, phase 1</td>
<td>245</td>
<td>S. senftenberg, 2282-58, phase 2*</td>
</tr>
<tr>
<td>l, v</td>
<td>S. london, phase 1</td>
<td>246</td>
<td>S. senftenberg, 840-59, phase 2*</td>
</tr>
<tr>
<td>l, w</td>
<td>S. dar-es-salaam, phase 1</td>
<td>247</td>
<td>S. mikawashima, 2547-60, z47 phase</td>
</tr>
<tr>
<td>l, z13</td>
<td>S. uganda, phase 1</td>
<td>249</td>
<td>S. infantis, 2783-61, z49 phase*</td>
</tr>
<tr>
<td>l, z28</td>
<td>S. javana, phase 1</td>
<td>250</td>
<td>S. mikawashima, 2547-60, z50 phase*</td>
</tr>
<tr>
<td>l, z40</td>
<td>S. rutgers, phase 1</td>
<td>252</td>
<td>S. tulear, phase 2</td>
</tr>
<tr>
<td>m, t</td>
<td>S. oranienburg</td>
<td>1, 2</td>
<td>S. paratyphi B, phase 2</td>
</tr>
<tr>
<td>r</td>
<td>S. rubislaw, phase 1</td>
<td>1, 5</td>
<td>S. thompson, phase 2</td>
</tr>
<tr>
<td>y</td>
<td>S. mikawashima, phase 1</td>
<td>1, 6</td>
<td>S. anatum, phase 2</td>
</tr>
<tr>
<td>z</td>
<td>S. poona, phase 1</td>
<td>1, 7</td>
<td>S. bredeney, phase 2</td>
</tr>
</tbody>
</table>

*S. senftenberg may occur with a variety of antigens in phase 2.

FIGURE 4

REFERENCES

A REVIEW

The eperythrozoa and hemobartonella parasites of animal erythrocytes do not, at the present time, appear to be credited with the economic significance and importance as disease producing agents which they deserve. The confusion and uncertainties of identification and classification, together with what is probably an inadequate record of low mortality, has contributed to a rather general unawareness of their prevalence and significance. It is believed that with better means of recognition and realization of their prevalence, appreciable mortality, economically significant values, and implications of influence on experimental work with animals will become evident.

The history of these blood parasites is rather interesting in that it originates in the intensive study of an endemic human disease seen for a long time. A monograph by Weinman (1944) gives details of the story covering a period of many years. Portions of his report bear reviewing to better understand the complexity of the group of blood parasites as it was originally organized. The human disease was known for a long time as verruga peruana, characterized by high morbidity, low mortality, but incapacitating cutaneous infection, and may be the disease referred to as a rather serious handicap to the Spanish in reports of their conquests in northwest South America in 1531.

The disease is also known as Carrion's disease in memory of an investigator who inoculated himself in 1885 in order to better understand the early symptoms and course of the disease. This experiment terminated fatally 37 days after the inoculation. In 1905 and 1909 the cause of Carrion's disease was described by Barton and the disease also became known as bartonellosis. For a time thereafter, what appeared to be similar blood infections in several species of animals were also referred to as bartonellosis. In the same areas where verruga was prevalent, what was apparently another disease was common. It was known as Oroya fever and was characterized by severe anemia and high mortality. In 1926 Noguchi and Battistini (1926) were able to show that cultures of either Oroya fever or verruga patients contained the same microorganism and a single agent as the cause of two clinically different diseases was finally proven in 1937 by the Harvard Expedition to Peru. Later work with the agent shows that it is a true bacterial organism (Peters and Wigand, 1955) although it is still classified with the Order Rickettsiales.

Eventually, the classification of non human, animal blood parasites other than protozoa had to be revised. Bergey's Manual (1957) includes them in the Order Rickettsiales. It should be pointed out that Dr. David Weinman prepared the revision of the Family Bartonellaceae in 1955 and he states that it is fundamentally
unsatisfactory because of inadequate knowledge. For further consideration this review will be concerned with only two genera, Haemobartonella and Eperythrozoan, of the Family Bartonellaceae.

Some significant features of these particular erythrocytic parasites can be found in Weinman's (1944) monograph. One of the features is that many of the type species of the genera were first found as a result of their presence in blood which was being studied for other, usually protozoan, blood parasites. Another feature is their pleomorphism and general similarity in appearance, and, to some extent, in staining properties. The distribution of the parasites is world wide and it is significant that numerous species of animals are hosts to some member of the parasite species. Eperythrozoa infects several species of animals. For example, Ohder (1967) cites references reporting the occurrence of eperythrozoons in sheep, cattle, deer, elk, swine, mice, dogs, and cats. Pigeons (cited by Weinman, 1944) appear to be susceptible to inoculation with the Haemobartonella of rats. Other references cited by Weinman (1944) describe the possible presence of haemobartonella in cold blooded animals such as tortoise, frog and lizard.

Latent infections and premunitive immunological states are the rule with these parasites. Clinically observable cases and detectable antibody response are of relatively short duration and recurrences are common. Latency can be activated by splenectomy but observations on the fluctuation of numbers of parasites present in the blood and hemoglobin levels indicate that other causes can also disturb the latency. One such cause is concurrent infection with other microorganisms (Ohder, 1967; Weinman, 1944; Wilson, et al 1968). Some cases of nonsplenectomized but otherwise activated latent infections terminate fatally with severe anemia. Eperythrozoan infection in swine is an excellent example of such cases. This is based on the fact that examination of blood from many newly farrowed pigs almost invariably shows the presence of at least a few parasitized cells. This implies that the infection is very common but latent in swine. But clinical evidence and mortality due to the infection is much less frequent than its implied incidence. The lower incidence of clinical and fatal cases also illustrates the previously mentioned feature of concurrent infections. A few years ago the presence of acute eperythrozoonosis and cholera was fairly common, but almost any systemic infection of swine may activate the latent eperythrozoons. The very high incidence in newly farrowed pigs also implies that intrauterine infection occurs in swine and the same type of evidence has been reported by Najarian (1961) for Haemobartonella infection in the Mongolian gerbil. Thurston (1954) also favors the possibility of intrauterine eperythrozoan infection in mice. Another example of nonsplectomized fatal eperythrozoan infection is that reported by Littlejohns (1960) in sheep. Eperythrozoan latency was noted by Howe, et al., (1965) in mule deer and elk and may have been responsible for many false positive complement fixation tests for anaplasmosis in antelope and elk.

In Iowa, instances of fatal termination of latency breaks have been observed in cattle, elk, sheep, and dogs. In each case the parasite involved was believed to be eperythrozoan species. Except for the elk, concurrent complications were not evident. Post mortem examination of the two year old male elk showed necrotic hepatitis, congestive nephritis, and an abdominal hernia which contricuted to his condition. Crocker and Sutter (1954) reported both mortality and economic loss in
eperythrozoon infected cattle. Although there are many reports of eperythrozoon infection in several species of animals, except for the generally recognized losses in swine and the previously mentioned report by Littlejohns (1960) involving sheep, mortality reports in animals under natural conditions are scarce. The features of latency and concurrent infections common to these parasites probably influences such scarcity but evaluation of these and other contributing factors is hampered by inadequate information.

An important characteristic of eperythrozoons is their ability to interfere with the normal course of other blood parasites or disease agents. In some instances the interference amounts to a replacement of some disease agent by the eperythrozoons. Ohrer (1967) reported E. ovis interference and replacement of Bovine Petechial Fever agent (a rickettsia-like organism) during experimental work with sheep. Tyzzer (1941) noted an antagonistic effect of E. coccoides in modifying the usually severe results of haemobartonella infection in the deer mouse. Foote, et al., (1957) reported a reciprocal interference between eperythrozoons and anaplasma in experimental calves. The summary of results of their work is quite clear and definite, but the reason for, and mechanism of, the interference is a matter for speculation. Information available up to this time does not warrant much speculation but the report section of this article may stimulate development of additional information to broaden our outlook on these parasites.

A REPORT

Bennett (1967) reported efforts to use the capillary agglutination test for anaplasmosis to distinguish between positive reactions due to anaplasmosis and eperythrozoanosis. This report is a continuation of observations toward the same objective. In order to show that such differentiation can be made several interrelated factors will be illustrated and discussed briefly. The factors can be listed as follows:

1. A group of individual bovine animals can be anaplasmosis free and show positive CA tests.
2. A particular bovine genetic blood type can influence some CA positive tests.
3. Positive test results due to eperythrozoons and/or blood type will vary over relatively short periods of time.
4. Positive test results due to anaplasma have a relatively high titer.
5. Positive test results due to eperythrozoons have a relatively low titer.
6. Agglutinin due to anaplasma infection is heat and time stable.
7. Agglutinin due to eperythrozoan infection is heat and time unstable.
8. Eperythrozoan agglutinins are stable to heating at 42°C.
9. Eperythrozoan infection is very common in cattle.

During a period of 11 months a small herd of dairy type cows was tested five times at irregular intervals by both CA and CF methods. At no time did the CF test results lead to interpretation of positive anaplasmosis, however, on three of the tests a single result was classified as suspicious and this involved a different individual each time. The next succeeding herd test gave negative results for two of the individuals while the third suspicious result occurred at the time of the final
herd test. Table 1 illustrates the series of tests by both CF and CA methods on the three animals.

INDIVIDUALS WITH SUSPICIOUS CF TEST RESULTS

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>CF</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>2+S</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>27</td>
<td>CF</td>
<td>N</td>
<td>1+S</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>CF</td>
<td>2+S</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1**

When the herd samples were tested by the standard CA method positive reactions were present in four of the five tests. The date of the test, the number tested, and the percentage of positive results are shown in Table 2.

EPERYTHROZOON HERD INCIDENCE

STANDARD CA TEST

<table>
<thead>
<tr>
<th>DATE</th>
<th>NUMBER TESTED</th>
<th>PER CENT POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 JUNE 1967</td>
<td>17</td>
<td>23.5</td>
</tr>
<tr>
<td>23 AUG. 1967</td>
<td>18</td>
<td>27.7</td>
</tr>
<tr>
<td>25 OCT. 1967</td>
<td>23</td>
<td>78.25</td>
</tr>
<tr>
<td>3 JAN. 1968</td>
<td>20</td>
<td>00.00</td>
</tr>
<tr>
<td>30 APR. 1968</td>
<td>21</td>
<td>9.5</td>
</tr>
</tbody>
</table>

**TABLE 2**
The same information rearranged to show seasonal incidence is graphically illustrated in Chart 1.

Some of the positive test results that occurred at the time of the October test are probably influenced by a genetic blood type. The commercial antigen used in the tests contained fragments of erythrocytes which were coated with bovine blood type "J" positive substance. Naturally occurring anti "J" serum of some individuals is at its highest antibody content at approximately this time of the year and it is known that several "J negative" animals were present in the herd. This does not mean that blood type is highly significant in the test results but whatever influence is exerted is more effective at this time of the year. After this annual peak the natural antibody level goes through its cycle of gradual decrease and gradual increase. There is a more frequent but irregular change in eperythrozoon antibody level in each infected individual. In other words, eperythrozoon antibody level follows an intermittent or fluctuating pattern as detected by test results. This is illustrated in Table 3 giving individual identification, "J" blood type status, and test results.
PATTERN OF EPERYTHROZOON TEST REACTIONS

<table>
<thead>
<tr>
<th>IDENTIFICATION</th>
<th>3</th>
<th>27</th>
<th>12</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAN. 3</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>APR. 30</td>
<td>P</td>
<td>NT</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>JUN. 1</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>AUG. 23</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>OCT. 25</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
</tbody>
</table>

N = Negative test reaction  
P = Positive test reaction  
NT = Not tested

In these examples the longest period that a positive reaction persisted was roughly 4 or 5 months in animal 22, while the shortest period in animal 12 was probably 1 or 2 months.

With a serum dilution system to establish end titers by the CA method, anaplasmosis infected individuals (all from field cases) have consistently shown higher titers than eperythrozoon infected cattle. With the 2 fold dilution system in use at the Iowa Diagnostic Laboratory the eperythrozoon titers are seldom more than a 1 in 4 dilution while the anaplasmosis titers are not less than a 1 in 8 dilution. More significantly, anaplasma antibody is quite stable while eperythrozoon antibody is very unstable. A bovine serum sample that gives a positive CA test for anaplasmosis can be stored frozen for several months, thawed and retested with positive results. Some samples can be kept for as long as four years and still give a good CA positive reaction. This is far from true with eperythrozoon antibody. Explanation of this fact requires additional information on the five herd tests. On each of two of the five tests (August and October) two or more blood films were prepared from each individual, stained with Giemsa and acridine orange (AO) stains and examined with a 97X oil immersion objective and appropriate bright field and dark field condensers. From these examinations each and every individual was found to be infected with eperythrozoons.

Standard technique in using the CA test requires that the serum be heated at 56°C for 30 minutes. The reason given for this requirement is elimination of non-specific reactions. This is alright but if there are non-specific reactions there should be some explanation for them. Routine testing of the herd samples before the serum was heated showed many positive reactions that disappeared after the required heating. Since all the animals were infected with eperythrozoons and there is some ill defined relationship between eperythrozoons and anaplasma, the "non-specific", or more accurately, the "nonanaplasmosis" reactions must be caused by eperythrozoon antibody. Most, but not all, of this antibody was apparently inactivated by heating at 56°C. However, this antibody is not similarly inactivated by heating at 42°C for 30 minutes and there is excellent agreement in test results of the serum samples before and after 42°C heating. The use of test procedures with unheated or 42°C heated serum is quite helpful in differentiating between anaplasma and eperythrozoon infections in cattle.
By showing test results of the unheated serum method in the same way as shown for the standard CA test results, Table 4 and Chart 2 can be compared with Table 2 and Chart 1. Animals and times are equal, the only difference between the two sets of data is 56°C heated serum (Table 2 and Chart 1) and unheated serum (Table 4 and Chart 1).

### EPERYTHROZOON HERD INCIDENCE

**UNHEATED SERUM CA TEST**

<table>
<thead>
<tr>
<th>DATE</th>
<th>NUMBER TESTED</th>
<th>PER CENT POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 JUNE 1967</td>
<td>17</td>
<td>58.8</td>
</tr>
<tr>
<td>23 AUG. 1967</td>
<td>18</td>
<td>88.8</td>
</tr>
<tr>
<td>25 OCT. 1967</td>
<td>23</td>
<td>73.9</td>
</tr>
<tr>
<td>3 JAN. 1968</td>
<td>20</td>
<td>70.0</td>
</tr>
<tr>
<td>30 APR. 1968</td>
<td>21</td>
<td>75.0</td>
</tr>
</tbody>
</table>

**TABLE 4**

The unheated test results gives a much different picture of eperythrozoon incidence. Remembering that an anaplasmosis free herd is being used and that
standard CA test results represent eperythrozoon antibody, the official CA test indicates a yearly average level of 27.8% incidence. The unheated test results show a yearly average level of 73.3% incidence which is much closer to the actual 100% shown by stained blood films. The test loss from 100% is largely explainable on the basis of characteristics of the eperythrozoon infection, including low pathogenicity, latency, and irregularity of antibody levels.

The 56°C serum heating procedure is not the most important reason for apparent inactivation of eperythrozoon antibody. The reason for a more important cause has not yet been investigated and is obscure but its action and effect can be perfectly illustrated by using results of the October herd test. Table 5 outlines the results obtained with serum from each animal. The fresh sera were first used to make CA tests with the unheated serum. A small portion of each serum was then identified and heated at 56°C for 30 minutes. The remainder of the sera was then stored in a 38°F refrigerator. The heated portion was used to make the CA test by standard procedure. As shown in Table 5 there was almost perfect agreement between the unheated and the heated test results. (This occurred only in the October test.)

HERD TEST, OCTOBER 1967

<table>
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<tr>
<th>IDENT.</th>
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<th>NOT HEATED</th>
<th>IDENT.</th>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>OCT. 27</td>
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<td>N</td>
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<td>P</td>
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<td>N</td>
</tr>
<tr>
<td>19</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = Negative test reaction  
P = Positive test reaction

TABLE 5

On October 27 the unused sera was removed from the refrigerator and used, without heating, to retest each sample. The retest gave negative results for each and every sample thus dropping the percentage of positive reactions from more than 70 to zero in a period of approximately 48 hours. This type of occurrence has been
observed frequently with routine field samples submitted for anaplasmosis testing. Many of these field cases will show a positive reaction by standard procedure only with undiluted serum and then give negative retests in approximately 24 hours. With these routine tests the serum used for retesting is not refrigerated and remains at room temperature between the time it is prepared for initial test and the time results are observed the following morning. In some instances weak positive or suspicious reactions may persist for 3 or 4 days before the agglutinins finally become visibly inactive. Whatever the reason for this apparent very short time agglutinability may be, it is evidently operative whether or not the serum is heated.

The eperythrozoon incidence in the herd tests previously mentioned does not represent field incidence, so a small survey of field samples was made. The samples used were all of bovine origin submitted for routine brucellosis testing. As far as could be determined the animals involved would be considered as in normal health. On six occasions between May 27 and August 8, 1968, random samples were taken and CA tested by three variations. The serum of each sample was divided into three portions, one was not heated, one was heated 30 minutes at 42°C, and the third was heated 30 minutes at 56°C. Table 6 shows the test results of each variation.

### RANDOM FIELD SAMPLES

**CA TESTS**

**SIX SAMPLINGS BETWEEN MAY 27 AND AUGUST 8, 1958**

<table>
<thead>
<tr>
<th>Total Tests</th>
<th>293</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive, not heated</td>
<td>144</td>
</tr>
<tr>
<td>Positive, heated 42°C</td>
<td>153</td>
</tr>
<tr>
<td>Positive, heated 56°C</td>
<td>21</td>
</tr>
</tbody>
</table>

**Table 6**

The total number of samples tested is not large enough to be highly significant but does indicate that the incidence is quite high. Even though eperythrozoon antibody can be detected with the CA anaplasma antigen a better test method and antigen is desirable for study of the infection in various species of animals.

**A SUMMATION**

Antigen used for the CA anaplasmosis test is reactive with serum of animals other than cattle. Positive test results have been observed at the Iowa Diagnostic Laboratory using serum from swine, deer, elk, sheep, cat, and dog. With cattle, deer, elk, and sheep the positive test might be attributed to anaplasma infection. With the finding of parasitized erythrocytes in stained blood films, the positive test with swine serum would be regarded as due to eperythrozoons. In the dog and cat the cause might be either eperythrozoons or hemobartonella but let's assume in this case that it is eperythrozoons. All of these animals are susceptible to eperythrozoon infection. Swine, cats, and dogs are never considered to have anaplasma infections. One of the characteristics of eperythrozoa is their occurrence in a wide variety of
animals. Anaplasma however, occurs only in a restricted group of the same animals. Explanation of why CA positive tests occur with the sera of swine, dogs, and cats might be that the antigen contains both anaplasma and eperythrozoons. In view of the propensity of eperythrozoons for mixed infections, such explanation might be correct but I think it is inadequate.

Some instances of eperythrozoon interference with other disease agents were mentioned earlier. In addition, Ishihara (1962) describes an interference and replacement of Gonderia (protozoa) by eperythrozoons during experimental work with calves. The reported instances of such interference involves four disease agents, Bovine Petechial Fever, (rickettsia), Hemobartonella, Anaplasma, and Gonderia, in three species of animal hosts, sheep, deer mouse, and calves, and all during experimental investigations.

The circumstances of having very few such reports, the widespread distribution of eperythrozoons, the variety of animals susceptible to these parasites, their usually high rate of incidence, their adaptability to mixed infections, and their interference properties, suggests that they may be responsible for effects which are not yet recognized. A common result of their interference is a significant delay in the expected results following inoculation with another agent in eperythrozoon infected individuals as compared with the non-eperythrozoon infected. Latency and the probability of its activation by concurrent infections can also cause failures in experimental work with infectious agents. When this occurs some cases may be severely anemic and fatal, while many will recover with a return to the latent state for recurrent activity at possibly short but indefinite times.

The full influence and effect of eperythrozoon infection in cattle is difficult to evaluate unless their presence or absence can be determined with a reasonable degree of assurance. One of the problems to be solved is due to the state of latency which can be rather lengthy. Foote, et al., (1957) reported a period of 243 days of latency in an experimental calf. Examination of stained blood smears from even a few animals needed for investigative work becomes a tedious, time consuming task. Another problem is the intermittency of serological test reactions. Although the currently used CA test for anaplasmosis can be used to identify eperythrozoon antibody, multiple tests of individual animals over a period of time may be required for the desired degree of assurance on presence or absence of the parasites. In the event a positive CA test is secured the differential serum dilution method which has been described is usable only with cattle serum. Positive test results with swine serum will give a higher average titer than that for bovine eperythrozoonosis.

Solutions to many of the problems created by the confusing and uncertain relationships between Hemobartonella, Eperythrozoon, and Anaplasma, could be aided by more specific methods of recognition and identification. The eperythrozoon is the most common member of this group and infects more economically important, wild and domestic animal species than the others. It deserves and has earned a reputation as a "bad actor" but has been able to mask or withhold sufficient evidence for conviction on the extent and even the nature of some of its damaging effects.
REFERENCES

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October 12-17, 1969
SHERATON-SCHROEDER
Milwaukee, Wisconsin

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October 18-23, 1970
HOTEL WARWICK
Philadelphia, Pennsylvania